STRUCTURE AND FUNCTION OF THE HUMAN MICROBIOME

by

Marina Lorna Ritchie

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
December 2011

DALHOUSIE UNIVERSITY

DEPARTMENT OF BIOLOGY

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "STRUCTURE AND FUNCTION OF THE HUMAN MICROBIOME" by Marina Lorna Ritchie in partial fulfilment of the requirements for the degree of Master of Science.

Dated: December 12th , 2011

Supervisor: _____

Readers: _____

_____

_____

DALHOUSIE UNIVERSITY

DATE: December 12th , 2011

AUTHOR: Marina Lorna Ritchie

TITLE: STRUCTURE AND FUNCTION OF THE HUMAN MICROBIOME

DEPARTMENT OR SCHOOL: Department of Biology

DEGREE: MSc CONVOCATION: May YEAR: 2012

_____

Signature of Author

In loving memory of my grandfather Dr. Wilhelm Esslinger who taught me that success comes from hard work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Humans harbour a diverse suite of microorganisms in and on their bodies. These microorganisms collectively amount to 10 times more cells than human cells in the body, and their combined genomes have more than 100 times more genes than the human genome does. Despite our understanding of the composition, diversity, and abundance of microorganisms of the human body, it is surprising how little we know about the structure and function of the human microbiome. Here, I use network structure to describe interactions among human-associated microbiota and the human body by exploring differences in structure of human microbiomes across five regions of the body and the robustness of these networks to perturbations. My results show that positive interactions among microbiota are extremely important in structuring microbiome networks and those structural aspects of microbiome networks play a major role in their response to perturbations.

LIST OF ABBREVIATIONS USED

AAD	Antibiotic Associated Diarrhea
a_w	Water activity
C	Connectance
CC	Clustering coefficient
CDD	*Clostridium difficile* Disease
CFU	colony-forming unit
GI	Gastrointestinal
GIT	Gastrointestinal tract
HPP	*Helicobacter pylori* infection
I^2	Heterogeneity
IBS	Irritable Bowel Syndrome
ID	Infectious Diarrhea
IPAA	ileal pouch anal anastomosis
L	Links
lc	Least connected
LGG	*Lactobacillus rhamnosus* GG
L/NS	Links/Node
mc	Most connected
NEC	Necrotizing Enterocolitis
NICU	neonatal intensive care unit
NS	Nodes
P	P value

Pouch	Pouchitis
R_{50}	robustness
ran	random
RR	Relative risk ratio
spp.	Species
TD	Traveler's Diarrhea

ACKNOWLEDGEMENTS

This thesis would not have been completed without the support of a number of incredible people. First and foremost, I would like to thank Dr. Tamara Romanuk for being such an amazing supervisor. You taught me so many things, gave me many great experiences, and are a role model and friend. I could not have asked for a better experience.

I would like to thank Karla Armsworthy for helping with the decisions on the methods and assembly of the microbiome networks. Your contribution to my research was very impressive for an undergraduate honors student. Likewise, I would like to thank Grace Murphy for helping me make it through this process. Your support as a lab mate and friend could not have been more perfect. Thanks to Mather Carscallen, Grace Murphy, and Jen Gauthier for their support as lab mates and providing helpful comments throughout these past two years. Thanks to all members of the Food Web Lab, for making the LSC a great working environment.

I would also like to thank Dr. Sina Adl, Dr. Song Lee and Dr. Dave Shutler for agreeing to be on my supervisory committee.

Thanks to all of my friends. You have kept me sane these past two years. Finally thanks to my family for all the love and support a person could ask for. Thank you so much Mom and Dad; I could not have completed this without your kindness, generosity and love. Thanks for being the best parents. Thanks to my brothers Owen and Daniel for always being there. Thanks to Gram and Gramp, and Grannie for being such supportive grandparents and thanks to Matt and Andy for providing me with helpful comments and support.

CHAPTER 1

Introduction

1.1 Introduction

Interest in the human microbiome began with the first observation of dental microbiota by Antonie van Leeuenhoek using a microscope in 1676 (Gonzalez et al., 2011). Since then, scientists have been studying the composition, abundance, and diversity of microbiota on and in the human body. My research takes this descriptive knowledge to the next level by looking at the structure and function of human-associated microbiota from a network perspective. How energy flows through an ecosystem is one of the most fundamental ways to view the structure and function of ecological communities. By integrating this ecological perspective into research on the human microbiome, I will be exploring a potentially powerful new paradigm in human health, which views the human organism as a superorganism (or ecosystem). The goal of this chapter is to provide the rational for studying structure and function of human microbiomes, to provide the necessary background information, and to highlight some of the implications of viewing the human microbiome in a network context.

In this chapter I present a comprehensive overview of human indigenous microbiota focusing on 1) describing functional roles of healthy microbiota in the human body, 2) environmental determinants of regional microbial colonization, 3) general patterns of species diversity, abundance, and composition for five regions of the human body, 4) reviewing previous research on human microbiota, 5) describing common bacterial and fungal microbes and 6) reviewing various types of biological networks.

1.2 Functional roles of microbiota in the human body

Human-associated microbiotas perform numerous important functions in the human body. The human indigenous microbiota aids in nutrition, contributes to pathogen resistance, and plays an adaptive role in functioning of immune systems (Costello et al., 2009; Wilson, 2008; Dethlefsen et al., 2007, Round et al., 2010). For example, in the large bowel of humans, diet-derived substrates such as plant structural material that cannot be directly digested by the host must be digested by microbiota through the process of fermentation (Tannock, 1999; Roediger, 1980). Microbiota aid in resistance to infectious disease by suppressing establishment of pathogenic bacteria, a phenomenon known as 'colonization resistance'(Tannock, 1999; Bohnhoff & Miller, 1962). Microbial cells can not only attain extremely high abundances in association with mucosal surfaces of the human body without triggering a marked inflammatory or immunological response from the host (Tannock, 1999; Kimura et al., 1997) but can also stimulate development of the human immune system (Tannock, 1999; Gordon & Pesti, 1971, Round et al., 2010). While it is widely acknowledged that microbiota are crucial to maintenance of life functions in humans and considerable literature exists on specific functional roles of human-associated microbiota, a holistic understanding of the role and function of the human microbiome is only beginning to be realized.

1.3 Environmental determinants

There are four main environmental determinants of microbial colonization in the human body: nutritional, physiological, mechanical, and biological. Colonization of indigenous microbiota in a region is determined by both the ability of organism to survive in specific environment, and the presence of adequate nutritional and physiochemical requirements (Gonzalez et al., 2011). Nutritional requirements describe specific nutrients microbiota need to optimize fitness, while physiological determinants describe environmental conditions microbiota need to survive (e.g., pH, salinity). Survival is also determined by ability of organisms to withstand host-defense operating systems (biological determinants) and various microbe-removing systems (mechanical determinants) such as urination, coughing, and mucus production (Wilson, 2008; Greene & Voordouw, 2003).

1.3.1 Nutritional requirements

Nutritional requirements of indigenous microbiota consist of a number of minerals and organic substrates (Samaranayake, 2006) that include, but are not limited to: carbon, oxygen, nitrogen, hydrogen, phosphorus, sulphur, potassium, sodium, calcium, magnesium, chlorine, and iron (Wilson, 2008). Small quantities of trace elements are also required as co-factors for various enzymes and constituents of proteins and other cell components which include: cobalt, zinc, cooper, manganese, and molybdenum (Wilson, 2008). There is a huge diversity of nutrients that bacteria can use as sources for each particular element and wide variation in the types of compounds that can serve as an energy source (McFarland, 2000) for example amino acids, vitamins, and fatty acids (Wilson, 2008).

There are two primary ways in which microbiota obtain nutrients in the human body. First, they may obtain them from the host. Host nutrients includes molecules excreted and secreted by host's cells, from interstitial fluid, from dead or dying host cells, in the mucous layer, and from the host's diet (pertaining to the oral cavity and the gastrointestinal tract) (Wilson, 2008). Host-derived nutrients that are available differ in different regions of the body. Host-derived nutrients are nutrients that are provided by the host itself and/or by the host's diet. For example, on the skin surface, lipids and proteins are provided by the host, whereas in the gastrointestinal tract, carbohydrates and proteins are provided by the host's diet (Wilson, 2008). In the respiratory mucosa, mucins and proteins are derived from the host's diet, and in the oral cavity mucins, proteins and dietary constituents are derived from the host's diet (Wilson, 2008).

Second, once a region has been colonized, molecules produced by microbiota can also serve as nutrients. For example, microbiota can receive nutrients from other microbiota by secreting or excreting them, or by extracting molecules from dead/dying microbiota (Wilson, 2008). Nutrients are often available as complex macromolecules, requiring hydrolysis by microbiota to obtain specific nutrients (e.g., elements). For example, *Streptococcus* spp. can use glucose as a carbon and energy source but *Veillonella* spp. cannot. However, *Streptococcus* spp. produce lactate by degrading glucose and *Veillonella* spp. use lactate as a carbon and energy source (Wilson, 2008).

1.3.2 Physiochemical determinants

Physiochemical determinants are also important in regulating composition of microbiota (Wilson, 2008). Because the human body is homeostatically regulated, environments that microbiota colonize are relatively constant (Wilson, 2008). The most

important physiochemical properties that affect indigenous microbiota of the human body are: temperature, pH, redox potential, atmospheric composition, water activity, salinity, and light (Wilson, 2008; Corthesy, 2007; Elson & Cong, 2002). Regions of the human body differ in homeostatic regulation of these physiological factors. For example, the skin experiences more dramatic fluctuations in temperature and humidity compared to internal regions (e.g., GIT, respiratory tract) (Li, 2002).

Temperature in the human body remains relatively constant around 37°C, whereas temperature is around 33°C on the skin and conjunctival surfaces (Marples, 1965). Microbiota must be able to tolerate these temperatures in order to colonize. In contrast to temperature, pH varies enormously across body regions ranging from 1-2 to alkaline values and plays a major role in species composition in different regions (Wilson M. , 2008). The stomach, duodenum, caecum, and skin regions are all highly acidic, whereas alkaline regions include tear film, the ileum, and subgingival regions of the oral cavity. Although pH of body site determines which microbiota are present, microbiota themselves also contribute to regional pH (Tannock, 1999; Harder et al., 2007; Hill & Marsh, 1990). For example, metabolic activities of microbiota on the skin and the vagina play a major role in lowering pH (Wilson, 2008; Tannock, 1999).

Another major role in determining which microbiota can survive in body regions is oxygen content. There are five different groups of microbiota that have specific oxygen gradients: 1) Obligate aerobes which require oxygen to grow (e.g., *Acinetobacter, Moraxella, Micrococcus, Brevibacterium*), 2) Capnophiles, aerobes that grow best at CO_2 concentrations between 5-10% (e.g., *Neisseria, Haemophilus, Aggregatibacter*), 3) Obligate anaerobes, that do not grow in the presence of oxygen (e.g., *Bacteriodes,*

Clostridium, Fusobacterium, Eubacterium), 4) Facultative anaerobes, that can grow in the presence or absence of oxygen (e.g., *Staphylococcus, Streptococcus, Enterococcus*), and 5) Microaerophiles, which grow best at low concentrations of oxygen (e.g., *Helicobacter, Lactobacillus, Campylobacter*) (Finegold, 2004). At birth, all surfaces of a human are aerobic, but once microbial communities become established the consumption of oxygen and production of CO_2 alters the regional oxygen levels (Wilson, 2008; Tannock, 1999). Another physiochemical determinant which is related to oxygen is redox potential. Redox potential is used to measure the reducing power of a system and has an important influence on the functioning of enzymatic reactions that entail the simultaneous oxidation and reduction of compounds (Samaranayake, 2006). The species composition of various regions depends on whether the environment has positive or negative redox potential (Samaranayake, 2006).

Water activity (a_w) is also a physiochemical determinant of microbe colonization. Water activity is the proportion of water available for microbial activity and is consistently less that the total amount of water present, since it is affected by the concentration of solutes and by the presence of surfaces (Wilson, 2008). Human cells require an a_w of 0.997 while pure water has an a_w of 1.0. Most of the microbiota common on the human body require an a_w of at least 0.96 for active metabolism and all the regions can satisfy this requirement except for regions of the skin (e.g., arm, leg, and palm of hand) (Wilson, 2008).

An additional physiochemical determinant is salt concentration. Most microbiota cannot tolerate high salt concentrations, since they cause denaturation of proteins and dehydration (Wilson, 2008). The skin is the main region affected by salt concentration

since humans sweat which causes high salt content on the skin. *Staphylococcus* spp. are halotolarant microbiota and an example of a microbe that can grow with high salinity and thus readily colonizes the skin. In other regions of the body the salt content remains relatively stable (Wilson, 2008). The final major physiochemical determinant that is important to colonization is exposure to sunlight. Sunlight contains potentially damaging ultra-violet radiation and on the human body the skin and eyes are most affected. Little is known about the effects of sunlight on microbiota except for in external regions of the body (Wilson, 2008; Aly & Maibach, 1977).

1.3.3 Mechanical determinants

Mechanical determinants can have a major influence on microbial colonization. Many regions of the human body have mechanics that cause microbiota not attached to a mucosal surface to become removed (Wilson, 2008). For example, in the oral cavity chewing, tongue and jaw movements, salivary flow and swallowing are all mechanical determinants. Other areas of the human body where mechanical determinants can affect microbial colonization are the eye, pharynx, esophagus, stomach, small intestine, large intestine, respiratory tract, and teeth (Wilson, 2008).

1.3.4 Biological determinants

In the context of the human microbiome, it is important to know the biological determinants that affect the composition of microbiota (e.g., hormones), and in turn how microbiota affect the human body (e.g., immune response). The immune systems (innate and acquired) of humans generate a variety of molecules and activated cells that inhibit the growth of microbiota, kill them, prevent their adhesion to epithelium, and neutralize the toxins they produce (Albiger, 2007; Beisswenger & Bals, 2005). To make a defensive

response against microbial pathogens the innate immune system must first be able to differentiate these pathogenic microbes from the indigenous microbes. Research in this area has only recently begun and very little is understood about the mechanisms underlying the recognition and discriminatory processes (Wilson, 2008). However, we do know that recognition is based on "microbe-associated molecular patterns" (MAMPs), which human cells use to detect conserved microbial structural components (Wilson, 2008).

Although the immune systems' exact role in the regulation of the indigenous microbiota is uncertain, evidence suggests that the indigenous microbiota is able to inhibit inflammatory responses (Mazmanian et al., 2008; Clavel & Haller, 2007; Wilson, 2008). This means that high abundances of microbial cells can exist in association with mucosal surfaces without inducing an inflammatory or immunological response from the host (Tannock, 1999), while unhealthy microbiota in the same region will (Tannock, 1999). For example, Berg & Savage (1972) found that when mice were injected with heat-killed cells of *E. coli* or *Bacteroides* spp. of murine origin, there were different immune responses of the strains on the mice. After 4 days inflammation on the murine strains was reduced, but it did not reduce for the *E. coli* strains. Round et al., (2010) stated that "human microbiota has a profound and long lasting effect on the development of our immune systems".

Another biological determinant that occurs in the human body is the production of hormones. Hormones can fluctuate in concentrations which can change the environment of many areas in the human body (Wilson, 2008). For example, at puberty hormones increase the amount of sebum which leads to dramatic changes in the skin regions. In

women, the production of estrogen and progesterone also alters the environment of the vagina at different life stages (Wilson, 2008).

1.4 General patterns of species diversity, abundance, composition

In this section, I describe general patterns of species diversity, abundance and composition for five regions of the human body (Table 1.1; Table 1.2). Understanding diversity and species abundance is important because they can relate to function. For example, in ecosystems, high diversity increases resistance to invasion, robustness to disturbances, and facilitates the efficient use of resources (Cardinale et al., 2002; Chapin et al., 2000). Community composition is important because it allows us to understand what organisms and organismal interactions make up a community (Gonzalez et al., 2011). Campbell et al. (2009) defines an ecosystem as a "biological environment consisting of all the organisms living in a particular area, as well as all the nonliving (abiotic), physical components of the environment with which the organisms interact, such as air, soil, water and sunlight". The functioning of these ecosystems in an ecological sense is related to structure. In the context of the human body, regions (e.g., GIT, oral cavity) and the associated microbiota can be viewed as ecosystems.

Differences in the composition of microbial communities in different regions of the body and inter-individual variability in regional microbial composition are currently a major research focus. For example, Costello et al. (2009) showed that *Actinobacteria* (36.6%), *Firmicutes* (34.3%), *Proteobacteria* (11.9%), and *Bacteroidetes* (9.5%) were the most abundant microbiota in the skin, oral cavity, and gut but that each region harboured a unique and different microbial community that was relatively similar across people and over time. Other research has suggested that inter-individual differences in microbial

composition are extremely high, and even that each human's microbiome may be unique as a fingerprint (Fierer et al., 2010; Wilson, 2005).

1.4.1 Skin

The human skin is a complex habitat. It is one of the largest organs on the body due to its surface area and weight (Wilson, 2008). During birth, the skin becomes host to resident microbiota. As a habitat, however, skin is highly heterogeneous. Moisture content ranges from very dry areas (forearm) to very moist areas (toe-webs). The composition of microbial communities on the skin are also heterogeneous and can be highly localized (Tannock, 1999). The human skin is made up of three main layers: the epidermis (outer layer), dermis (middle layer), and the subcutaneous layer (inner layer). The epidermis contains a stratum corneum which consists of dead cells and these cells are sloughed off taking microbiota with them. The stratum corneum is replaced every 15 days (Wilson, 2008). The dermis contains hair follicles, sebaceous glands, and sudoriferous (sweat-producing) glands. The subcutaneous layer contains hair, follicles and apocrine and eccrine glands. The apocrine glands are associated with hair follicles and less common compared to the eccrine glands which are not associated with hair follicles (Wilson, 2008). The most common site of microbe colonization in the skin is the surface and hair follicles (Tannock, 1999).

Since there are many different regions for microbiota to inhabit on the skin; distinguishing the microbiota present in these areas requires site-specific sequencing. To date the most common genera identified in the skin microbiota are: *Corynebacterium, Staphylococcus, Propionibacterium, Micrococcus, Mallassezia, Brevibacterium, Dermabacter, Actinetobacter and Methylobacterium* (Wilson, 2008; Tannock, 1999;

Chiller et al., 1991). Grice et al., (2008) compared relative abundance of microbiota sequenced on human skin in 5 healthy humans. They found that *Proteobacteria* (*Pseudomonas spp, Janthinobacteria spp, Alphaproteobacteria spp, Gammaproteobacteria spp,* and *Betaproteobacteria spp.*) were the most common, making up 85-90% of the total abundance, while *Actinobacteria* comprised 0-7%, and *Firmicutes* 0-5% (Grice et al., 2008). Skin has a more diverse microbial community than the gut or the mouth (Costello et al., 2009; Table 1.1; Table 1.2).

1.4.2 Eye

The human eye is made up of several distinct parts that microbiota colonize: the cornea, the outermost layer of the eyeball, the sclera which is a clear layer in the front and back of the cornea, the conjunctiva, which is a clear layer of skin that covers the cornea and lines the eyelid and eyelid margin, the choroid which is a layer of vascular tissue behind the eye, the retina which covers three-quarters of the inner surface of the eyeball, and the lens of the eye which is covered by a region called the anterior cavity and is filled by a liquid called the aqueous humor (Wilson, 2008). In the eye nutrients are supplied to the sclera by the choroid, while the aqueous humor supplies nutrients and oxygen to the iris, cornea, and the lens (Wilson, 2008). Another region of the eye is a region behind the lens called the vitreous humor, which is a clear gel. When a person blinks the vitreous humor provides tears to the glands, canals and ducts behind the eye called the lacrimal apparatus. Tears provide the eye with lubrication, moistening, protective and cleaning functions (Wilson, 2008).

The conjunctiva of the eye is the only area of the eye that is exposed to the environment that does not contain adjacent skin regions. For the conjunctiva and eyelid

margin, all the microbiota present is similar to skin species except for *Viridians streptococcus*, *Streptococcus pneumoniae*, *Peptostreptococcus* spp., and *Haemophilus influenza* (Wilson, 2008). In culture studies done on the eye, 65% of species ever found in the eye can be cultured, although sometimes the eye can be reported as sterile (Soudakoff, 1954; Evans et al., 2007). Capriotti et al., (2009) sequenced swabs of 276 people's right eyes from Sierra Leone. They found that the most common organisms were coagulase-negative *Staphylococcus* (28.6%), fungus (26.0%), *Staphylococcus aureus* (19.9%), gram negatives other than *Pseudomonas/Haemophilus* (9.8%), *Nocardia/Actinomyces* (6.5%), and *Pseudomonas aeruginosa* (6.2%) (Capriotti et al., 2009). The microbial community of the human eye is not nearly as diverse as other areas of the human body, and the abundance these species can range from low (100 colony forming units (cfu) to high abundances (5x 10^4 cfu) (Wilson, 2008) (Table 1.1; Table 1.2).

1.4.3 Respiratory tract

The human respiratory tract is composed of several sections which can be broken up into two main regions. The upper respiratory tract includes the nasal cavity, pharynx and the larynx. The lower respiratory tract includes the trachea, primary bronchi and the lungs (Wilson, 2008). The upper respiratory tract is heavily colonized by microbiota while the lower respiratory tract is relatively free of indigenous microbiota although small numbers of microbiota have been isolated from these areas (Wilson, 2008; Tannock, 1999). Microbiotas colonize the lower respiratory tract during fluid aspiration from the upper respiratory tract which can carry concentrations (up to 10^8cfu/ml) of bacteria to the lower respiratory areas (Wilson, 2008). The amount of nutrient that is

supplied to the microbiota in the respiratory tract is highly dependent on the area of colonization. All of the regions contain a fluid that is on the mucosal surface and has plasma transdudate (low protein content, primary cells with mononuclear cells: macrophages, lymphocytes and mesothelia cells) (Heffner, 1997). Saliva and food flowing through the pharynx may also provide nutrients for microbiota in the pharynx (Wilson, 2008). Nutrients can also be provided by the nasal fluid, airway surface liquid, and the alveolar lining fluid. Nasal fluid provides microbiota with more than 1000 different proteins along with other nutrients. The airway surface liquid is produced daily and consists mainly of water, mucins and proteins. The alveolar lining fluid is a mixture that contains proteins and lipids (Wilson, 2008).

Since there are many different resources and regions for microbiota to colonize in the respiratory tract, wide ranges of microbiota are present in these regions. The most common species are: *Viridians streptococci, Streptococcus pyogenes, Neisseria* spp. *Haemophilus* spp. *Moraxella* spp. *Staphylococcus aureus, Streptococci, Corynebacterium* spp., *Propionibacterium* spp., *Prevotella* spp., *Porphyromonas* spp. *Mollicutes* and *Kingella* spp. (Wilson, 2008). In the nasal vestibule, microbe abundances can range from 10^6 and 10^7 cfu in each nostril (Glück & Gebbers, 2000; Lina et al., 2003; Wilson, 2008) while in the nasophayrnx the density of microbe colonization ranges from 10^4 to 10^8 cfu (Konno et al., 2006; Wilson, 2008) (Table 1.1; Table 1.2).

1.4.4 Oral cavity

The human oral cavity is made up of three main regions; the cheeks (hard and soft plates), the tongue, and the teeth. In total the oral cavity has a surface area of around 200 cm^2. The oral cavity has keratinized (hard plate, gingivae), non-karitinized (soft palate,

cheek, floor of mouth, inside the lips, and the underside of the tongue) and both keratinized and non-keratinized areas (tongue) (Wilson, 2008). In the oral cavity the main nutrients are provided by the saliva, compounds produced by host cells, host's diet, microbial metabolism, and gingival crevicular fluid. The oral cavity provides a wide variety of habitats for microbiota to colonize that differ in environmental selection factors. For example, the oral cavity contains both shedding and non-shedding surfaces as well as strong mechanical forces. The microbiome of the oral cavity has been extensively studied due to ease of access and the prevalence of caries and periodontal disease (Wilson, 2008).

Since the oral cavity is one of the most studied regions of the human body the microbiota inhabiting it are well known. The oral cavity alone contains an extremely diverse resident bacterial community, consisting of 100-200 species at any one time in healthy individuals (Rasiah et al., 2005; Wilson, 2008). Ghannoum et al. (2010) found six different fungal species in the oral cavity of healthy individuals. The seven genera observed by % frequency across subjects were *Candida* (75), *Cladosporium* (65), *Aureobasidium* and *Saccharomyces* (50), *Aspergillus* (35), *Fusarium* (30), and *Cryptococcus* (20) (Ghannoum et al., 2010). Other regions of the human body that have fungal microbiota are the gastrointestinal tract and vagina (NIH HMP Working Group et al., 2009). The mouth harbours at least six billion bacteria representing more than 700 phylotypes, but 50% of these phylotypes are still unknown (Aas et al., 2005) (Table 1.1; Table 1.2).

1.4.5 Gastrointestinal tract

The human gastrointestinal tract (GIT) is a very complex system made up of many functionally distinct regions: esophagus, stomach, small intestine (duodenum, jejunum, and ileum), and the large intestine (cecum, colon, and rectum) (Wilson, 2008; Tannock, 1999). Within each region of the GIT the environmental conditions differ. The GIT is basically a tube going from the pharynx to the anus, and has four main layers, 1) the mucosa (epithelium surrounded by connective tissue and a thin layer of muscle), 2) submucosa (connective tissue), 3) muscularis (muscles), and 4) serosa (connective tissue covered by squamous epithelium). The mucosa is the largest surface area on the human body that is exposed to the environment (Wilson, 2008). The major problem with detecting the microbiota in the GIT is that most of the areas require the individual to go under anesthesia or undergo discomfort. In the GIT the upper regions (stomach, duodenum, and jejunum) are colonized by fewer species than the lower regions, likely due to strong mechanical forces such as saliva and mucus which travels faster in the upper compared to areas lower in the GIT (Wilson, 2008; Tannock, 1999). In the lower GIT microbiota receive nutrients from the host diet, mucus, and other microbiota. The lower GIT (colon and ileum) provides a suitable habitat for large and diverse microbiota (Wilson, 2008).

It is estimated that the number of microbial cells in the GIT out numbers our body cells by a factor of at least 10 (Zoetendal et al., 2008). In the upper GIT lower abundances of microbiota (10^3-10^5 bacteria) are found relative to the lower GIT (10^{10}-10^{11} bacteria) (Mackie et al., 1999). It has been estimated that around 400 species are present in the human colon (Mackie et al., 1999; Eckburg et al., 2005). Up to 80% of

species found in the colon of the GIT have not yet been cultured and are novel phylotypes (Wilson, 2008) (Table 1.1; Table 1.2).

1.5 Human microbiome studies to date

The advancement of DNA sequencing has contributed significantly to our understanding of the human microbiome. It has been estimated that less than 1 percent of bacterial species can be cultured (Staley & Konopka, 1985). The development of through-put sequencing and meta-genomics has been a major technological advancement and has led to a focus on functional relationships and interactions among communities, taxa and genomics (Gonzalez et al., 2011). The scientific community is now poised to explore the importance of the human microbiome in human health and disease. Examples are provided below (also see Table 1.3-1.7) of studies on the human microbiota for five regions (eye, skin, oral cavity, the respiratory tract, and the gastrointestinal system) of the body.

1.6 Common bacterial and fungal taxa

While many bacterial and fungal taxa are found only in particular regions, a number of genera are present in all regions of the human body that are typically colonized by microbiota. Bacterial genera common across the human body regions are: *Stapylococcus, Propionibacterium, Micrococcus, Corynebacterium, Streptococcus, and Neisseria*. Common fungal species found in the human body are: *Candida* and *Malassezia.* Listed below are the characteristics of each main genus (Table 1.8).

1.7 Networks

Networks are a series of nodes interconnected by communication paths (Harbeck, 2000), and are a powerful way to look at the structure of systems. My research focuses on creating functional structural networks for the human body called human microbiome

networks. Viewing interactions in a system from a network approach provides a highly general framework by which to compare systems based on their topology or structure and can be used to determine how systems respond to perturbations (Foster et al., 2008). Network approaches have been used to explore chemical (e.g., chemical compounds), social (e.g., Facebook), economic (e.g., markets), geographic (e.g., GIS), biological (e.g., gene-protein) and food webs (Strogatz, 2001; Albert & Barabási 2002). In relation to the human body, network approaches have been applied to the genome, proteome, metabolome, yeast-protein interactions, disease transmission pathways, and neural systems. For example, Vazquez et al., (2003) assembled a protein-protein interaction network for the yeast *Saccharomyces cerevisae* to look at the robustness of the network when proteins are deleted or inserted in the network. Villadsen et al. (2011) explored the network structure of biochemical reaction networks to understand how raw material of oil interacts. Disease transmission networks have been explored for Syphilis (Rothenberg et al., 1998a) and HIV (Rothenberg et al., 1998b) to understand the likelihood of contracting disease. Lastly, neural networks have been assembled to explore human brain function (Kosko & Burgess 1998).

Ecological networks are used to describe interactions between species in ecosystems (Dunne, 2006). Food-webs, which describe energy flow in communities based on predator-prey interactions, are the most common type of ecological network (Dunne, 2009). Food web approaches have been used to analyze and measure direct and indirect interactions among different species (Dunne, 2009), to explore the roles of connectance and species richness on food web structure and stability (Dunne et al., 2002a), and to explore the consequences of extinctions and how communities collapse

(Dunne & Williams, 2009; Dunne et al., 2002a), among others. Of particular interest from the standpoint of the human microbiome, network structure can be related to function in that the structure of a network affects how robust a network is to perturbations (Dunne et al., 2002a) such as broad spectrum antibiotics.

Network analysis is based on graph theory (Albert & Barabási, 2002; Strogatz, 2001). In an ecological network, species are represented by vertices (nodes) and interactions between species are represented by edges (links) between vertices (Dunne, 2009). All networks can be characterized by their topological properties (Dunne, 2009). In an ecological network topological properties are determined by the number of taxa in the network and their interactions. I used seven different topological properties to characterize and compare the structure of human microbiome networks (Chapter 2 and 3): the number of nodes (NS), the number of links (L), the number of links per node (L/NS), connectance (L/NS^2), clustering coefficients (CC), fraction cannibal (or self-facilitation), and path length (path). The two most fundamental measures used to characterize ecological networks are the number of nodes in the web (NS), and connectance (L/NS^2), which is the number of links/number of nodes squared (Dunne, 2009) and is a standard measure of complexity (Williams et al., 2002). Previous studies have found that networks that are more highly connected tend to be more robust to node loss (Dunne et al., 2002a). The clustering coefficient (CC) is the probability that two taxa linked to the same taxon are also linked. Path length (path) is the mean shortest set of links (undirected) between species pairs. These two properties are important as they are associated with network redundancy (Dunne, 2009). Fraction cannibal (or self-facilitation) is the fraction of species that feed on themselves. In the context of the human

microbiome networks I term this interaction self-facilitation, which is when one species feeds off of the by-products of its own species.

1.7.1 Differences between predator-prey and facilitative networks

Food webs are a type of network that only includes consumer resource links (negative feeding interactions), while networks can include other types of links both trophic and non-trophic (Williams & Martinez, 2000). In human microbiome networks, there are two main types of interactions (links) that can occur: consumer-resource (negative interactions) and facilitative (positive interactions) (Ings et al., 2009). In consumer-resource networks, the links can relate to a wide variety of feeding interactions including predator-prey, herbivore-plant, or parasite-host (Dunne, 2009). For example, in a predator-prey feeding link, a predator preys on a prey (e.g., wolf on a rabbit). In herbivore-plant links, an herbivore feeds on a plant (e.g., horse on a grass), and in parasite-host interactions, a parasitoid feeds on a host (parasitic wasp on a terrestrial insect). These consumer-resource interactions usually involve consumers that are bigger than their resources (Cohen et al., 1993; Brose et al., 2005; Brose et al., 2006) and are antagonistic interactions. In the context of the human microbiome networks, consumer-resource links are represented by microbiota (e.g *Bifidobacterium*) on the human body (consumers) and source-specific nutrients (resource) (e.g., sugars provided by the host`s diet).

The second type of interaction that can occur is a facilitative (positive) one. These interactions are commonly known as mutualistic or commensal (Ings et al., 2009). Mutualism is when two organisms interact and each individual gains a fitness benefit (Breton & Addicott, 1992), while commensalism is when only one side gains a benefit

but the other side experience no negative effect (Hooper & Gordon, 2001). Some examples of mutualistic and commensal networks in the literature are pollination and seed dispersal networks. For example, pollination networks include interactions between plants and their animal pollinators, and frugivore networks interactions between plants and their animal seed dispersers (Ings et al., 2009).

In the context of the human microbiome, facilitative interactions can be syntrophic or mechanical. Syntrophic facilitative interactions occur when species feed off the by-products (source-specific resources the species produce) of other species. For example, in the human eye *Candida* spp. feed on amino acids that are produced by *Serratia* spp., but not *Serratia* spp. itself. Mechanical facilitative interactions are when a micro-organism needs to physically attach to another organism to obtain specific resources. For example, in the oral cavity *Fusobacterium* spp., which form part of the plaque surrounding teeth in the oral cavity, physically attach to *Neisseria* spp., which attach directly to the teeth. This is because *Fusobacterium* spp. lacks the adhesions to attach directly to the tooth surface so they fasten indirectly by attaching onto *Neisseria* spp. In chapter 2 and 3, I discuss assembly and comparison of both consumer-resource and facilitative networks for regions of the human body.

1.7.2 Details of how networks were assembled

Networks were assembled by constructing a data set of all the micro-organisms and their source-specific nutrients that have been identified for five regions of the human body: the surface of the skin (including hair follicles but excluding genital skin), the globe of the eye, the oral cavity (limited to the oral mucosa, tongue, and teeth), the gastrointestinal tract (from the esophagus to the rectum), and the respiratory system

(including sinuses, trachea, and lungs). These data sets were compiled from primary and secondary literature (e.g., Wilson, 2008; Bojar & Holland, 2002; Hayashi et al., 2005). Data on species composition is typically provided in the form of species abundance in an individual or population and includes both culture-based and sequence-based studies. Although data is provided on particular species abundant in the human body regions, not enough information is found on the specific metabolic properties of the species; therefore species were categorized into genera (Table 1.9).

I focused specifically on assembling genera lists for healthy humans between the ages of 20-40. The microbiota genera lists did not include macro-organisms such as hexapods, because they are not as common and have lower abundances on humans therefore only micro-organisms were included. We did not include microbiota that are only pathogenic and are not found in healthy hosts. Macro-parasites and micro-parasites were also excluded. Predator/prey links for consumer-resource (negative) networks and facilitative (positive) links for facilitative networks were assembled for the different regions. Since resources can be available from different sources (i.e., glucose in the eye microbiome is available from two specific sources: tears and sweat), nutrients from different sources were included as they represent different pathways of energy transfer.

1.7.3 Details on source-specific resources

In food webs, the bottom trophic level is usually represented by basal resources (Dunne, 2009) such as algae or plants. In the context of the human microbiome networks the basal resources are source-specific resources. The source-specific resources (i.e., resources provided by more than one source) were identified for each region (Table 1.10). Source-specific resources can either be provided by the host or by other micro-

organisms (Wilson, 2008). They also can be provided by the host in different ways. For example, in the skin, amino acids secreted as part of the individual's sweat is distinguished from amino acids available directly from the host's epidermal cells (Wilson, 2008). Networks representing microbiome consumer-resource networks will have the source-specific resources as their basal trophic level, whereas in the facilitative networks the source-specific nutrients are not included as links are made between the organisms providing the resource to the other organism.

1.7.4 Robustness in Networks

Understanding how structural human microbiome networks respond to node removal is important because it allows us to understand the structural properties that affect robustness and how the network responds to primary and secondary extinctions. Extinction in the context of the human microbiome could be caused by perturbations such as the loss of species following broad spectrum antibiotics, or the loss of species from pathogens and therefore is of major significance.

The response to simulated node loss has been examined for a number of network types including the internet (Albert et al., 2000) and metabolic and protein networks (Jeong et al., 2000). These networks all display highly skewed power-law degree distributions, and are extremely sensitive to the loss of highly connected nodes but comparatively robust to the loss of randomly chosen nodes (Dunne, 2009). In contrast, random networks with Poisson degree distributions, which are relatively unskewed since nodes, have similar numbers of connections, exhibit similar responses to loss of highly connected and random chosen nodes (Strogatz, 2001).

In food webs, similar patterns have been shown in response to node loss (Solé & Montoya, 2001; Dunne et al., 2002a; Dunne et al., 2004), despite the fact that most food webs do not display power–law degree distributions. Moreover, loss of high-degree nodes results in more rapid fragmentation in the networks (Solé & Montoya, 2001). The magnitude of secondary extinctions that occur in networks when species are removed depends on the connectance of the networks (Dunne et al. 2002a). For example, when removing high-degree nodes (i.e., degree represents the number of edges that a vertices has) in a network the fraction of secondary extinctions can be greater or comparable to networks that have low-degree nodes removed (Dunne, 2009; Solé & Montoya, 2001). Therefore, the nodes (NS) and the connectance (C) of networks are important in determining their robustness to node removal.

In ecological networks, ecologists have used network simulation modeling to explore the potential for secondary extinctions in response to perturbations in food webs (Srinivasan et al., 2007; Dunne & Williams, 2009; Roopnarine, 2006; Roopnarine et al., 2007). Secondary extinctions occur when a non-basal species or a cannibalistic species, loses all of its prey except for itself. Basal species in networks may experience primary species removal but may not experience secondary extinctions. In the context of the human microbiome networks, removal simulations can be performed to relate them to 'real world' primary removals and secondary extinctions. Robustness is defined as the proportion of primary species removal that leads to approximately 50% total species loss. The maximum possible robustness that a network can have is a value of 0.5 and the minimum is 1/NS (Dunne et al., 2002a).

1.8 Thesis outline

Links between the compositions of human microbiomes have now been made with disease, obesity, and allergies, among others (NIH HMP Working Group et al., 2009). We now know that our microbiomes play a much larger role in human health and disease than has previously been recognized. In Chapter 2, I describe the network structure for five regions of the human body. The objective of this research was to assemble and compare the topological structure of functional core microbiome networks for human-associated micro-organisms for five regions of the adult body: the eye, the oral cavity, the skin, the gastrointestinal track (GIT), and the respiratory system and determine the robustness of the networks to node loss.

In Chapter 3, I examine ontogenic and regional patterns in network topology for the oral cavity and gastrointestinal tract microbiomes. I assembled networks for the oral cavity for different life stages (newborn, child, adolescent, adult, and elderly) and networks for four regions of the gastrointestinal tract (esophagus, stomach, small intestine, and large intestine) to compare their topology and robustness to node loss.

In Chapter 4, I used a meta-analysis to determine the efficacy of probiotics on the gastrointestinal tract. I conducted a meta-analysis on the efficacy of probiotics on the gastrointestinal tract to determine their role in sustaining a healthy microbiota of the gut. This meta-analysis was designed to determine whether probiotics are more or less effective in the prevention and treatment of eight different gastrointestinal diseases across 11 species or species mixtures of probiotics. Furthermore, I determined whether factors such as patient age, dose, length of treatment, and single vs. multiple probiotic species affect efficacy.

Finally, Chapter 5 discusses the strengths and limitations of my research. It also emphasizes the relevance of the main results from each chapter in the context of the main goal of my research, which was to compare the topological structure of regional microbiome networks in the human body.

Table 1.1. The species richness, genera richness, and common genera from five regions of the human body (skin, eye, oral cavity, gastrointestinal tract, and respiratory tract). The regions are shown in the following order: A) skin and eye, B) oral cavity, C) gastrointestinal tract and respiratory tract.

A)

Region	Species Richness (healthy and non-healthy numbers)	Genera Richness (healthy numbers)	Common Genera	References
Skin	100 to 182 species in one area, ~500 to ~1000 species in total	18 genera	*Actinetobacter, Brevibacterium, Corynebacterium, Dermabacter, Mallassezia, Methylobacterium, Micrococcus, Propionibacterium, and Staphylococcus.*	Aly & Maibach, 1977; Berlau et al., 1999; Chiller et al., 1991; Dekio et al., 2007; Fierer et al., 2008; Gao et al., 2007; Grice et al., 2008; Lee et al., 2006; McGinley et al., 1978; Rocha et al., 1999; Sugita et al., 2001; Tannock 1999; Taylor et al., 2003; and Wilson 2008.
Eye	221 species	9 genera	*Candida, Corynebacterium, Micrococcus, Propionibacterium, Pseudomanas, Serratia, Staphylococcus, Streptococcus, and Stenotrophomona*	Albietz & Lenton, 1996; Capriotti et al., 2008; Dong et al., 2011; Evans, 2007; Graham et al., 2006; Graham et al., 2007, Maria et al., 2000; Soudakoff, 1954; Stapleton et al., 1997; and Wilson, 2008.

B)

Region	Species Richness (healthy and non-healthy numbers)	Genera Richness (healthy numbers)	Common Genera	References
Oral cavity	100 to 200 species at any one time, 700 species in total	66 genera	*Abiotrophia, Actinomyces, Atobium, Bifidobacterium, Capnocytophaga, Eikenella, Eubacterium, Fusobacterium, Gemella, Granulicatella, Haemophilus, Kingella, Lactobacillus, Leptotricha, Mycoplasma, Neisseria, Peptostreptococcus, Porphyromonas, Provotella, Propionibacterium, Rothia, Staphylococcus, Streptococcus, Treponema, and Veillonella.*	Aas et al., 2005; Diaz et al., 2006; Ghannoun et al., 2010; Mahmoud et al., 2010; Nakano et al., 2008; Paster et al., 2006; Rasiah et al., 2005; Tanner et al., 2006; Wilson, 2008; and Zaura et al., 2009.

C)

Region	Species Richness (healthy and non-healthy numbers)	Genera Richness (healthy numbers)	Common Genera	References
Gastrointestinal tract	400 species in colon along, ~1000 species in the GIT	32 genera	*Actinomyces, Bacteriodes, Bifidobacterium, Candida, Citrobacter, Clostridium, Desulfovibrio, Enterobacter, Enterococcus, Escherichia, Eubacterium, Fusobacterium, Gemella, Helicobacter, Lactobacillus, Peptostreptococcus, Peptococcus, Porphyromonas, Propionibacterium, Proteus, Provotella, Ruminococcus, Serratia, Streptococcus, and Veillonella.*	Eckburg et al., 2005; Finegold et al., 1987; Gavini et al., 2001; Hill & Marsh, 1990; Johansson et al., 1993; Macfarlane et al., 2004; Mackie et al., 1999; Pajecki et al., 2002; Pei et al., 2003; Skinner & Carr, 1974; Stojanović et al., 2007; Sullivan et al., 2003; Tannock, 1999; van Nipsen et al., 1998; Wantanabe et al., 2003; Wilson, 2008; and Woodmansey et al., 2004.
Respiratory tract	243 species	39 genera	*Corynebacterium, Haemophilus, Kingella, Mollicutes, Moraxella, Neisseria, Porphyromonas, Prevotella, Propionibacterium, Staphylococcus, and Streptococcus.*	Gluck & Gebbers, 2000; Golin et al., 1998; Graevenitz et al., 1989; Jousimies-Somer et al., 1989; Knapp & Hook, 1988; Konno et al., 2006; Kuklinska & Kilian, 1984; Rasmussen et al., 2000; Willner et al., 2009; and Wilson, 2008.

Table 1.2. The range of abundances for microbiota found in five regions of the human body (skin, eye, oral cavity, gastrointestinal tract, and respiratory tract).

Region	Abundances (cfus)	References
Skin	estimated up to 10^{12}	Li, 2011
Eye	100 cfu to 5 x 10^4	Wilson, 2008
Oral cavity	6 x 10^9	Aas et al., 2005
Gastrointestinal tract	10^3- $10^5$to 10^{10}-10^{11}	Mackie et al., 1999
Respiratory tract	nasal vestible: $10^6$to 10^7 nasopharynx 3 x 10^4 to 4 x 10^8	Glück, 2000; Konno, 2006; Lina, 2003; and Wilson, 2008.

Table 1.3. Human microbiome studies of the skin, including studies with a specific area of focus, and the information and results for the area of focus.

Area of focus	Information/Results	References
Microbes present on the human skin.	Similarity in species composition between individuals and in regions of the skin.	Grice et al., 2008; Taylor et al., 2003
Effects of disease on the skin microflora and how species differ in abundance and diversity.	High variablility in the species abundance and major differences in diversity.	Levy et al., 2003; Chiller et al., 2001; Sugita et al., 2001; Fredricks, 2001; Elsner, 2006; Noble, 1992; Leyden & McGinley, 1992; Sugita et al., 2001; Aly & Maibach, 1977; Lee et al., 2006; Grice et al., 2008; Bruggemann et al., 2004; Frank et al., 2003
Regional differences in the skin microbiomes.	Depending on region microbes can differ immensely, which is mostly due to the skins physiochemical determinants.	Bojar & Holland, 2002; Funke et al., 1997; Paulino et al., 2006; Webster, 2007; Wilson, 2008; Tannock, 1999; Hill & Marsh, 1990; Skinner & Carr, 1974; Gao et al., 2007
Functional roles of specific species in areas of the skin.	Abiotic and biotic factors required for optimal growth (details in Chapter 1.3).	Crespo-Erchiga & Florencio, 2006; Joly-Guillou, 2005; Kazmierczak et al., 2005; Mack et al., 2007; Morishita & Sei, 2006; Perry & Lambert, 2006; Rosen, 2007; Tauch et al., 2005; Ashbee, 2007; Kloos et al., 1975
Antimicrobial clothing and effects on the skin microflora.	No adverse effects on the skin flora.	Hoefer & Hammer, 2011
Bioemulsifier production by *Actinetobacter* strains isolated from the human skin.	Higher emulsification activity on healthy human skin compared to burn wounds and soil isolates.	Patil & Chopade, 2001
Temporal variation in the composition of skin microbes.	Skin microbiota varies systematically across time.	Hopwood et al., 2005; Costello et al., 2009; Jung et al., 1998

Table 1.4. (A, B) Human microbiome studies of the eye, including studies with a specific area of focus, and the information and results for the area of focus.

A)

Area of focus	Information/ Results	References
Diseased eyes compared to healthy eyes.	Differences in the diversity of species isolated.	Albietz & Lenton, 2006; Graham et al., 2007; Gritz et al., 1997; Yamauchi et al., 2005; Campos et al., 1994; Perkins et al., 1975; Bilen et al., 2007; Chaidaroon et al., 2006
Effects of contact lenses.	Fewer microbes colonizing the eyes of contact lens wearers compared to non-contact lens wearers.	Stapleton et al., 1995; Iskeleli et al., 2005
Microbial composition resulting from ocular surgery such as cataract surgery.	Arantes et al., (2006) looked at antibiotic resistance patterns in eyes of patients undergoing cataract surgery and showed that in the case of the bacteria coagulase-negative *staphylococcus* there was low susceptibility rate to neomycin, and high susceptibility rates to cephalotin, vancomycin, chloramphenicol, ofloxacin and gatifloxacin.	Arantes et al., 2006; Locatelli et al., 2003; Chisari et al., 2004; Rubio, 2004; Srinivasan et al., 1999; Kato & Hayasaka, 1998

B)

Area of focus	Information/ Results	References
Compositional changes during ontogeny	The microflora in adult and children's eyes were compared and Singer et al., (1988) found that adults had higher abundances of species compared to the children, and a significantly higher number of anaerobic species were found in the adult eyes.	Singer et al., 1988
Metabolism of ocular microbe species.	Bacteria in the healthy ocular surface possess mucinolytic activity on both intact and surface processed mucins, targeted to discrete sites in the mucin molecule.	Berry et al., 2002
Effects of eye drops on the human eye microflora.	Topically applied dexamethasone did not cause a significant change in colony counts of the healthy conjunctiva.	Ermis et al., 2004
Indigenous ocular microbes through culture and sequenced based methods.	Providing details on common species, diversity and abundances.	Capriotti et al., 2009; Soudakoff, 1954; Evans et al., 2007; Fung et al., 2002; Kirkwood, 2007; Ueta et al., 2007; von Graevenitz et al., 2001; Zhao et al., 2001; Wilson, 2008; Tannock,

Table 1.5 (A, B). Human microbiome studies of the oral cavity, including studies with a specific area of focus, and the information and results for the area of focus.

A)

Area of focus	Information/ Results	References
Sequences from saliva samples of 120 individuals taken from 12 world-wide locations to determine global trends in diversity of individual salivary microbiomes.	High diversity within and between individuals but little geographic structure.	Nasidze et al., 2009
Changes in the oral microbiome with diet, age, and systemic health.	Stable ecological conditions were maintained long-term with the microflora but that transient fluctuations also occur and the variety of microbes between individuals increased with maturity.	Rasiah et al., 2005
Ontogeny of oral microflora.	Microflora diversity and composition changes with time.	Percival, 2009; Gusberti et al., 1990; Nakano et al., 2008; Kumar et al., 2002
Microflora changes due to disease.	For example, Paster, et al., (2002) compared microbes in individuals with HIV infected with necrotizing ulcerative periodontitis compared to healthy individuals. They found that patients with HIV and patients that had necrotizing ulcerative periodontitis differed from individuals that were HIV-negative, in bacterial pathogens.	Sakamoto et al., 2003; Ashely et al., 1988; Ledder et al., 2007; Tanner et al., 2006; Ximénez-Fyvie et al., 2000; Slots, 1977; Rudney et al., 2005; Kurata et al., 2008; Kazor et al., 2003; Dale et al., 2005; Hintao et al., 2007; Kumar et al., 2005, Paster et al., 2002
Microflora of plaque and dental carries compared to microflora on individuals without plaque or carries.	Differences in their microflora abundances and composition.	Hintao et al., 2007; Könönen et al., 1999

B)

Area of focus	Information/ Results	References
The indigenous microflora of the oral cavity.	Microbial composition, abundances and diversity (Chapter 1: Section 1.4).	Li et al., 2004; Sakamoto et al., 2005; Ramberg et al., 2003; Aas et al., 2005; Hori et al., 1999; Bowden et al., 1975; Theilade et al., 1982; Hartley et al., 1996; Diaz et al., 2006; Duncan, 2005; Suzuki et al., 2005; Wang et al., 2007; Hintao et al., 2007; Hu et al., 2007; Marsh, 2005; Marsh, 2006; Marshall, 2004; Robinson et al., 2006
The oral microflora of patients with cleft lip/ or palate.	They are more susceptible to dental caries.	Cheng et al., 2007
The effects of sucrose-contaning foods on the oral microbiota.	Sucrose-containing foods can enhance plaque accumulation in the oral cavity.	Beighton et al., 1999
Distribution of microflora species on the intra-oral surface.	Bacterial species marked differently on different oral surfaces.	Mager et al., 2003
Specific microbial species found in the oral cavity and their prevalence.	Generally these studies focused on where in the oral cavity the species is commonly found, and its abundance. In Al-Ahmad et al., (2007), the temporal dynamics of the four bacteria (*Actinomyces naesulundii*, *Fusobacterium nucleatum*, *Streptococcus* spp. and *Veillonella* spp.) were studied *in vivo* and they found significant changes in the abundances of *Streptococcus* spp. and *F. nucleatum* spp..	Al-Ahmad et al., 2007; Edwards et al., 2006; Kreth et al., 2005; Palmer et al., 2003; Ruhl et al., 2004; Suntharalingam et al., 2005

Table 1.6 (A, B). Human microbiome studies of the respiratory tract, including studies with a specific area of focus, and the information and results for the area of focus.

A)

Area of focus	Information/ Results	References
Differences in microbial communities between healthy individuals and individuals with respiratory diseases.	Significant change in the microbial composition with disease (Konno et al., 2006), differences in the abundances of the species, and differences in composition between healthy and disease individuals.	Konno et al., 2006; Diggle & Clarke, 2006; Mitchell, 2006; Cantin, 2001; Wertheim et al., 2005; Trotter et al., 2006; Kellner et al., 1998; De Lencastre & Tomasz, 2002; Stjernquist-Desatnik & Holst, 1999; Gordts et al., 1999; Bals & Hiemstra, 2004; Snyder et al., 2005; Courtney et al., 2002; Garcia-Rodriguez & Fresnadillo Martinez, 2002; Karalusa & Campagnaria, 2000; LeVine & Whitsett, 2001
Individual's nasal vestibules and cavities were swabbed to distinguish the composition of potential bacterial pathogens.	There were differences in the flora of the two sites, and the potential bacterial pathogens were found more commonly in the vestibule compared to the cavity of the patients.	Glück & Gebbers, 2000
The ingestion of probiotics on the bacterial flora of the nose.	Regular intake of probiotics can reduce potential bacterial pathogens in the upper respiratory tract.	Glück & Glebbers, 2003
Specific microbial species and their prevalence in the respiratory tract.	There is a common set of genera in the respiratory tract of healthy humans.	Gunnarsson et al., 1998; Gordts et al., 2000; Kononen et al., 2002; Hood, 2003; Laval et al., 2006; Liñares et al., 1992; Jousimies-Somer et al., 1989; Soriano & Rodriguez-Cerrato, 2002; Saez et al., 1998; Frandsen et al., 1991; Von Graevenitz et al., 1998; Bernardini et al., 2007; Bullard et al., 2007; Clark & Foster, 2006; Cole et al., 2001; Erwin & Smith, 2007; Lopez, 2006; Lux et al., 2007; Verduin et al., 2002

B)

Area of focus	Information/ Results	References
Characterization of the indigenous microflora of the respiratory tract.	Mostly these studies have focused on describing species richness and composition (Chapter 1: Section 1.4) in the respiratory tract and differences between individuals.	Trotter, et al., 2006; Ylikoski, et al., 1989; Choi, et al., 2006; Mukundan, et al., 2007; van Belkum, 2006; Wilson, 2008; Tannock, 1999; Hill & Marsh, 1990; Skinner & Carr, 1974; Rasmussen, et al., 2000
Bacterial interactions in the respiratory tract have been studied to look at the competition of bacteria in the human nasal cavity.	*Corynebacterium* spp. and *S. epidermidis* decrease *S. aureus* colonization.	Lina et al., 2003
Metabolism occurring in the respiratory tract.	Rose & Voynow (2006) published a review on the role and regulation of mucin glycoproteins.	Rose & Voynow, 2006; Randell & Boucher, 2006; Travis, et al., 2001
Microbial differences in the oropharynx of alcoholics and non-alcoholics.	Alcoholics have more anaerobic micro-organisms (84.5%) present compared to non-alcoholics (30.5%), and the alcoholics have a higher risk of lower airway infections.	Golin et al., 1998
Differences in microflora due to method of feeding in children.	Breastfed infants had higher abundances of bacteria compared to formula fed infants.	Hokama et al., 1999
Physiology of airway surface liquid.	Boucher (1999) reviews two ways that epithelia that line the airways of the lung function, which are:1) defending against infections and 2) replenish water loss from airway surfaces.	Boucher, 1999; Kozlova et al., 2006
Differences in the microflora of smokers and non-smokers.	Smokers contain fewer aerobic and anaerobic organisms, than those of non-smokers.	Brook & Gober, 2005

Table 1.7 (A, B). Human microbiome studies of the GIT, including studies with a specific area of focus, and the information and results for the area of focus.

A)

Area of focus	Information/ Results	References
Sequencing of the microbiota in the human gastrointestinal tract in patients with and without eczema.	Abundances of *Bifidobacterium* spp. and *Enterobacteriaceae* spp. were different for the two groups.	Hong et al., (2010)
Microbiota of individuals with and without disease.	Major differences in the relative abundance of different groups of microbes between diseased and healthy patients.	Lepage et al., 2005; Macfarlane et al., 2004; Macfarlane et al., 2007; Andoh et al., 2007; Blaser & Atherton, 2004; Blaut & Clavel, 2007; Gilmore & Ferretti, 2003; Guarner & Malagelada, 2003; Lucke et al., 2006; Magalhaes et al., 2007; Manichanh et al., 2006; Thompson-Chagoyan et al., 2007; Clavel et al., 2007
Effects on microbial composition from probiotics.	Probiotics associated with an increase in healthy microflora.	Johansson et al., 1993; Carey et al., 2007; Tuohy et al., 2007
Effects on microbial composition from antibiotics.	Microflora composition is typically strongly affected by antibiotics, which leads to a decrease in abundances and diversity.	Finegold et al., 1987
Across-subject variability in composition, region variability and healthy microflora.	Eckburg et al., (2005) detected the diversity of the human intestinal microbial flora by using ribosomal RNA gene sequences from colonic mucosal and fecal sites. They showed that there was high across-subject variability and differences between stool and mucosa communities.	Eckburg et al., 2005

B)

Area of focus	Information/ Results	References
Studies on the microbial composition for differnt locations in the GIT.	GIT composition is location specific. The microflora change in composition and abundance depending on which region of the GIT was sequenced.	Zoetendal et al., 2008; Marteau et al., 2001; Zoetendal et al., 2002a; Pajecki et al., 2002; Sullivan et al., 2003; Pei et al., 2004; Hayashi et al., 2005; Bik et al., 2006; Booijink et al., 2007; Xu et al., 2007; Delgado et al., 2006; Donskey et al., 2003; Hill 1998; Duncan et al., 2007; Egert et al., 2006
Indigenous microflora of the gastrointestinal tract.	They have found what microbes are commonly detected, their abundances and diversity (Chapter 1: Section 1.4).	Gill et al., 2006; Marchesi & Shanahan, 2007; Lee, 2008; Berg, 1996; Manson et al., 2008; Mahowald et al., 2009; Wang et al., 2003; Wang et al., 2005; Zilberstein et al., 2007; Rajilić-Stojanović et al., 2007; O'Hara & Shanahan, 2006; O'Hara & Shanahan, 2007; Savage, 1977; Wilson, 2008; Tannock, 1999; Hill & Marsh, 1990; Skinner & Carr, 1974; Abell & McOrist, 2007; Ben-Amor et al., 2005
Impact of diet and nutrition on the GIT microbiota.	Differences in diet change the composition of the indigenous microflora.	Flippo et al., 2010; Louis et al., 2007; Langlands et al., 2004; Gostner et al., 2006; Cummings, 1998; Flint et al., 2007
Ontogeny of microbiota of the GIT.	Composition of the microbes changes with age.	Mshvildadze et al., 2008; Mackie et al., 1999; Woodmansey, 2007; Andrieux et al., 2002; Edwards & Parrett, 2002
The gut microbiome differences in obese and lean twins.	The microflora was extremely different between obese and lean twins.	Turnbaugh et al., 2009
Specific microbes and their prevalence in the gastrointestinal tract.	Studies have shown what specific habitats species need for their optimal growth and at what abundances the species occur.	Tannock, 2002; Neilsen et al., 2003, Zoetendal et al., 2002b; Belenguer et al., 2006; Go, 2002
Studies on people sequenced from different geographical areas.	The environment of individuals corresponds to the species composition of their GIT microbiome.	Lay et al., 2005; Mueller et al., 2006

Table 1.8. Characteristics (region of human body found, genera details e.g. nutrition) of common fungal and bacterial genera found on the human body. A) Fungal genera (*Candida* and *Malassezia*). B) Bacterial genera: I) *Staphylococcus* and *Propionibacterium*, II) *Micrococcus* and *Corynebacterium*, III) *Streptococcus* and *Neisseria*.

A)

Fungal Genera

Genus	Region found on the body	Details
Candida	The genus is commonly found in eyes, skin, oral cavity, and the respiratory tract of the human body.	*Candida* is a yeast that is oval in shape, is dimorphic, an anaerobe and reproduces by budding (Wilson 2008). *Candida albicans* is the most common species found on the human body and is found in the eye, skin, respiratory tract and the oral cavity. It can use a wide range of sugars including glucose, maltose, surcrose, galactose and xylose for a carbon and energy source. It is able to partially degrade mucins, oligiosaccharides, and proteins to provide nutrients for an energy source (Wilson 2008, Skinner and Fletcher 1960). *Candida* can also use alcohols, carbohydrates and amino acids for energy (Skinner and Fletcher 1960). It can get resources from the host such as, hyaluronate, and uses sugars and amino acids that it makes itself (Wilson 2008).
Malassezia	The genus is commonly found on the skin of the human body.	*Malassezia* is dimorphic yeast that inhabits the human skin. The genus consists of 11 species, and ten of these have been found on healthy human skin (Wilson 2008). The ten species commonly found are: Mal. furfur, Mal. pachydermatis, Mal. sympodialis, Mal. globosa, Mal. slooffiae, Mal. restricta, Mal.obtuse, Mal. dermatis, Mal. japonica and Mal. yamatoensis (Wilson 2008). Malassezia sp. at the highest abundances is found on several skin sites of the body which are: the chest and upper back, the scalp, the forehead, the face and neck, and the perineum (Lee et al. 2006). Malassezia can inhabit on any area of the skin because it can survive aerobically and anaerobically given that the other conditions are suitable (Wilson 2008). The genus cannot ferment sugars but it can use lipids as its sole energy source (Wilson 2008). They use amino acids from sweat as a nitrogen source, and lipids from skin sebum as a carbon source and do not require vitamins as an energy source (Wilson 2008). From other microorganisms they can obtain NH4, amino acids and fatty acids and they also can use proteins and lipids from the host (Wilson 2008).

B. I)

Bacterial Genera

Genus	Region found on the body	Details
Staphyloccus	The genus is commonly found in the eye, skin, respiratory tract, and the gastrointestinal tract of the human body.	Staphylococci are gram-positive cocci that can be found single, in pairs or in clusters. This genus is comprised of 35 species (Wilson 2008). The species *S. aureus, S. intermedius,* and *S. delphni* produce an enzyme called coagulase which converts fibrinogen to fibrin. Most of the species in this genus ferment sugars to produce lactic acid (Staley et al. 2007) are halotolarant, produce a variety of hydrolytic enzymes and grow with low water activities (Wilson 2008). *Staphylococci* are facultative anaerobes which use lactate, amino acids, NH_4, and sugars from sweat of the host (Wilson 2008). They also use proteins and lipids (glycerol) from the host for nutrition. Furthermore, they get amino acids, lactic acid, NH_4, urea, and sugars from other microbes in their community (Wilson 2008, Tannock 1999, Staley et al. 2007).
Propionibact-erium	The genus is commonly found on five areas (eye, skin, oral cavity, respiratory tract, and the gastrointestinal tract) of the human body.	*Propionibacterium* are gram-positive bacilli that often have a branching structure (Wilson 2008). They are anaerobic/microaerophilic organisms and therefore can colonize in areas with low oxygen such as hair follicles. However, they can be found on areas where high oxygen levels are present (when other microbes use up all the oxygen). They require vitamins such as biotin, nicotinamide, pantothenate, and thiamine for growth (Wilson 2008). *Propionibacterium* use amino acids, glycerol, sugars, and fatty acids as carbon and energy sources. The human host provides them with glycerol, lipids, fatty acids (from sebum in skin), and proteins. Also from human sweat and dying cells, they can obtain vitamins sugars and amino acids (Wilson 2008). *Propionibacterium* can also obtain nutrients from other organisms such as O_2 amino acids, fatty acids, lactic acid and sugars (Wilson 2008, Staley et al. 2007).

B. II)

Genus	Region found on the body	Details
Miccrococcus	They are commonly found on the human skin, eyes, respiratory tract and the gastrointestinal tract.	The genus *Micrococcus* is gram-positive cocci that are found mostly in clusters and are obligate aerobes. The species in this genus need amino acids for growth factors, and most of the strain produce proteases and keratinases themselves (Wilson 2008). The genus can be halotolerant (Wilson 2008). They receive nutrients such as amino acids, lactate, sugars, and NH_4 from the hosts sweat. They also receive carbohydrates, proteins, glycerol, and fatty acid (in skin from sebum) from the host (Wilson 2008). Other organisms in their niche can provide them with amino acids, fatty acids, lactate, acetate and sugars (Wilson 2008).
Corynebacterium	The genus is commonly found on five areas (eyes, skin, oral cavity, respiratory tract and gastrointestinal tract) of the human body.	*Corynebacterium* is gram-positive rods that are non-branching and non-sporing and can be aerobic (Collins 1987) or anaerobic, and are halotolerant (Wilson 2008). There are 59 species in the genus but not all of them are found on the human body (Wilson 2008). They are able to use amino acids and carbohydrates as sources of carbon (Wilson 2008). *Corynebacterium* obtains amino acids, sugars, vitamins, and urea from other microorganisms in their habitat (von Graevenitz and Bernard 2006). They also receive nutrients such as carbohydrates, sugars, and amino acids from their host (von Graevenitz and Bernard 2006, Wilson 2008). *Corynebacterium* species also produce urea themselves and can feed off of it (von Graevenitz and Bernard 2006).

B. III)

Genus	Region found on the body	Details
Streptococcus	The genus is commonly found in the skin, respiratory tract, oral cavity, eyes and the gastrointestinal tract.	*Streptococci* are gram-positive cocci that can be spherical or ovoid in shape. There are at least 39 different species in this genus. They are facultative anaerobes that ferment carbohydrates and sugars to produce lactate and other nutrients (Staley et al. 2007). The acquire nutrients such as proteins, amino acids (on skin region from sweat), vitamins (from sweat and dying skin cells), sugars, mucins, hyaluronate, and carbohydrates from the host (Wilson 2008, Staley et al. 2007). They also, obtain sugars and amino acids from other micro-organisms in their habitat (Wilson 2008, Tannock 1999).
Neisseria	The genus is commonly found in the skin, respiratory tract, gastrointestinal tract and the oral cavity.	The genus Neisseria is gram-negative cocci, which are aerobes, but have anaerobic growth when in the presence of nitrates (Wilson 2008). There are 16 species that are classified in this genus and the most common ones in the human body are *N. perflava* , *N. sicca* , and *N. lactamica* (Wilson 2008). *Neisseria* produce acetate which can be used by other organisms as a resource. They utilize proteins and sugars from other organisms in their habit and obtain carbohydrates, proteins, and sugars from their host (Wilson 2008).

Table 1.9. The number of bacteria, fungi, and archean genera are present for five areas of the human body (eye, skin, oral cavity, respiratory tract, gastrointestinal tract), and the number of species that are present in each region. These numbers are specific to the numbers of my microbiome networks.

Region	# of bacteria	# of fungi	# of archean	# of species detected
Eye	9	1	0	16
Skin	16	2	0	44
Oral cavity	48	1	0	104
Respiratory tract	30	1	0	54
Gastrointestinal tract	29	0	1	108

Table 1.10 (A, B). Source-specific resources in five regions of the human body: eye, skin, oral cavity, gastrointestinal tract (GIT), and respiratory tract. The resource, resource source, and region are included.

A)

Resource	Resource Source	Region
amino acids	component of host's sweat	skin, eye
amino acids	host's epidermal cells	skin
amino acids	secreted in mucus	respiratory, oral, GIT
amino acids	part of host's diet	oral, GIT
amino acids	part of host's saliva	oral, GIT
ammonium	component of host's sweat	eye, skin
carbohydrates	secreted in airway surface liquid	respiratory
carbohydrates	part of host's diet	oral,eye, skin, GIT
citrate	host's citrate	eye, oral, respiratory, skin, GIT
chondroitan sulfate	chondroitan sulfate from host	respiratory, GIT
DNA	DNA from host	respiratory, GIT
fatty acids	component of host's sweat	skin
fatty acids	host's epidermal cells	skin
fatty acids	host's sebum	eye, skin
glycerol	host's epidermal cells	skin, eye, GIT
hematin	hematin from host	oral, respiratory, GIT
hydrocarbons	part of host's diet	eye, oral, respiratory, skin
hyaluronate	hyaluronate from host	eye, oral, respiratory, skin, GIT
lactic acid	component of host's sweat	skin, eye
lipids	secreted in airway surface liquid	respiratory
lipids	part of host's diet	oral, eye, skin, GIT
lipids	acquired enamel pellicle	oral, skin
methanol	methanol in host	skin
mucin	secreted in mucus	respiratory, oral, GIT
mucin	secreted in airway surface liquid	respiratory
mucin	secreted in nasal fluid	respiratory
nitrates	from hosts diet	respiratory, skin, GIT
protein	host's epidermal cells	skin, eye
protein	secreted in mucus	respiratory, oral
protein	secreted in tears	eye
protein	secreted in nasal fluid	respiratory
protein	secreted in airway surface liquid	respiratory
protein	part of host's diet	oral, GIT
protein	part of host's saliva	oral
protein	gingival crevicular fluid	oral

B)

Resource	Resource Source	Region
sugars	sugars from host's diet	oral, GIT
sugars	component of host's sweat	skin, eye
sugars	host's epidermal cells	skin
sugars	secreted in mucus	respiratory, oral
sugars	secreted in tears	eye
sugars	sugars from gingival crevicular fluid	oral
vitamins	component of host's sweat/dying skin cells	eye, skin
vitamins	from hosts diet	oral, respiratory, GIT
urease	from host	GIT

CHAPTER 2

Network Structure of the Human Microbiome

2.1 Abstract

The human body contains 10 times more non-human cells than human cells, and the human colon has the highest density of microbiota recorded in any habitat of the planet. While links have been identified between the composition of the human microbiome and obesity, inflammatory bowel disease, cancer, sexually transmitted diseases, allergies and asthma, and cardiovascular disease, very little is known about how the species on and in our bodies interact. Network approaches, which have been widely applied to natural ecosystems, represent a promising approach to describing the complex interactions that exist within organisms. I assembled consumer-resource and facilitative interaction networks for human-associated microbial species and source-specific nutrients for five regions of the human body: eye, skin, oral cavity, respiratory tract, and the gastrointestinal tract to compare the topological structure of the networks and their robustness to node removal. The networks were composed of 72 bacteria, 1 archaea (*Methanobrevibacter*), and 2 fungal genera (*Malassezia* and *Candida)* along with 39 source-specific nutrients. In total, 115 nodes and 2335 links were summed over all five

regions. Across all site-specific networks, 67% of links were facilitative. Complexity, defined as connectance of facilitative networks was on average 46% higher than the complexity of the consumer-resource networks. The networks were more robust to random and least-connected node removals than most-connected node removals. Facilitative networks were more robust overall than consumer-resource networks or composite networks of the human body regional networks. The oral cavity was the most robust region for the composite (complete) network. Robustness was driven by node richness for the consumer-resource and composite networks, but not for the facilitative networks. As connectance decreased, robustness increased for the consumer-resource networks. This research represents the first attempt to assemble and compare microbiome networks for the human body.

2.2 Introduction

There are 10 times more non-human cells in the human body than human cells (Turnbaugh et al., 2007), 100 trillion microbial cells in the human gut (Bäckhed et al., 2005; Ley et al., 2006), and 100,000 million cells per mL in the human colon, the highest density of microbiota recorded in any habitat on the planet (Bäckhed et al., 2005; Ley et al., 2006). Microbiota colonize the human host during vaginal birth and within the first weeks of life and by senescence, 99% of DNA in the human body is non-human (Wilson, 2008). Inter-individual differences in the composition of microbiota are extremely high and it has been suggested that each human‘s microbiome may be as unique as a fingerprint, with less than 5% of species shared between any pair of individuals (Wilson, 2008; Fierer, 2010). Despite the high inter-individual difference in human microbiomes, some microbiota, such as Enterobacteriacae spp., *Staphylococcus* spp., and *Streptococcus*

spp. (Bojar & Holland, 2002; Wilson, 2008), which make-up the core microbiomes, are shared by most individuals.

The functional significance of the human microbiome is currently a major focus of research by scientists in a variety of professions or specialties including geneticists, immunologists, and ecologists. The National Institute of Health (NIH) recently announced the Human Microbiome Project as part of the NIH Roadmap for medical research (NIH HMP Working Group et al., 2009). The goal of this project is to sequence all microbiota in and on the human body (NIH HMP Working Group et al., 2009). This project has resulted in the identification of links between the composition of the human microbiome to obesity, inflammatory bowel disease, cancer, sexually transmitted diseases, allergies and asthma, and cardiovascular disease (Arumugam et al., 2011; Mazmanian et al., 2008; Li et al., 2008; Ley et al., 2006; Ordovas & Mooser, 2006). However, to date, the bulk of this research has been descriptive, focusing on identifying the composition (microbial diversity and abundances) of different regional microbiomes.

Because network structure affects function of the human body, a potentially more relevant way to characterize the human microbiome is not to just look at species that are present, but to look at the topology of the networks that arise when the species are linked together into interaction networks. Networks are a powerful way to explore the structure of systems and have been used to explore the structure and robustness of a wide variety of systems including: power grids, the internet, social networks, contacts between people at risk of HIV, and food webs which are networks of predator-prey interactions (Dunne, 2009). The strength of using a network approach in exploring the structure of ecological communities such as human-associated microbiomes is that network approaches can

provide relevant information on how a system might, for example, respond to disturbances (Foster et al., 2008; Dunne & Williams, 2009). In a human microbiome context I could explore how a system will respond to a disturbance, such as the loss of species following broad spectrum antibiotics, or how the system would respond to an invasion event such as a pathogen (Foster et al., 2008).

Structural ecological networks, based on predator- prey (negative) and facilitative (positive) interactions, were used to describe and compare network topology. Human microbiota, like all ecological systems, can be described by both its species composition (the identity of bacteria, archea, and fungi that live on and in the human body) and its structural topology based on interactions between species (Ings et al., 2009; Dunne, 2009). Since the human microbiome project began in 2006, considerable effort has been expended to assemble comprehensive species lists for regional microbiomes (NIH HMP Working Group, 2009). By assembling these species lists into structural networks, I am able to explore differences in the topological properties across regions. Structural properties of networks, or network topology, can affect many aspects of the dynamics and function of systems including temporal stability, resistance to invasions, resilience to disturbance, and persistence (Williams & Martinez, 2008; Romanuk et al., 2009; Dunne, 2009; Solé & Montoya, 2001; Dunne et al., 2002a; Dunne et al., 2002b; Dunne & Williams, 2009).

Microbiome networks are somewhat different from most other networks types that are studied. There are two broad classes of energetic links that occur in the microbiome: consumer-resource (feeding/negative interactions) links and facilitative (positive) interactions. Consumer-resource links include predator-prey, herbivore-plant,

and parasite-host interactions (Dunne, 2009). Facilitative links may be syntrophic or mechanical. Syntrophic facilitative links, or cross-feeding links, correspond to interactions where a species lives off the products of another species (Ings et al., 2009). For example, in the human respiratory tract, *Brevibacterium* spp. feed on sugars from *Micrococcus* spp., but not on *Micrococcus* spp. itself (Wilson, 2008). Mechanical facilitative interactions occur when a micro-organism needs to physically attach to another organism to obtain specific resources and thus are indirect energetic links. For example, in the oral cavity *Fusobacterium* spp., which form part of the plaque surrounding teeth in the oral cavity, physically attach to *Neisseria* spp., which attach directly to the teeth. This is because *Fusobacterium* spp. lacks the adhesions to attach directly to the tooth surface so they fasten indirectly by attaching onto *Neisseria* spp (Wilson, 2008).

There are a wide range of topological properties that can be used to describe the nodes and links in a network. The main structural properties I used are: the number of nodes (NS), number of links (L), number of links/nodes (L/NS), connectance (L/NS^2), clustering coefficient (CC), self facilitation, and path length (path) (Dunne, 2009; Watts & Strogartz, 1998). These properties that have been reported in several comparative, model-based studies of structure of biological networks that contain both negative (consumer) (Dunne et al., 2008; Dunne et al., 2002b) and positive (facilitative) (Jordano, 1987; Paine, 1980; Olesen et al., 2007) interactions.

Since it is very difficult to compile detailed long term empirical data for dynamics of many species interactions, research on these interactions usually relies on analytical or simulation modeling (Dunne et al., 2008). Ecologists have used network simulation

modeling to explore the potential for secondary extinctions in response to perturbations in food webs (Srinivasan et al., 2007; Dunne & Williams, 2009; Roopnarine, 2006; Roopnarine et al., 2007). Previous studies on removing species from empirical food webs have shown that the results of complex link topologies correspond to those of actual ecological communities (Sole´ & Montoya, 2001; Dunne et al., 2002a; Allesina & Bodini, 2004; Memmott et al., 2004; Srinivasan et al., 2007). Like all networks, human microbiome networks can be modeled using removal simulations to relate them to real world primary removals and secondary extinctions. Node removals are simulated to 1) assess how robust the networks might be to node loss; and 2) to determine whether robustness differs among networks (Dunne et al., 2002b; Dunne & Williams, 2009).

The objective of this chapter is to determine the composition and topological structure of the functional human microbiome for five regions (the eye, the oral cavity, the skin, the gastrointestinal track (GIT), and the respiratory system) of the human body and to determine the robustness of the networks to node removals (e.g. that might occur following the use of antibiotics).

2.3 Methods

I constructed a data set of all the micro-organisms and their source-specific nutrients that have been identified for five regions of the human body: the surface of the skin (including hair follicles but excluding genital skin), the globe of the eye, the oral cavity (limited to the oral mucosa, tongue, and teeth), the gastrointestinal tract (from the esophagus to the rectum), and the respiratory system (including sinuses, trachea, and lungs). These data sets were compiled from primary and secondary literature from approximately 150 sources (e.g., Wilson, 2008; Bojar & Holland, 2002; Hayashi et al., 2002). Data on species composition is typically provided in the form of species

abundance in an individual or population from both culture-based and sequence-based studies. Although data was provided on particular species abundant in the human body regions, not enough information was found on the specific metabolic properties of the species; therefore species were aggregated into genera-level nodes. This was done by first creating links between species and their source-specific resources, then aggregating the links to genera. The types of individuals included in the study were healthy humans between the ages of 20 and 40 years, due to the microbiota structure being dependant on factors such as age, geography, ethnicity, gender, etc. Macro-organisms and microbiota that are only pathenogenic were excluded as well as macro-parasites and micro-parasites.

Source-specific resources, i.e. resources provided by more than one source, were identified for each region. For example, in the skin, amino acids secreted as part of the individual's sweat were distinguished from amino acids available directly from the host's epidermal cells (Wilson, 2005). Predator/prey links for consumer-resource (negative) networks and facilitative (positive) links for facilitative networks were assembled for the different regions. This was done first by distinguishing the genera, then identifying the source-specific resources they use and produce in the five regions of the body. Next, consumer-resource and facilitative links between the microbiota were compiled based on the source-specific resources they require and produce. For example, in the oral cavity *Actinomyces* spp. feed on proteins provided by the host, this is an example of a consumer-resource link (Wilson 2008). An example of a facilitative link in the oral cavity is when *Actinomyces* spp. obtains sugars produced by *Propionibacterium* spp. (Wilson, 2008).

Three different networks were created based on these links for each of the five regions: 1) composite networks (with both consumer-resource and facilitative links), 2) consumer-resource networks, and 3) facilitative networks. Networks for each region were analyzed using Network 3D (software written by R. J. Williams).

Seven different topological properties for the consumer-resource networks and the facilitative networks were calculated and compared. number of nodes (NS), the number of links (L), the number of links per node (L/NS), connectance (L/NS^2), clustering coefficient (CC), self facilitation, and path length (path). The number of nodes represents the number of genera in the networks, while the number of links represents the links among the genera. Connectance measures complexity in the networks and is the proportion of possible links in the network that actually occur, and the clustering coefficient is the possibility that two taxa linked to the same taxon are linked (Dunne, 2009). Self facilitation is when a taxon uses a resource that they themselves produce. Path length is a calculation of the mean shortest set of links between nodes (Dunne, 2009).

To compare the topology of different regional microbiomes I aggregated genera that share 100% of their links into functional nodes. These functional nodes represent groups of genera and nutrients that contain identical sets of interactions with other nodes. In the context of these host microbial networks this leads to aggregation not just of taxa with similar links, but aggregation into functional nodes where groups of microbiota all perform a similar function. Aggregating nodes based on similar links is a common approach used in food-web ecology that minimizes bias due to uneven resolution, incomplete sampling effort, or differences in sampling effort across different food-webs (Martinez, 1991; Hall & Raffaelli, 1991; Dunne et al., 2005). Topological properties of

the composite networks (combined consumer-resource and facilitative), the consumer-resource networks, as well as, facilitative networks were calculated for each region.

To determine the magnitude of secondary extinctions (node loss) that results from primary node removal, I conducted three sets of node removals. Removal scenarios included removing the most connected (mc), least connected (lc), and random (ran) nodes to: 1) assess how robust the functional networks were to primary node loss; and 2) to determine whether robustness differs across the five microbiomes. For the random removals, 1000 iterations were simulated for each regional network. Robustness is defined based on the R_{50}, the proportion of nodes that need to be removed to collapse a network to 50% of its initial size. Basal species (species with predators but no prey), which in this case were source-specific nutrients, were excluded from removals. The most connected and least connected nodes at each removal step were determined based on the network remaining after all previous primary and secondary extinctions. The robustness of the composite networks, the consumer-resource networks, as well as, facilitative networks was determined for each region. After the R_{50} was determined for these three scenarios, I used linear regression analysis to examine the relationship between R_{50}, and three measures of food web complexity: the number of nodes (NS), number of links (L), and connectance ($C=L/NS^2$).

2.4 Results

2.4.1 Network topology

The human microbiota networks included 75 genera: 72 bacteria, two fungi (*Malassezia* and *Candida*), one archean, *Methanobrevibacter* (Table 1.4), and 39 source-specific resources (e.g., proteins, carbohydrates: Table 1.5) for a total of 115 nodes and 2335 links across all five regions. The composite human microbiome had a functional

node richness of NS=107, and 2169 links, and an average of NS=40 across the five regional networks (Figure 1, Table 2A). Thirteen nodes were aggregated into functional nodes for the composite functional network. The nodes that were aggregated were: 1) *Aggrebacter* spp. and *Simonsiella* spp., 2) amino acids in sweat and amino acids in host epidermal, 3) fatty acids in sebum, in host sweat, and in host epidermis, 4) sugar in diet and sugar in saliva, 5) protein from host nasal fluid and protein from the host airway surface liquid, 6) protein in mucus and protein in gingival crevicular fluid.

In the composite functional networks of the five regions, the oral cavity had the highest number of links (957), while the eye had the fewest (91) (Table 2.1A). The oral cavity also had the highest connectance (0.27), while the GIT had the lowest (0.17) (Table 2.1A). The fraction of self-facilitation ranged from 0.19 to 0.29, and the path length ranged from 1.63-1.87 in the regional composite networks (Table 2.1A). The composite eye network had the largest clustering coefficient (0.59), and the respiratory tract (0.39) and GIT (0.4) composite networks had the smallest (Table 2.1A). Visual representations of the five regions and their composite, consumer-resource and facilitative functional networks are shown in Figures 2.1-2.6.

For the consumer-resource networks, node richness ranged from NS 20 for the eyes to NS 45 for the respiratory network. Connectance ranged from 0.05 to 0.1, and was highest in the eye network. Path length ranged from 2.39 to 2.76 and was highest in the oral network. Clustering coefficient and self-facilitation for the regional networks is zero (Table 2.1B).

For the facilitative networks, node richness ranged from 5 for the eye to 47 for the oral cavity network. Connectance ranged from 0.30 to 0.72, and was highest in the eye.

Path length ranged from 1.0 to 1.54, and was highest in the GIT. The clustering coefficients ranged from 0.45 in the respiratory network to 0.73 in the eye network. The fraction of self-facilitation ranged from 0.29 (respiratory tract) to 0.60 (eye) (Table 2.1C).

Overall, facilitative links dominated the networks for all regions except for the eye. In total, facilitative links make-up 67% of the links in the network (Figure 2.7, Table 2.1B, 2.1C). The oral cavity links had the highest difference between the fractions of consumer-resource (7%) versus facilitative links (93%) (Figure 2.7). The eye had the lowest difference between the numbers of consumer-resource (68%) versus facilitative links (32%), and it was the only region that had a lower fraction of facilitative links than consumer-resource links (Figure 2.7).

As node richness increased, the difference between the fraction of consumer-resource and the percent facilitative links increased (Figure 2.7). Node richness was lowest in the eye, followed by the skin, respiratory tract, GIT and oral cavity for the facilitative networks. For the consumer-resource networks node richness was lowest in the eye, followed by the skin, GIT, oral, and respiratory tract (Table 2.1B, 2.1C). Consumer-resource networks had a higher number of nodes, except for in the oral cavity networks, than the facilitative networks. Consumer-resource networks also had higher path lengths than the facilitative networks. Facilitative networks had higher connectance values, higher clustering coefficients, and higher self-facilitation than the consumer-resource networks (Table 2.1B, 2.1C). The consumer-resource networks had a mean connectance of 7%, while the facilitative networks have a mean connectance of 46%.

2.4.2 Node removals

When nodes were removed randomly (ran) from the composite regional networks, 38% of the non-basal nodes needed to be deleted to collapse the networks to 50% of their initial size on average, suggesting that the composite microbiomes were robust to random node removals. Random deletions triggered the highest number of secondary extinctions in the eye, with 24% of primary removals needed to reduce the network to half its initial size, followed by the skin (35%), GIT (42%), respiratory tract (44%), and the oral cavity (45%). Less than a 13% difference in robustness was observed based on whether removals occurred in ascending (least connected= lc) or descending order (most connected= mc) of connectivity for the eye (mc= 19%, lc= 29%), skin (mc= 29%, lc= 37%), GIT (mc= 32%, lc= 41%), respiratory tract (mc= 34%, lc= 47%), and the oral cavity (mc= 44%, lc= 44%). Overall, the oral cavity (ran= 45%, mc= 44%, lc= 44%) was the most robust to node removals for the composite networks (Figure 2.8A).

For the random removal scenario in the consumer-resource regional networks, 36% of the non-basal nodes needed to be deleted to collapse the networks to 50% of their initial size on average, suggesting that the consumer-resource microbiomes were generally robust to random node removals. This result is similar to what was observed in the composite networks, except that networks with the least connected (lc) removals were more robust compared to the networks with most connected (mc) removals for the oral cavity (mc= 32%, lc= 41%). There was less than a 10% difference between the most connected and least connected removals for the respiratory tract (mc= 36%, lc= 42%), GIT (mc= 31%, lc= 41%), skin (mc= 29%, lc= 37%), and the eye (mc= 15%, lc= 25%) regions. Overall, the respiratory tract (ran= 44%, mc= 36%, lc= 42%) was the most robust to node removals for the consumer-resource networks (Figure 2.8B).

For the facilitative networks, all of the regions were highly robust across all removal scenarios with an average robustness of 44%. When nodes were removed randomly from the facilitative regional networks, 45% of the non-basal nodes needed to be deleted to collapse the networks to 50% of their initial size on average, suggesting that the facilitative networks were highly robust to random node removals. Random deletions triggered the highest number of secondary extinctions in the eye, with 36% of primary removals needed to reduce the network to half its initial size, followed by the GIT (43%), skin (48%), respiratory tract (49%), and the oral cavity (50%). Less than a 6% difference in robustness was observed based on whether removals occurred in ascending (least connected) or descending order (most connected) of connectivity for the eye (mc= 40%, lc= 40%), skin (mc= 40%, lc= 46%), and respiratory tract (mc= 46%, lc= 50%). In contrast, connectivity of node loss strongly affected robustness for the GIT (mc= 32%, lc= 46%), and the oral cavity (mc= 34%, lc= 49%) with a difference of more than 14%. The respiratory tract was highly robust across all three methods of node removals (ran= 49%, mc= 46%, lc=50%), while the oral cavity was highly robust to the random (50%) and least connected (49%) removals, but was highly susceptible to collapse following removal of the most connected nodes (35%)(Figure 2.8C).

When comparing the composite, consumer-resource and the facilitative network removals, removing the least connected nodes and the nodes randomly led to less secondary extinction than removing the most connected nodes except for the eye facilitative network (Figure 2.8A, 2.8B, 2.8C). Furthermore, the facilitative networks were more robust in general than the corresponding consumer-resource and composite networks. However there were two exceptions, first removing most connected taxa in the

oral cavity region facilitative network caused more secondary extinctions than in the oral cavity consumer-resource and composite network. Second, removing most connected taxa in the GIT regions facilitative network caused more secondary extinctions than in the GIT consumer-resource and composite networks. The oral cavity (44%) composite network was the most robust across all the removal scenarios and the eye (24%) was the least, while in the consumer-resource networks, the respiratory tract (40%) was the most robust across all the removal scenarios and the eye (21%) was the least. In the facilitative networks, the respiratory tract (49%) was the most robust to node removal across all the scenarios and the eye (39%) was the least (Figure 2.8A, 2.8B, 2.8C).

Across the three types of networks (composite, consumer-resource, facilitative), the robustness of networks under the three removal criteria did not vary significantly with the number of links. In contrast, robustness was strongly related to the number of nodes and connectance in the composite and consumer-resource networks but not for the facilitative networks (Table 2.1). Robustness increased as node richness increased in the composite (r^2= 0.91, p= 0.01); and consumer-resource networks (r^2= 0.87, p= 0.02) (Table 2.1). Robustness decreased with increasing connectance in the consumer-resource networks (r^2= 0.95, p= 0.0049) (Table 2.1). In the facilitative networks, there was no significant relationship between the number of nodes, number of links, or connectance to the average robustness (Table 2.1).

2.5 Discussion

Studying complex interactions in biological systems and understanding their structure and robustness is the goal of systems biology. The ultimate goal of the human microbiome project is to develop a 'super-organism' theory of human health and disease (NIH HMP Working Group, 2009).

My analysis of the topological structure of human microbiome networks has shown that despite the differences in scale between microbiome networks, and there are some surprising topological similarities between consumer-resource microbiome networks and composite food webs of whole ecosystems such as similar levels of connectance. There are also some potentially fundamental differences between microbiome networks and ecosystem food webs. In particular, the inclusion of facilitative links in microbiome networks results in extremely high clustering coefficients and connectance. Below I discuss similarities and differences in the topology of consumer-resource microbiome networks and composite food webs and the topological consequences of the inclusion of facilitative links in microbiome networks. Lastly, I discuss how the topology of microbiome networks affects how robust microbiome networks are to perturbations such as node removal and how robustness of microbiome networks to perturbations differs from ecosystem food webs and mutualistic networks.

2.5.1 Consumer-resource/facilitative networks vs. food webs

Despite the scale-related differences between microbiome networks (organismal) and composite food-webs (entire ecosystems), topological properties of human microbiome networks showed a number of similarities to the structure of ecosystem food webs. Consumer resource-networks only have two trophic levels, whereas food webs (e.g., Little Rock Lake) generally have more than three trophic levels (Williams & Martinez, 2000). Microbiome consumer-resource networks have connectance values (0.05-0.1) that fall within the range of 16 ecosystem food webs (0.026-0.315), path lengths (2.39-2.76) that fall in the ranges of the food webs (1.33-3.74), and links per node (1.88-2.34) that fall in the ranges of the food webs (1.59-25.13). These comparisons show

that the consumer-resource networks values were at the lower end of the connectance, path length, and links per node ranges but were generally similar to the properties observed in composite food webs. Microbiome networks generally had fewer nodes due to aggregation of species into genera (20-45) than composite food webs (33-586), and lower clustering coefficients for the consumer-resource networks (microbiome networks 0, food webs 0.02-0.43) (Dunne et al. 2002a).

The properties of the facilitative microbiome networks can be compared to mutualism networks such as networks between plants and their pollinators. When I compared 29 mutualistic networks to the facilitative microbiome networks several differences were found. First, the number of taxa in plant-pollinator networks (22-952) was higher than the number in the facilitative networks (5-47) (Olesen & Jordano, 2002). Second, the number of links in mutualistic networks was larger (27-2933) than the number of links observed in the microbiome facilitative networks (18-848) (Olesen & Jordano, 2002). Third, connectance in mutualistic networks is smaller (0.02-0.29) than in facilitative microbiome networks (0.3-0.72). These differences may have arisen as facilitative microbiome networks differ from other mutualism networks in the nature of the interactions between the taxa. For example, in microbiome facilitative networks, facilitative interactions include two types of facilitation (mutualism and commensalism). In plant-pollinator networks only mutualistic interactions occur such that both the organisms gain fitness (Dash, 2001). In the context of the microbiome networks, syntrophic facilitation can be mutualistic or commensal depending on whether the microbe gains resources for itself and other micro-organisms or just for other microbiota. Furthermore, mechanical interactions occur in the facilitative networks and these

represent commensal interactions because one species uses another species to physically attach to a substrate.

2.5.2 Consumer-resource vs. facilitative networks

There are a number of important differences between consumer-resource microbiome networks and facilitative microbiome networks. First, consumer-resource microbiome networks contain two trophic levels (microorganisms and their resources) while in facilitative networks all nodes are at the same trophic level. Second, the facilitative links differ from the consumer-resource links in that they do not represent the death of an individual following energy transfer but rather, can be energetic (energy is transferred between individuals as is shown by syntrophic links) or non-energetic (links occur due to obligate relationships such as mechanical attachment). Consumer-resource links show feeding interactions between predator and prey. Therefore there is only one type of interaction occurring in consumer-resource networks compared to the two in facilitative networks.

Whether the topology of food webs differs from other ecological networks such as mutualism networks is of considerable interest (Ings et al., 2009; Solé & Montoya, 2001; Montoya et al., 2006). For example, Ings et al. (2009) compared the number of links and species richness for host-parasitoid networks and food webs. They showed that food webs have more links for the same number of species than do host-parasitoid networks (Ings et al., 2009). Between the topological properties of the consumer-resource microbiome networks and the facilitative microbiome networks, five main differences were found: 1) as node number increased the fraction of links in consumer-resource networks decreased, whereas in the facilitative networks the fraction of links increased 2)

Facilitative links dominated the links in the consumer-resource networks, with 67% of links representing facilitative interactions. 3) The facilitative networks had a higher mean connectance of 40%, compared to the consumer-resource networks. 4) The clustering coefficient was zero in the consumer-resource networks and ranged from 0.45 to 0.73 in the facilitative networks, suggesting that there is more redundancy in facilitative networks. 5) Self facilitation only occurred in the facilitative networks, and was highest in the eye region. These results suggest that facilitation between microbial species is an important component of the function of microbiome networks. The study of positive interactions (facilitation) is receiving increasing attention in ecological research (Stachowicz, 2001; Callaway, 1995). My results suggest that facilitative interactions in the microbiome networks are highly complex and must be incorporated to develop an understanding of the function of microbiota within the human body.

2.5.3 Robustness

The robustness of microbiome networks to node removal is of particular interest as many diseases are treated with broad spectrum antibiotics that reduce abundance and diversity of pathogenic microbiota as well as indigenous microbiota (Guarner et al., 2003; Tagg & Dierksen, 2003). One main pattern observed related to robustness to node removal was that networks are more robust to random and least connected removals than most connected removals. This pattern held for each of the regional composite, consumer-resource, and facilitative networks except for the oral composite and the eye facilitation networks. Comparing the result to other networks (e.g., food webs), a similar trend was observed with removal of most connected nodes generally leading to a greater fraction of secondary extinctions than removal of more weakly connected or random

nodes (Dunne, 2009; Strogatz, 2001). This could be because when highly connected nodes are removed from networks, the average path length tends to increase quickly, and the networks rapidly partition into isolated clusters. Therefore, networks are simply more disrupted by loss of nodes that are directly connected to an unusually large number of nodes (Dunne, 2009).

2.5.4 Robustness for network types (composite, consumer-resource, facilitative)

When comparing the mean robustness of the composite, consumer-resource, and facilitative network types for the five microbiome networks, I found facilitative networks were more robust than consumer-resource and composite networks. Memmott et al. (2004) determined the robustness of pollinator networks (mutualistic networks) to node removals. The loss of plant diversity associated with removals of pollinators was not as extreme as the loss of pollinators, which may be due to the redundancy and the nested topology of those networks (Memmott et al., 2004). The results of our node removal simulations in the microbiome networks are similar to the plant-pollinator removals in that the facilitative networks were more robust than the consumer-resource networks.

2.5.5 Robustness for regional networks (eye, skin, oral cavity, GIT, respiratory tract)

Mean robustness was highest for the oral cavity in the composite networks. The robustness of the composite networks is particularly important because it includes all the interaction types (facilitation and consumer-resource), thus reflecting changes that would occur in both facilitative and consumer-resource interactions. Since the oral cavity was the most robust to removals for the composite regional networks, in a 'real world' context this suggests that the oral cavity may be highly robust to perturbations. This could be due to aspects of the environment. For example, the oral cavity is exposed to many pathogen

invasions, and it may be structured (network topology of microbiota and source specific resources) in a way as to inhibit invasions. The eye was the least robust to node removals and robustness of the eye was significantly lower than in the other four network types. This was likely a result of the eye having the fewest nodes of all networks.

2.5.6 Robustness compared to network topology

Robustness is, in general, strongly related to network topology (Dunne et al., 2002a). Dunne (2009) stated that from a topological perspective, food webs with more densely interconnected taxa are more robust to species loss, because it takes greater species loss for consumers to lose all their resources. In the human microbiome context, high connectance was not positively related to robustness. The eye region has the highest connectance compared to the other regions across all the network types and was the least robust. Instead, for the composite and consumer-resource networks robustness increased as the number of nodes increased. Interestingly, robustness increased with decreasing connectance (Fig 2.9). This latter result differs from previous analyses of the robustness of food webs to primary removals where robustness generally increases with connectance (Dunne et al., 2002a; Dunne et al., 2004). One potential reason for this discrepancy lies in the range of connectance values of microbiome networks relative to food web networks. Connectance in the consumer-resource networks ranged from 0.06-0.1 while in whole food-webs connectance typically ranges from around 0.03 to 0.3.

Robustness to node loss in other ecological networks is strongly dependent on connectance, with higher connectance generally leading to high robustness (Dunne et al., 2002a). This mechanism relates to structure. However from the perspective of community succession higher connectance is often a result of a community reaching a

climax state, containing both specialists and generalists, and experiencing lower turnover. In the human microbiome robustness to node loss is proximally determined by aspects of structure. But from an evolutionary standpoint the structure of microbial communities is determined by the interplay between ecology and evolution. Thus the ultimate determinant of robustness may be linked to environmental conditions in different regions and in particular aspects of homeostatic regulation. For example, in a region with only minimal mechanical action such as the respiratory tract, community composition is likely to better reflect the characteristics of a climax community, where biotic interactions become much more important than abiotic conditions after the initial community development. Likewise, a region that suffers from strong mechanical action, such as the skin, where the upper layer in replaced every 15 days, may be structured more similarly to a community at an early succession stage that is experiencing frequent invasions and extinctions and thus might have a structure that is optimized for low homoeostatic regulation and ease of turnover.

2.5.7 Final remarks

Looking at regional human microbiomes from a network approach has a number of prospective benefits, even at this early stage of investigation. Mathematical network theory is ideal for studying the interactions between species in ecological networks, allowing us to understand both normal and disturbed microbial community functions, from the standpoint of systems biology (Foster et al., 2008). Systems biology has also strong theoretical groundwork relating to potential trade-offs between structural robustness, resilience, and redundancy. A homeostatic view related to regulation of the internal and external environment and the robustness and resilience of microbial

networks has great potential for exploring mechanisms that maintain function in human health. Furthermore, a network perspective may also provide the key to developing a more complete understanding of diseases.

Table 2.1. Structural properties for five regional networks (eye, skin, respiratory tract (Resp), gastrointestinal tract (GIT), and oral cavity (oral)) of the human body. A) Composite networks, B) Consumer-resource networks, and C) Facilitative networks. The structural properties are number of nodes, number of links, links/nodes, connectance, self facilitation, path length, and clustering coefficient.

A) **Composite networks (Consumer-resource and Facilitaive interactions)**					
	Eye	**Skin**	**Resp**	**GIT**	**Oral**
Number of nodes	21	35	43	44	59
Number of links	91	271	381	321	957
Links/Nodes	4.33	7.74	8.86	7.29	16.22
Connectance	0.21	0.22	0.21	0.17	0.27
Fraction Cannibal	0.29	0.25	0.19	0.27	0.25
Path length	1.87	1.84	1.70	1.76	1.63
Clustering Coefficient	0.59	0.58	0.39	0.4	0.47
B) **Consumer-resource networks**					
	Eye	**Skin**	**Resp**	**GIT**	**Oral**
Number of nodes	20	35	45	36	34
Number of links	38	82	105	70	64
Links/Nodes	1.9	2.34	2.33	1.94	1.88
Connectance	0.1	0.07	0.05	0.05	0.06
Fraction Cannibal	0	0	0	0	0
Path length	2.39	2.39	2.63	2.6	2.76
Clustering Coefficient	0	0	0	0	0
C) **Facilitative Networks**					
	Eye	**Skin**	**Resp**	**GIT**	**Oral**
Number of nodes	5	15	28	28	47
Number of links	18	121	263	235	848
Links/Nodes	3.60	8.07	9.39	8.39	18.04
Connectance	0.72	0.54	0.34	0.3	0.38
Fraction Cannibal	0.6	0.47	0.29	0.43	0.32
Path length	1	1.28	1.43	1.54	1.4
Clustering Coefficient	0.73	0.7	0.45	0.46	0.49

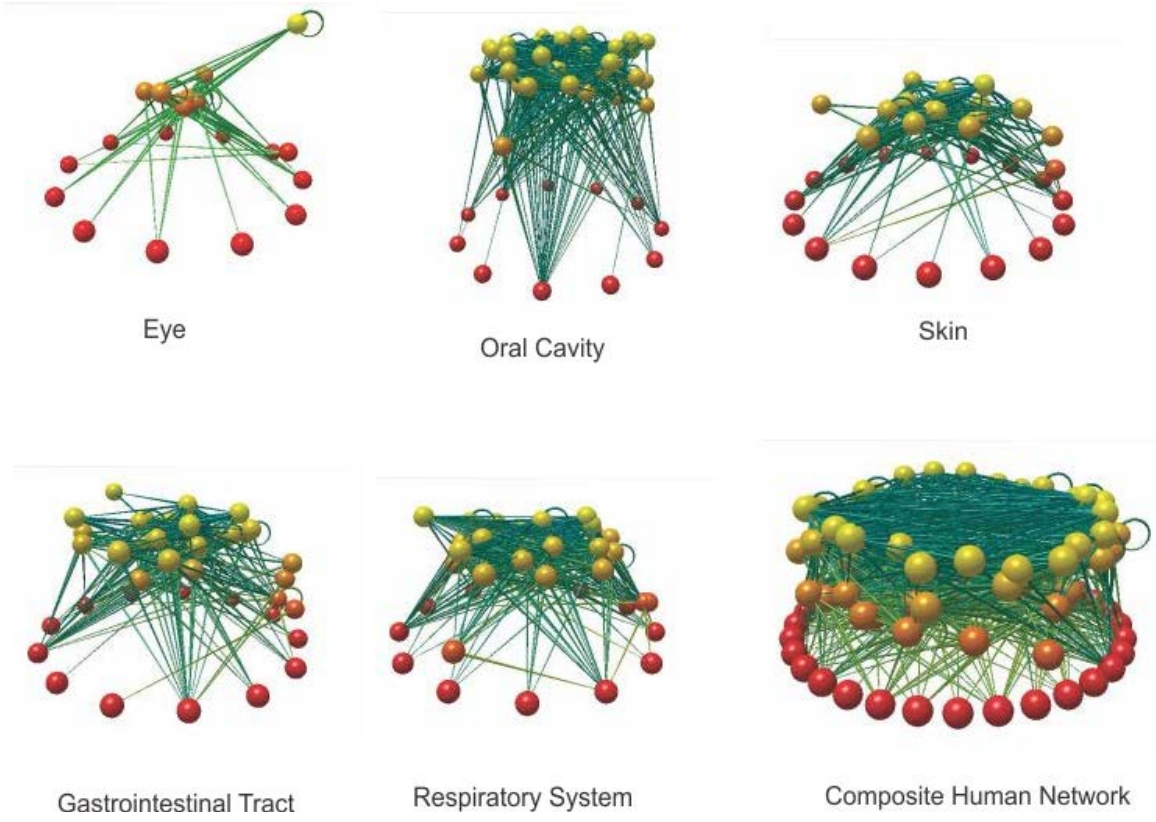

Figure 2.1. Visual representations of the complete human, eye, skin, oral cavity, respiratory, and gastrointestinal composite networks. Circles represent nodes, with red nodes representing source-specific nutrients, and orange and yellow nodes representing genera. Links are represented by the green/blue lines between the nodes. Images are from Network 3D (software written by R. J. Williams).

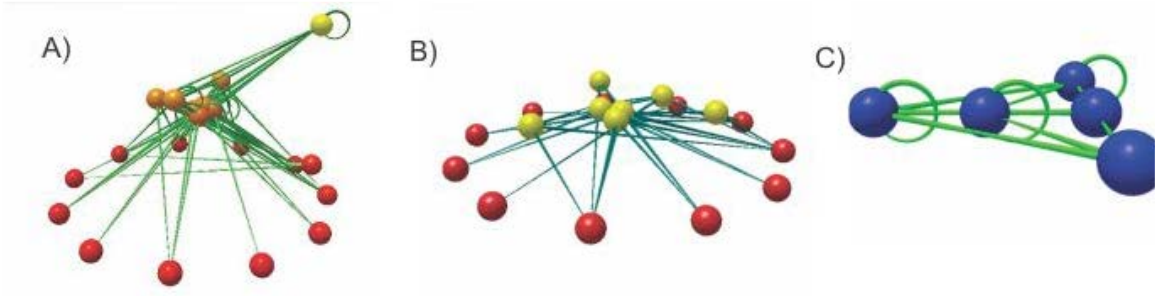

Figure 2.2. Visual representation of the human eye networks: A) composite, B) consumer-resource, C) facilitative. Circles represent nodes, with red nodes representing source-specific nutrients, and orange and yellow nodes representing genera for composite and consumer-resource networks. For the facilitative network the blue nodes represent genera. Links are represented by the green/blue lines between the nodes. Images are from Network 3D (software written by R. J. Williams).

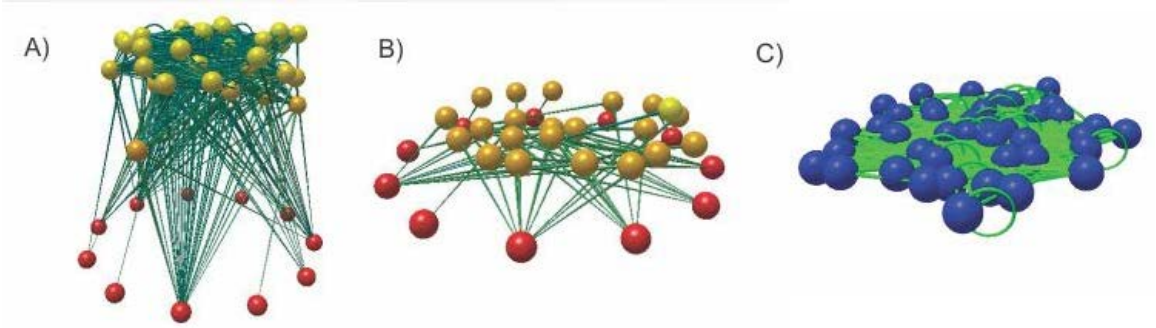

Figure 2.3. Visual representation of the human oral cavity networks: A) composite, B) consumer-resource, C) facilitative. Circles represent nodes, with red nodes representing source-specific nutrients, and orange and yellow nodes representing genera for composite and consumer-resource networks. For the facilitative network the blue nodes represent genera. Links are represented by the green/blue lines between the nodes. Images are from Network 3D (software written by R. J. Williams).

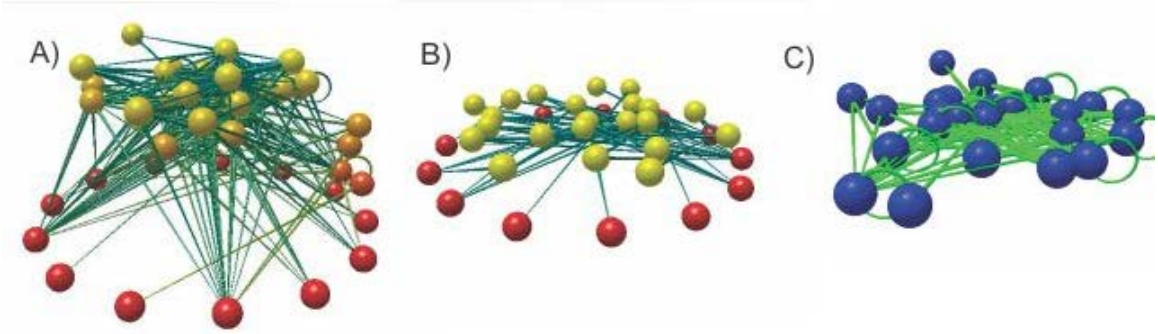

Figure 2.4. Visual representation of the human gastrointestinal networks: A) composite, B) consumer-resource, C) facilitative. Circles represent nodes, with red nodes representing source-specific nutrients, and orange and yellow nodes representing genera for composite and consumer-resource networks. For the facilitative network the blue nodes represent genera. Links are represented by the green/blue lines between the nodes. Images are from Network 3D (software written by R. J. Williams).

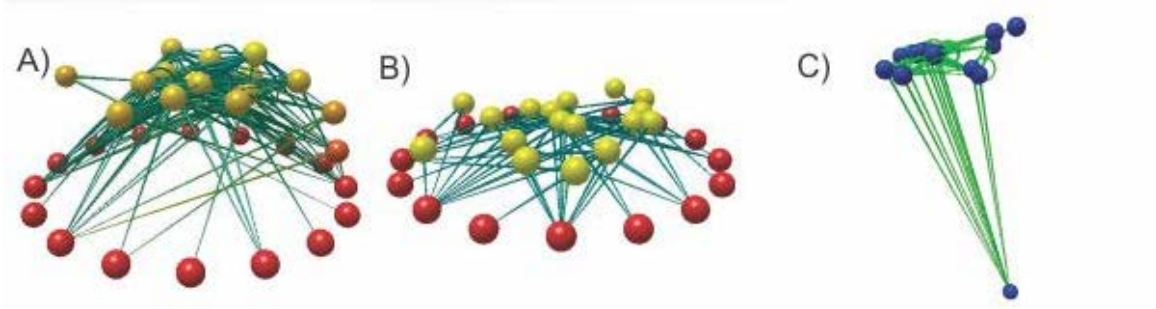

Figure 2.5. Visual representation of the human skin networks: A) composite, B) consumer-resource, C) facilitative. Circles represent nodes, with red nodes representing source-specific nutrients, and orange and yellow nodes representing genera for composite and consumer-resource networks. For the facilitative network the blue nodes represent

genera. Links are represented by the green/blue lines between the nodes. Images are from Network 3D (software written by R. J. Williams).

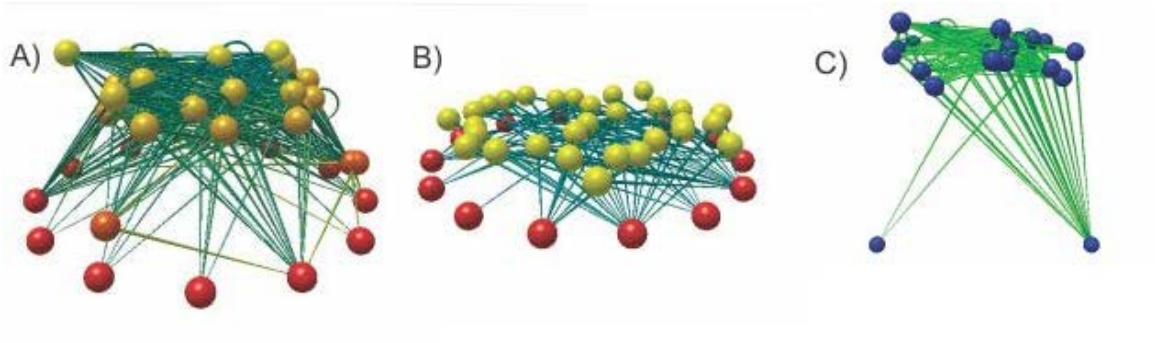

Figure 2.6. Visual representation of the human respiratory tract networks: A) composite, B) consumer-resource, C) facilitative. Circles represent nodes, with red nodes representing source-specific nutrients, and orange and yellow nodes representing genera for composite and consumer-resource networks. For the facilitative network the blue nodes represent genera. Links are represented by the green/blue lines between the nodes. Images are from Network 3D (software written by R. J. Williams).

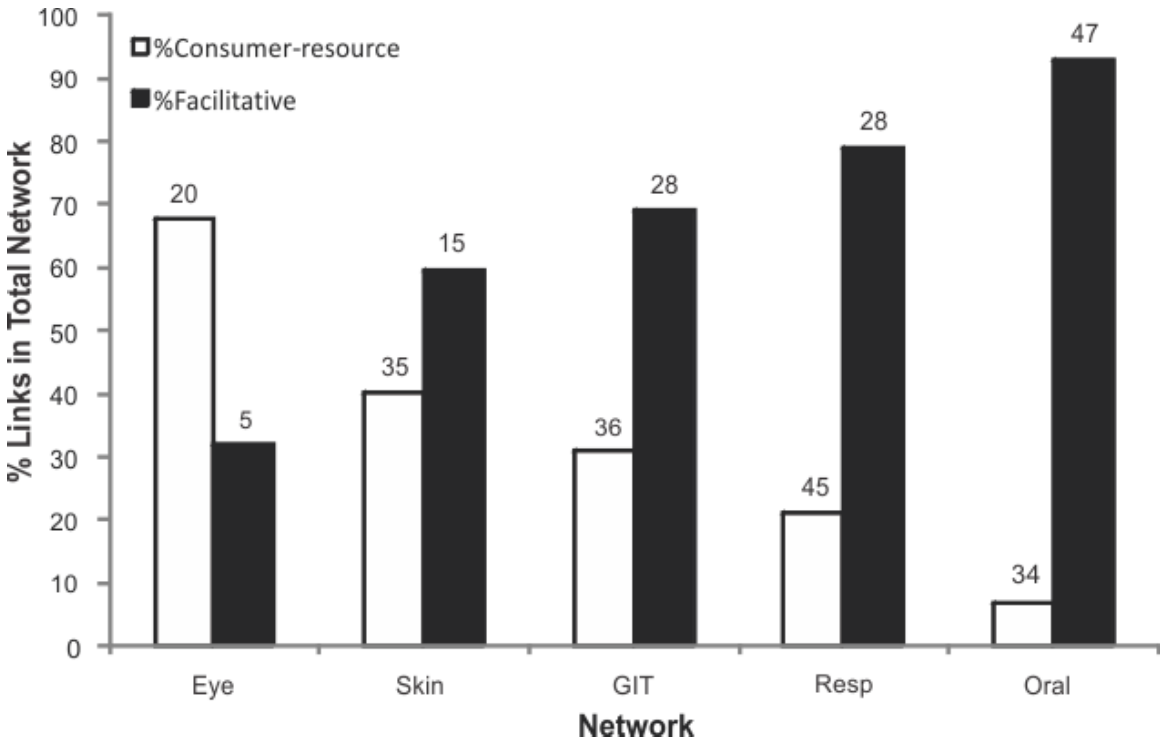

Figure 2.7. Regional (eye, skin, gastrointestinal tract (GIT), respiratory tract (Resp), and oral cavity) networks verses the percent of links in the total network (composite), to compare the difference in consumer-resource and facilitative links in the networks. Percent consumer-resource links are shown in white, while percent facilitative links are shown in black. The sample size is shown above each bar.

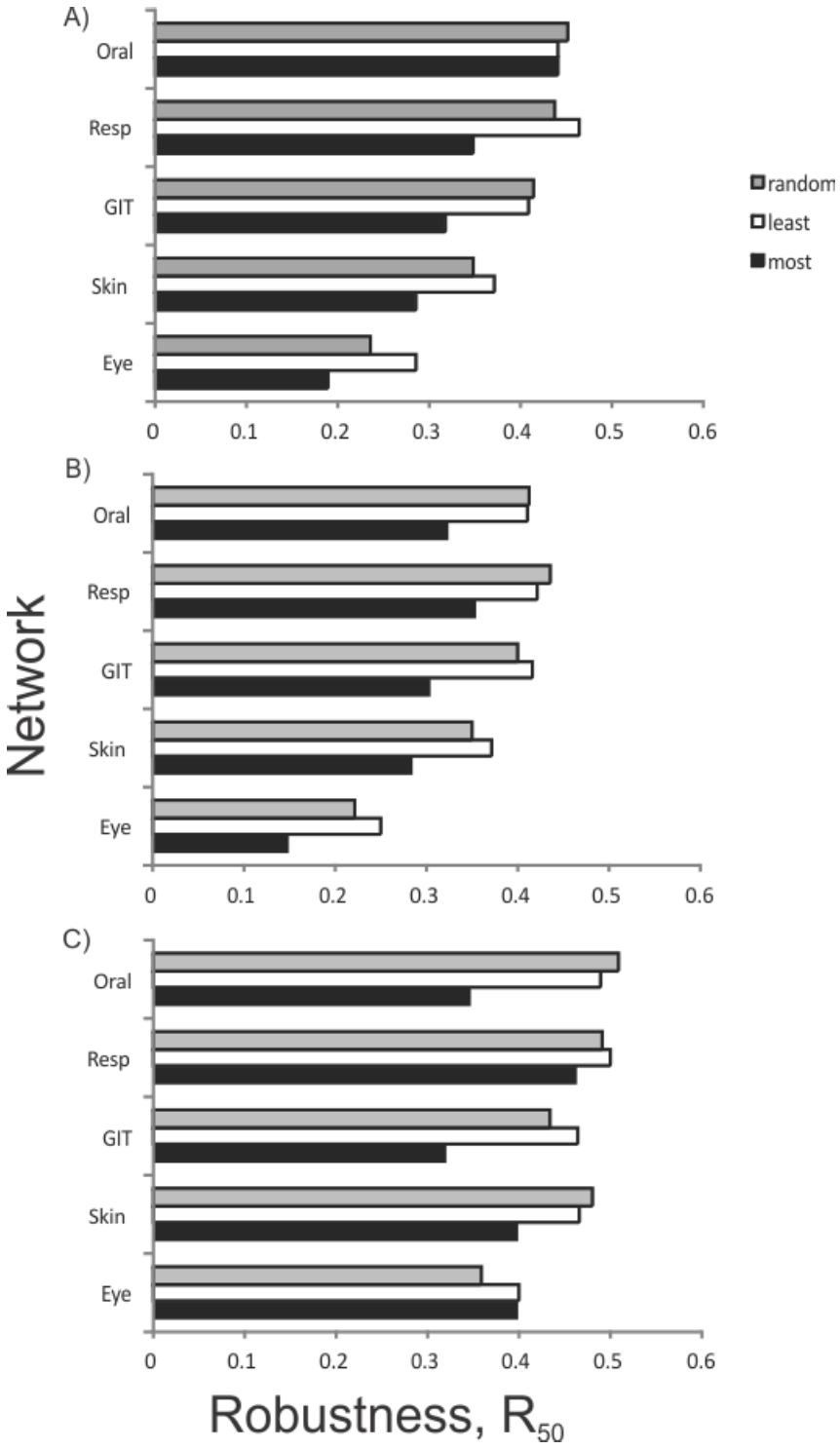

Figure 2.8. Robustness of networks to node removals for five different regional networks (eye, skin, oral cavity, gastrointestinal tract and the respiratory tract). Three methods of removals are shown, most connected (black), least connected (white) and randomly connected (shaded), for each regional network. Three main network types are shown, A) composite, B) consumer-resource and C) facilitative networks.

Table 2.2. Regression analysis for the composite, consumer-resource (C-R) and facilitative (Fac) networks to test if average robustness is driven by either the number of nodes, the number of links or the connectance of the networks. Bold indicates significant results.

	Number of Nodes			**Number of Links**			**Connectance**		
Network	Slope	r^2	P	Slope	r^2	P	Slope	r^2	P
Composite	**0.006**	**0.909**	**0.012**	0.0002	0.641	0.104	0.759	0.112	0.583
C-R	**0.008**	**0.868**	**0.021**	0.002	0.64	0.104	**-3.699**	**0.949**	**0.005**
Fac	0.001	0.239	0.403	0	0.139	0.537	-0.118	0.275	0.363

CHAPTER 3

Ontogenic and regional patterns in network topology of human oral cavity and gastrointestinal tract microbiomes

3.1 Abstract

I assembled consumer-resource, facilitative and composite networks for human-associated microbial genera and source-specific nutrients for the oral cavity and the gastrointestinal tract (GIT) to test whether topology and robustness to node removal change ontogenically in the oral cavity and within regions of the GIT. These oral cavity networks were assembled for five developmental stages: newborn, child, adolescent, adult and elderly. The GIT networks were assembled for four regions: esophagus, stomach, small intestine, large intestine. In total, GIT regional networks contained 29 bacterial genera, 1 archean genus, and 15 source-specific nutrients. Of the 30 total genera in the GIT regional networks, only one genus, *Streptococcus*, was present in all the regions. Oral cavity ontogenic networks in total contained 58 bacterial genera, 1 fungi genus, and 13 source-specific nutrients. Of the 59 total genera in the oral ontogenic networks, seven (*Actinomyces*, *Capnocytophaga*, *Fusobacterium*, *Lactobacillus*,

Peptostreptococcus, *Prevotella*, and *Veillonella*) were present at all the ontogenic stages. For both the oral ontogenic and the GIT regional networks, facilitative links dominated (oral 86%, GIT 64%). Node richness and connectance increased with ontogenic stage in the oral cavity and in regions further down the gastrointestinal tract. Further down the gastrointestinal tract, node richness increased, as well when the oral ontogenic stages increase by age, so did the connectance of the networks. For the GIT regions the small and large intestine were more robust on average to node removals, while in the oral ontogenic networks the child, adult and elderly networks were more robust. Many different biological and developmental factors affected the topological structure of the oral ontogenic networks and the GIT regional pattern networks. This research represents the first attempt to assemble and compare microbiome networks through developmental stages and regional patterns within a specific system.

3.2 Introduction

Structural ecological networks, based on predator- prey (negative) and facilitative (positive) interactions, can be used to describe and compare the network topology of ecological systems (Ings et al., 2009). Characterizing human microbiota is complicated because the human body contains many unique microbial niches, and for some of the niches, the composition of the microbial community is so different across individuals that it has been suggested that the composition of certain skin sites could be used in forensic identification (Fierer et al., 2010). While this may be true for some regions such as the skin, other studies have shown that inter-individual differences in composition can be relatively constant when sampling is limited to specific areas (Costello et al., 2009; Grice et al., 2008; Caporaso et al., 2011).

In addition to microbiome differences across individuals, the composition of microbiomes also differs in different regions of the body (Costello et al., 2009). From a functional perspective, understanding the composition of microbiomes for different regions of the body is important as each region (e.g., GIT, skin) performs specific functions. When composition of microbiome communities is compared within regions there appears to be much less inter-individual variability. For example, Costello et al., (2009) showed that although personalized, our microbiome varies systematically across body habitats and time with microbial composition determined primarily by body habitat (Costello et al., 2009). Likewise, Grice et al., (2008) found that within the skin, bacterial communities are more similar at sites with similar physiology than in spatially adjacent sites.

In Chapter 2, I reported genera-level human microbiome networks associated with skin, the eye, the gut, the respiratory system, and the oral cavity. Here I explore two of these networks in more detail, asking two main questions. First, how does topology of gut microbiomes change spatially in four regions of the gastrointestinal tract (GIT: esophagus, stomach, small intestine and large intestine)? Second, how does the topology of the oral cavity microbiome change temporally during ontogeny?

3.2.1 Spatial distributions

The human body is an ecological landscape, harbouring unique ecosystems which arise due to a variety of biotic and abiotic determinants as well as barriers and corridors that prevent and facilitate dispersal (Gonzalez et al., 2011). A predominant theory in microbial ecology is that "everything is everywhere, but the environment selects" (Baas-Becking, 1934; O'Malley, 2007). Since dispersal is mostly limitless for microbiota, a

realized niche might be controlled by abiotic and biotic factors instead of dispersal ability (Gonzalez et al., 2011). For example, Nemergut et al., (2011) found that micro-organisms within local habitats were determined by biotic interactions, exhibiting specific co-occurrence patterns. In the human GIT, there are several factors that are constantly challenging the stability of microbial communities: 1) rapid turnover of intestinal epithelium and overlying mucus, 2) peristaltic activity, food molecules, and gastric, pancreatic, and biliary secretions, and 3) exposure to transient bacteria from the oral cavity and esophagus (Manson et al., 2008).

Human microbiota in the GIT has received much attention in recent years especially in terms of how species composition of the GIT might affect human health. The GIT has four main regions that microorganisms colonize: the esophagus, the stomach, the small intestine, and the large intestine. Each of these regions has different gut functions and thus their microbiomes are likely to differ. Different GIT regions also differ in environmental and physiological properties such as pH, transit time of food, amount of mucus, bile, peristalsis, and oxygen content (Wilson, 2008); therefore the regions microbial compositions likely differ. For example, the pH of the small intestine ranges from 5.7 to 6.4, while the stomach has a pH of 1.4 (Wilson, 2008). To develop a greater understanding of the functional differences in gut microbiomes, structural networks for each area were assembled and the topological properties across the regions of the GIT were compared.

3.2.2 Ontogenic changes

Microbial communities in the human body also change with development. Ontogeny, termed by Ernst Haeckel, describes the life history of an individual, including

the somatic growth and development from conception to adulthood (Mai et al., 2005). Through development, small changes have effects on a wide range organismal characteristics, including disease resistance (Round et al., 2008; Waterland & Jirtle, 2004), behaviour (Fujiwara et al., 1987), and fitness (Dasilao et al., 2002). The exploration of ontogenic changes in the microbiome of humans has only begun to be explored. In adults, there is evidence that microbiomes are relatively constant. For example, Costello et al. (2009) found minimal temporal variability within body habitats in adults over one year. However, major ontogenic changes that include hormonal changes can be accompanied by major changes in microbial composition. For example, Koenig et al., (2011) found microbial diversity in the gut steadily increased from birth until two and a half years.

The oral cavity contains a very diverse resident bacterial community, consisting of 100-200 species at any one time in healthy adult individuals (Rasiah et al., 2005; Wilson, 2008). Humans are gnotobiotic in the womb, or 100% human (Tlaskalová-Hogenová et al., 2004). The development of a complex community of oral microbiota begins within eight hours after birth (Percival, 2009). Differences in our oral microbiota have been related to a variety of diseases, including root caries (Preza et al., 2009) and periodontitis (Kurata et al., 2008); thus the composition of the oral microbiome is an active area of research, especially in dentistry.

The particular combination of microbial species and the resource environment that exists at any one time in a human host is an intricate association determined by a broad range of environmental and physiological determinants. In the oral cavity, there are various mechanical (e.g., chewing), nutritional (e.g., gingival crevicular fluid), and

physiochemical determinants (e.g., pH) that affect whether microbial colonization occurs. Ontogenic changes, such as the eruption of teeth, hormonal changes, and continual exposure to different microbiota can also affect which species become established in and on human hosts (Gusberti et al., 1990; Percival, 2009). For example, the hard surface of teeth provides a niche that *Streptococcus* and *Actinomyces* species typically inhabit (Percival, 2009); thus tooth eruption is a major determinant of whether or not particular species will be present in the oral cavity. Similarly, hormonal changes during puberty directly and indirectly affect oral microbiota by increasing the permeability of blood vessels to the gingival and periodontum, consequently altering the suitability of the environment for particular microbiota (Gusberti et al., 1990).

The objective of this chapter is to assemble and compare structural networks for four regions of the gut and five ontogenic stages of life for the oral cavity and to determine how robust they are to node removals. I assembled four subset regions of the GIT, the esophagus, the stomach, the small intestine, and the large intestine to compare their topological properties and their robustness. I further assembled five networks for the oral cavity based on major ontogenic changes including: colonization after birth, emergence of teeth, puberty, and a weakened immune system associated with old age. These networks provide a basis from which to explore the nature and patterns of disease associated with ontogenic changes and spatial differences within a specific region.

3.3 Methods

I assembled a data set of all the micro-organisms and their source-specific nutrients that have been identified for two regions of the human body: the oral cavity (limited to the oral mucosa, tongue, and teeth), and the gastrointestinal tract (from esophagus to rectum). The oral cavity data sets were categorized into five ontogenic

stages. The five life stages included: newborns (birth to one year of age), child (one year to seven years), adolescent (seven to seventeen years of age), adult (seventeen to ~60 years of age), and the elderly (~60 years of age and above). The gastrointestinal tract data set was categorized spatially into four main regions: the esophagus, the stomach, the small intestine (duodenum, jejunum, ileum), and the large intestine (cecum, colon). These data sets were compiled from primary and secondary literature from approximately 70 sources (e.g., Wilson, 2008; Bojar & Holland, 2002; Hayashi et al., 2002). Data on species composition was compiled from culture-based and sequence-based studies that typically report species abundance in an individual or a population. To date there has not been enough research on the metabolic properties of specific species to assemble species specific networks, thus species were aggregated into genera-level nodes. These taxa lists were used to assemble consumer-resource, facilitative, and composite networks (i.e., combined consumer-resource and facilitative networks). For the GIT networks data on species composition was included for an age range of 20-40 years, and data was only included if the genera had been cultured or sequenced from healthy humans. For all of the networks, macro-organisms, macro- and micro-parasites, and pathogenic microbiota were excluded.

For each network, source-specific resources, i.e. resources provided by more than one source, were identified. For example, in the oral cavity glucose can be secreted as part of the gingival crevicular fluid, as part of the saliva, or can be obtained directly from food obtained by the host (Wilson, 2008). Each of these three sources was considered a separate source. For the oral cavity networks, although they had microhabitat taxa differences, I included all the taxa as part of the oral cavity microbiota. Although the oral

cavity consists of a series of sub-networks, genera were aggregated across these sub-networks due to the migration of genera from one sub-network to another, as a result of mechanical perturbations (i.e., swallowing, chewing, and movement of saliva). For example, *Simonsiella* that only inhabit the hard palate and *Rothia* that live on the teeth both use sugars (Holt et al., 1994**;** Brown et al., 1969) that could come from any number of species (e.g. *Candida*) that add to this resource pool (Wilson, 2008). For the GIT networks, the microhabitats were not aggregated to determine the structural differences across the GIT regions.

For the oral cavity and the GIT networks, predator/prey links for consumer-resource (negative) networks and facilitative (positive) links for facilitative networks were assembled. To do this, genera were distinguished, and then the source-specific resources they use and produce were identified. Subsequently, consumer-resource and facilitative links between the microbiota were compiled based on the source-specific resources they require and produce. For example, in the GIT *Bifidobacterium* spp. feed on carbohydrates provided by the host; this is an example of a consumer-resource link (Wilson, 2008). An example of a facilitative link in the GIT is when *Clostridium* spp. obtains amino acids produced by *Prevotella* spp. (Wilson, 2008).

Three different networks were assembled based on these links for the oral cavity and the GIT: 1) composite networks (with both consumer-resource and facilitative links), 2) consumer-resource networks, and 3) facilitative networks. Networks for each region were analyzed using Network 3D (software written by R. J. Williams). Seven different topological properties were calculated and compared for the composite, consumer-resource and the facilitative networks: number of nodes (NS), the number of links (L), the

number of links per node (L/NS), connectance (L/NS^2), clustering coefficient (CC), self facilitation, and path length (path).

Nodes were aggregated, based on 100% similarity in their links, to compare the topology of the networks. These aggregated nodes represent functional nodes, each with its own unique function. This type of aggregation is a common approach used in food-web ecology because it minimizes bias due to: uneven resolution, incomplete sampling effort, or differences in sampling effort across different food-webs (Martinez, 1991; Hall & Raffaelli, 1991; Dunne et al., 2005). Topological properties of the composite networks (combined consumer-resource and facilitative), the consumer-resource networks, as well as, facilitative networks were calculated for each oral ontogenic network, and each GIT spatial network.

Node removals were simulated in order to determine the response of the networks to primary species loss, in terms of secondary extinctions. Nodes were sequentially removed based on three criteria, including the removal of the most connected (mc), least connected (lc), and random (ran; 1000 iterations) nodes to: 1) assess how robust the functional composite networks were to primary node loss; and 2) to determine whether robustness differs spatially and temporally. Robustness is defined based on the R_{50}, the proportion of nodes that need to be removed to collapse a network to 50% of its initial size (Dunne, 2009). Basal species (species with predators but no prey), which in this case were source-specific nutrients, were excluded from the removals. The robustness of the composite ontogenic oral networks and the composite GIT spatial networks were determined for each region. Separate comparisons were made between the spatial

networks and the ontogenic oral networks in their robustness to removals using t-tests for robustness.

3.4 Results

3.4.1 Spatial networks (GIT)

I constructed four GIT microbiome networks for each region of the GIT: esophagus, stomach, small intestine, and large intestine (Figure 3.1). In total, the GIT microbiome networks contained 29 bacterial genera, 1 archea genus, and 15 source-specific nutrients. Of the 30 total genera, only *Streptococcus* was present in all regions. Topological properties for the 100% aggregated GIT spatial networks are shown in Table 3.1. The number of genera present at each spatial stage was 6 for the esophagus, 13 for the stomach, 17 for the small intestine, and 18 for the large intestine (Table 3.2).

In the composite functional networks of the four regions, node richness ranged from NS 12 to NS 29, and the large intestine network had the highest (L 178), while the esophagus network had the fewest (L 24). The number of nodes, and number of links, increased from the esophagus, stomach, small intestine, large intestine, (Table 3.1A). The small intestine network had the highest connectance (0.22), while the esophagus had the lowest (0.17). The fraction of self-facilitation ranged from 0.08 to 0.29, and the path length ranged from 1.71 to 1.83 (Table 3.1A). The clustering coefficient for the composite spatial networks ranged from 0.31 to 0.50 (Table 3.1A). Visual representations of the composite networks are shown in Figure 3.1.

In the consumer-resource spatial networks, node richness ranged from 11 in the esophagus to 26 in the stomach and small intestine (Table 3.1B). Connectance ranged from 0.06 to 0.09, and was highest in the esophagus. Path length ranged from 2.0 to 2.7

and was highest in the small intestine network. The number of links was highest in the small intestine (44) and lowest in the esophagus (11) (Table 3.1B).

In the facilitative networks, the large intestine network had the highest number of nodes (20), and the esophagus had the lowest number (5) (Table 3.1C). Connectance ranged from 0.33 to 0.54, and was highest in the stomach. Path length ranged from 1.20 to 1.46, and clustering coefficient ranged from 0.41 to 0.61 (Table 3.1C). The fraction of self facilitation was highest in the stomach (0.50) and lowest in the esophagus (0.20) (Table 3.1C).

Facilitative links dominated all networks for the four regions. In total 65% of the links in the networks were facilitative links. The consumer-resource networks had a mean connectance of 7%, while the facilitative networks had a mean connectance of 41%. Consumer-resource networks had higher path lengths, while the facilitative networks had higher clustering coefficients and fraction of self facilitation (Table 3.1).

When nodes were removed randomly (ran) from the composite GIT spatial networks, 36% of the non-basal nodes needed to be deleted to collapse the networks to 50% of their initial size on average, suggesting that the composite GIT spatial microbiome networks were generally robust to node removals of the networks (Figure 3.2). Random deletions triggered the highest number of secondary extinctions in the esophagus (26%), followed by the stomach (31%), small intestine (39%), and large intestine (40%) (Figure 3.2). Less than a 16% difference in robustness was observed based on whether removals occurred in ascending (least connected=lc) or descending order (most connected=mc) of connectivity for the esophagus (mc= 17%, lc= 33%),

stomach (mc= 31%, lc= 35%), small intestine (mc= 32%, lc= 42%), and large intestine (mc= 31%, lc= 45%).

Overall, the large intestine (ran= 40%, mc=31%, lc= 45%) was the most robust to node removals. Average robustness of the large intestine network was 39%, and differed by 14% between random, most and least connected removals (Figure 3.2). The large intestine and esophagus had a significant difference in their mean robustness (t=15.13, p= 0.004) and the small intestine and esophagus had a significant difference in their means (t= 7.29, p= 0.018). The large (mean robustness 39%) and small (mean robustness 38%) intestine were more robust compared to the esophagus (mean robustness 25%) (Figure 3.2).

3.4.2 Temporal networks (Oral cavity)

I constructed five oral microbiome networks for each ontogenic stage: newborn, child, adolescent, adult, and elderly (Figure 3.3). In total, the microbiome networks contained 58 bacterial genera, 1 fungi genus, and 13 source-specific nutrients. Of the 59 total genera, only seven were present at all ontogenic stages: *Actinomyces*, *Capnocytophaga*, *Fusobacterium*, *Lactobacillus*, *Peptostreptococcus*, *Prevotella*, and *Veillonella*. Topological properties for the trophic ontogenic networks are shown in Table 3.3. The number of genera present at each ontogenic stage was 13 for the newborns, 36 for children, 14 for adolescents, 41 for adults, and 40 for elderly (Table 3.4).

In the composite functional networks of the five ontogenic stages, node richness ranged from 18 to 59, and the adult had the highest number of links (957), while the newborn had the fewest (84) (Table, 3.3A). The elderly had the highest connectance (0.31), while the newborn and child had the lowest (0.23). The fraction of self-facilitation

ranged from 0.19 to 0.26, and the path length ranged from 1.52 to 1.75 in the oral ontogenic networks (Table 3.3A). The clustering coefficient for the composite ontogenic networks ranged from 0.43 to 0.47 (Table 3.3A). Visual representations of the composite networks are shown in Figure 3.3.

In the consumer-resource ontogenic networks, node richness ranged from 16 in the newborn to 34 in the adult (Table 3.3B). Connectance ranged from 0.06 to 0.09, and was highest in newborns. Path length ranged from 2.26 to 2.83 and was highest in children. The number of links was highest in adults (64), and lowest in the newborn, and adolescent (24) (Table 3.3B).

In the facilitative networks, adults had the highest number of nodes (47), and newborns had the lowest (13) (Table 3.3C). Connectance ranged from 0.31 to 0.44, and was highest in the elderly. Path length ranged from 1.33 to 1.44, and clustering coefficient ranged from 0.48 to 0.55 (Table 3.3C). The fraction of self facilitation was highest in the newborn (0.38) and lowest in the elderly (0.27) (Table 3.3C).

Topological properties of the oral ontogenetic consumer-resource and the facilitative networks, facilitative links dominated the networks for the five life stages. In total facilitative links made up 86% of the links in the networks. The consumer-resource networks had a mean connectance of 7%, while the facilitative networks had a mean connectance of 39%. Consumer-resource networks had higher path lengths, while the facilitative networks had higher clustering coefficients and fraction cannibal (Table 3.3A, B, C).

When nodes were removed randomly (ran) from the composite oral ontogenetic networks, 43% of the non-basal nodes needed to be deleted to collapse the networks to

50% of their initial size on average, suggesting that the composite oral ontogenetic microbiome networks were generally robust to node removals (Figure 3.4). Random deletions triggered the highest number of secondary extinctions in the newborn (37%), followed by the adolescent (41%), child (43%), adult (45%), and elderly (47%) (Figure 3.4). Less than a 11% difference in robustness was observed based on whether removals occurred in ascending (least connected=lc) or descending order (most connected=mc) of connectivity for the newborn (mc= 33%, lc= 44%), child (mc= 42%, lc= 40%), adolescent (mc= 33%, lc= 43%), adult (mc= 44%, lc= 44%), and elderly (mc= 43%, lc= 50%). Overall, the elderly network (ran=47%, mc=43%, lc= 50%) was the most robust to node removals. Average robustness of the elderly network was 47%, and differed by 7% between random, most and least connected (Figure 3.4). When comparing the mean robustness across oral life stages, I found that the elderly and adolescent networks had a significant difference in their means ($t=6.3$, $p= 0.02$) and the elderly and newborn networks had a significant difference in their means ($t= 6.1$, $p= 0.03$). The elderly network (mean robustness 47%) was significantly more robust than the adolescent (mean robustness 39%) and the newborn networks (mean robustness 38%) (Figure 3.4).

3.4.3 Ontogenic vs. regional patterns

I discovered several trends in the ontogenic and regional networks. First, the number of links and nodes increased further down the GIT. A linear increase was not observed in the ontogenic (temporal) networks, due to the oral adolescent life stage network having a smaller number of nodes and links compared to the child and adult (Figure 3.5 A, B). Second, connectance in the oral ontogenic networks increased with life stage. There was a trend towards an increase in connectance as the networks went further down the GIT

(Figure 3.5 C). Thirdly, the path length across the ontogenic and GIT regional networks, path length decreased with life stage in the oral ontogenic networks, while in the GIT networks path length remained relatively stable (Figure 3.5 E). No ontogenic or spatial trends were found for self-facilitation or the clustering coefficient (Figure 3.5 D, F).

3.5 Discussion

The objective of this research was to assemble and compare the structural properties and robustness of microbial networks at five oral ontogenic stages and for four regions of the gastrointestinal tract. The ultimate goal of the Human Microbiome Project is to apply a 'super-organism' theory to human health and disease. The approach to this goal should not only involve identification of species, but also the links between them and the emergent properties of the resulting networks. Topological properties such as connectance, clustering coefficients, and path lengths have been linked to the robustness of networks to disturbance and their resistance to invasion (Watts, 2002; Romanuk et al., 2009). Alteration in our oral microbiota has been linked with various diseases and hence the composition of the oral microbiome is an active area of research, particularly in dentistry. Specific oral microbial species are associated with dental caries, periodontitis, cardiovascular diseases, osteomyelitis in children, aspiration pneumonia, and preterm low birth weight (Aas et al., 2005). The oral microbiota can also be used as a diagnostic marker for cancer (Lazarevic et al., 2009). Changes in our GIT microbiota are of particular interest because they have been linked to many diseases including: Irritable Bowel Syndrome, Crohn's disease, and Ulcerative Colitis (Manson et al., 2008; Knight et al., 2008). Furthermore, scientists have also linked GIT microbiota to obesity (Turnbaugh et al., 2009). Alterations in the gut flora have emerged as a leading mechanism for the increased prevalence of certain GIT diseases (Isolauri et al., 2001, Sanderson et al., 1993,

Carol et al., 1998). Recently, it has been hypothesized that a disturbance in a whole microbial community may cause a disease, rather than an invasion of a single organism (Lazarevic et al., 2009) but relatively little is known about the healthy microbiomes of humans (Aas et al., 2005).

Most knowledge of the human microbiome has come from 16S rRNA studies due to the fact that 20% to 60% of human-associated microbiota cannot be cultured (Peterson et al., 2009). The oral cavity differs from most body sites by having a higher proportion of culturable research on oral microbiomes, and thus has a longer research history than other regional microbiomes. Two of the most common infections in humans (caries and periodontal diseases) are caused by oral microbiota, and these infections have been heavily investigated for many years (Wilson, 2008). Samples of oral microbiota are also relatively simple to obtain as sampling the oral microbiota does not cause discomfort or embarrassment (Wilson, 2008).

In contrast, the GIT microbiome is much more difficult to sample. Sampling some regions of the GIT requires anesthesia and can lead to discomfort (Wilson, 2008; Rajilić-Stojanović et al., 2007). Since the application of molecular techniques, scientists have discovered that the GIT microbiota is significantly more complex than previously thought, and that only fractions of the bacteria living in the GIT have been cultured (Suau et al., 1999).

A number of potentially informative trends were observed in how topology changes ontogenically in the oral cavity and spatially within the GIT. In particular, the number of nodes and links increases further down the GIT and connectance increases as life stage increases for the ontogenic networks. Below I discuss how ontogenic network

structure correlates to the developmental factors, and how biological factors affect the structure of the GIT regional pattern networks. Lastly, I discuss how the topology of microbiome networks affects how robust microbiome networks are to perturbations such as node removal and how robustness of microbiome networks to perturbations differs for regional pattern networks and for ontogenic networks.

Facilitative links dominated both the oral ontogenic and the spatial GIT networks (facilitative: oral 86%, GIT 64%; consumer-resource: oral 14%, GIT 35%). Average connectance was higher for the facilitative (oral 39%, GIT 41%) networks than the consumer-resource networks (oral and GIT 7%). Facilitative links are an important component of microbiome interaction networks and outnumber consumer-resource interactions by a factor of 6 and 2.

An interesting trend was an increase in the number of nodes and links from the esophagus to the large intestine. This increase in node richness and links could be due to many physiological factors such as peristalsis of the host's food slowing down further down the GIT, and the pH becoming more desirable for the microbiota colonizing in the small and large intestine. In the oral ontogenic networks there was also a trend in that as nodes and links increased, networks increased in developmental stages. This trend in node and link increases could be related to biological factors, such as hormones, teeth shedding, etc. Another interesting trend is that connectance increases with age suggesting an increase in complexity of the interactions with age. Moreover, in the GIT regional pattern networks the same trend was observed with the exception of the small intestine.

3.5.1 Ontogenic patterns in the oral microbiome

Humans undergo many changes throughout their ontogeny that can be associated with changes in the oral microbiome. Newborns go through many major events that lead to changes in the oral microbiome. Firstly, as early as eight hours after birth, microbes begin to inhabit the human body. The identity of the microbiota that initially colonize an infant depend on several different variables, including the mode of delivery (e.g., vaginal or cesarean; Kelly et al., 2007), the mother's microbial community, the immediate environmental conditions, and early feeding practices (e.g., breast-feeding vs. formula-feeding; Salminen & Isolauri, 2006). Secondly, tooth eruption occurs around six months of age (Percival, 2009). In the context of the oral microbiome this is an import stage because the hard, non-shedding surface of the teeth provides a new microhabitat necessary for certain species to survive (Wilson, 2008). Although data is not available on variability in microbial communities in the oral cavity from birth to one year, there is on the GIT. In the GIT, bacterial species become established approximately one week, and the microbiota remains relatively stable until an adult-like equilibrium state is reached by the end of the first year (Mshvildadze et al., 2008). This study showed an increase in tooth-associated genera (e.g., *Abiotrophia*, *Enterococcus*, *Haemophilus*, *Neisseria*; Wilson, 2008) after the newborn stage, the large difference in total genera between newborns and adults does not demonstrate a relatively stable adult-like equilibrium at the end of the first year of life. This could be due to the oral microbiome having more ontogenic stages than the GIT, such as gain and loss of teeth (Wilson, 2008).

During the child ontogenetic stage, the microbiotas remain relatively stable once teeth have emerged, although shedding teeth does occur (Wilson, 2008). I found a clear increase in the number of nodes ontogenically from 19 in the newborns (as teeth emerge),

to 47 in children, to 59 in adults for the composite networks. The largest increase occurred from newborns to children, with an addition of 28 genera. From children to adult only 12 genera were added. The similarity between children and adults was unexpected as hormonal changes associated with puberty were expected to lead to somewhat different microbiomes. It has previously been shown that children have a higher diversity of anaerobic bacteria compared to newborns since the occurrence of anaerobic bacteria has increased from 18-40% to over 90% after the age of five (Percival, 2009). This expected increase in the diversity of anaerobic bacteria during childhood was not observed in the composite networks. Both newborns and children have six of the 21 anaerobic species of the composite network, although the identity of the six genera differed between ontogenic stages.

Adolescents represent a second important ontogenic stage as hormones begin to surge during this developmental stage. Hormones augment the permeability of blood vessels of the gums and perdiodontum, altering the chemical microhabitat for microbiota (Wilson, 2008). Gusberti et al., (1990) showed that microbiota of dental plaque react directly in response of increased concentrations of hormones in oral fluids. The fact that the oral microbiotas are affected by the increase of hormones in the oral fluid was observed in adolescent networks. The number of nodes decreased rapidly from the children network (47) to the adolescent network (21). Furthermore, this was the first developmental stage in which the genus *Campylobacter* occurred, meaning that the hormones may have affected colonization of *Campylobacter* spp.

Once established, the adult oral microbiome remains relatively stable over time in healthy individuals (Percival, 2009). The adult microbiomes maintain the human immune

system by providing a barrier against the colonization of pathogenic species. Nonetheless, as adults age there are several physiological changes that occur which can collapse the homeostatic relationship between the host and its microbiome (Percival, 2009). Elderly humans are at risk for many changes in their oral microbiome. As humans age increases, tooth loss occurs and this can deplete a critical microhabitat for certain genera within the oral cavity (Wilson, 2008). To restore this microhabitat, dentures can be used, but there are several downfalls in that they decrease salivary flow which is already occurring in elderly people due to the natural aging process, and the effect of increased medications which generally cause dry mouth (Percival, 2009). Decreased salivary flow is linked to decreases in the supply of nutrients and the flow of innate antimicrobial substances which normally prevent colonization of potential pathogens. In support of this I found that *Candida* only occurred in the adult and elderly ontogenic stages. The reason adults could have *Candida* spp. is because some adults lose their teeth earlier and rely on dentures.

Diet is another factor that affects the maintenance of the oral host-microbial homeostatic relationship during adulthood. Elderly people tend to change their diet from when they were adults, and switch from hard, fiber-rich foods (fruits and vegetables) to softer and easier to swallow foods like sugary carbohydrates. The increased sugar intake directly impacts which microbiota can colonize the oral cavity, and the decrease of fruits and vegetables indirectly influences microbial populations because of lowered vitamins and minerals which normally protect against immunodeficiency and infection (Percival, 2009).

3.5.2 Spatial networks (GIT microbiome)

In the human body, different regions of the GIT differ in their functions therefore their microbiomes differ. While the human GIT is an open system, physiological conditions differ among regions (Manson et al., 2008). In the esophagus the lumen differs from other regions of the GIT, because it is a passage way and only contains material for short periods of time (Wilson, 2008). The mucus in the esophagus and the esophagus itself are the only areas where microbiota can colonize. Since the esophagus is close to the oral cavity the microbial community composition could be similar however, we found that the esophagus lacks many of the genera indigenous to the oral cavity, and has a much smaller diversity (6). The esophagus microbiome is thus distinctively different from the oral cavity microbiome (Wilson, 2008). In the regional GIT composite networks the esophagus had the smallest number of nodes (12), links (24), and connectance (0.17) (Table 3.1). The low diversity of genera in the esophagus was expected as nutrients provided by the host diet are moving fast through the esophagus from peristalsis, and there are only two areas were the microbiota can colonize (the mucus and esophageal wall).

In the stomach the prevalence and diversity of microbiota is influenced by several factors, including pH, redox potential, mucin secretion and nutrient availability (Manson et al., 2008). The stomach microbiome networks only contained 13 genera. This low diversity could be due to low pH, swift peristalsis, and gastric juice (Manson et al., 2008). Furthermore many microbiota from the human diet can colonize in the human stomach and persist over long periods of time (Wilson, 2008) therefore identifying the indigenous microbiota of the stomach is difficult to distinguish. Furthermore, in the stomach gastric juices and microbiota break down food into specific molecules, whereas in the

esophagus, since peristalsis is very fast, not nearly as much food is degraded. This could explain why the number of genera increases from the esophagus to the stomach.

The small intestine is composed of the duodenum, the jejunum, and the ileum. The GIT small intestine network included all studies of indigenous microbiota found in these three main regions. The duodenum and the jejunum make up the upper region of the small intestine, while the ileum makes up the lower region (Wilson, 2008). There are several physiological differences in these regions such as, 1) the pH which is lower in the upper small intestine, 2) the upper region having high bile concentrations, and 3) peristalsis being quicker in the upper regions compared to the lower regions (Manson et al., 2008). Since there are different physiological differences in the upper and lower small intestine regions it would make sense to separate them, but it was not possible to obtain community composition data on these separate areas. The small intestine composite network (28) had a similar number of nodes as the large intestine (29) composite network, although the composition differed. The small intestine and the large intestine only shared ten genera. Compositional differences (and the 10 genera in common) are likely due to physiological factors as the upper small intestine is physiologically different from the large intestine, yet the lower region of the small intestine (ileum), is similar to the upper region of the large intestine. For example, the lower small intestine and the large intestine both have slower peristalsis, the content of the bile acid is reduced and pH rises, and it is largely anaerobic (Wilson, 2008, Manson, 2008).

The robustness to node removal of the spatial GIT networks is of particular importance as many diseases are treated with antibiotics to reduce the abundance of pathogenic microbiota while they also reduce the abundance and diversity of the

indigenous microbiome. The large intestine was more robust to node removal compared to the other networks, and the small intestine was the second most robust. The reason these two networks were more robust was likely due to the fact that the genera diversity is much higher for these networks compared to the esophagus and stomach networks. Furthermore, since the large intestine harbours the majority of the micro-organisms in the GIT and achieves the highest cell densities recorded from any ecosystem (Whitman et al., 1998), I would predict that it would be the most robust area of the GIT.

3.5.3 Summary

The data presented in this paper suggests that due to biotic and abiotic factors the difference in microbial community composition can differ ontogenically in the oral cavity and regionally within the GIT. Understanding how human microbiomes change spatially within body regions and with ontogeny is important to understanding the functions of human-associated microbial communities. My results show that there are some consistent changes with age in topology, associated with increased complexity. Changes with region along the GIT are associated with increased node richness, links, and higher connectance consistent with the increased functional importance of lower verses higher regions in terms of digestion.

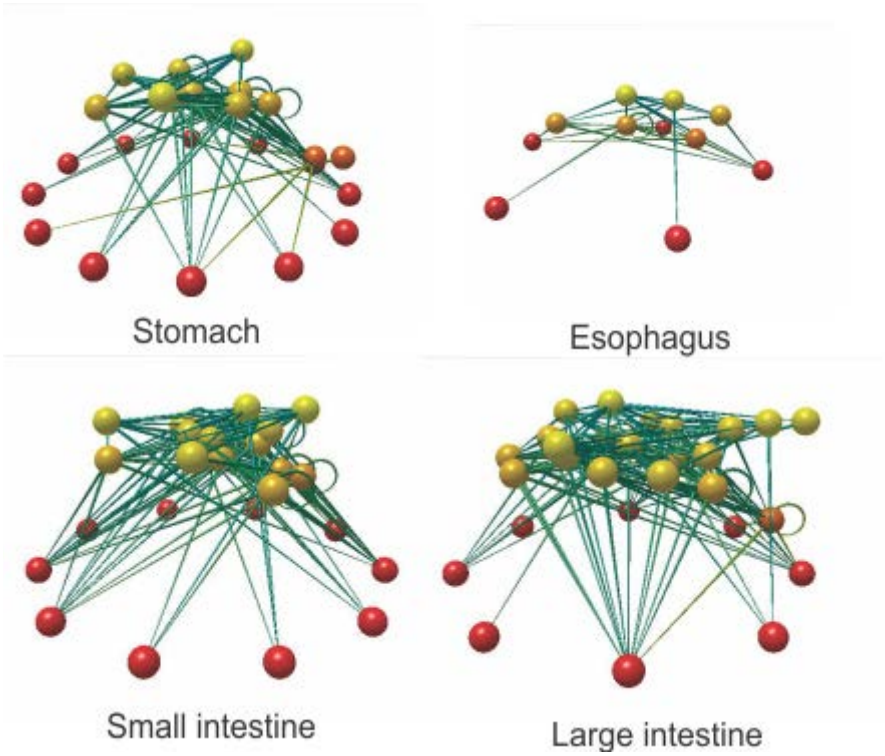

Figure 3.1. Visual representation of the composite 100% aggregated networks for four regions of the gastrointestinal tract (stomach, esophagus, small intestine, and large intestine). Circles represent nodes, with red nodes representing source-specific nutrients, and orange and yellow nodes representing genera. Links are represented by the green/blue lines between the nodes. Images from Network 3D (software written by R. J. Williams).

Table 3.1. Structural properties for four regional networks of the gastrointestinal tract (esophagus, stomach, small intestine, and large intestine) of the human body. A) Composite networks, B) Consumer-resource networks, and C) Facilitative networks. The structural properties are number of nodes, number of links, links/nodes, connectance, self facilitation, path length, and clustering coefficient.

A) Composite Networks (consumer-resource and facilitative interactions)				
	Esophagus	Stomach	Small intestine	Large intestine
Number of nodes	12	26	28	29
Number of links	24	121	169	178
Links/Node	2	4.65	6.04	6.14
Connectance	0.17	0.18	0.22	0.21
Self facilitation	0.08	0.23	0.29	0.24
Path length	1.83	1.74	1.77	1.71
Clustering coeficient	0.31	0.48	0.5	0.44
B) Consumer-resource networks				
	Esophagus	Stomach	Small intestine	Large intestine
Number of nodes	11	26	26	24
Number of links	11	43	44	39
Links/Node	1	1.65	1.69	1.63
Connectance	0.09	0.06	0.07	0.07
Self facilitation	0	0	0	0
Path length	2	2.22	2.7	2.62
Clustering coeficient	0	0	0	0
C) Facilitative networks				
	Esophagus	Stomach	Small intestine	Large intestine
Number of nodes	5	12	17	20
Number of links	9	78	118	131
Links/Node	1.8	6.5	6.94	6.55
Connectance	0.36	0.54	0.41	0.33
Self facilitation	0.2	0.5	0.47	0.35
Path length	1.2	1.21	1.38	1.46
Clustering coeficient	0.41	0.61	0.56	0.47

Table 3.2. List of genera in the four (esophagus, stomach, small intestine, large intestine) gastrointestinal networks.

Genera	Esophagus	Stomach	Small intestine	Large intestine
Actinomyces			X	
Bacteroides		X	X	
Bifidobacterium		X	X	X
Clostridium			X	X
Corynebacterium		X		
Egerthella				X
Enterobacter			X	X
Enterococcus		X	X	X
Escherichia			X	X
Eubacterium				X
Fusobacterium			X	X
Granulicatella	X			
Haemophilus		X	X	
Helicobacter pylori		X		
Klebsiella			X	X
Lactobacillus		X	X	X
Megasphaera	X			
Methanobrevibacter				X
Micrococcus		X	X	
Neisseria		X	X	
Peptostreptococcus				X
Porphyromonas				X
Prevotella	X	X		X
Propionibacterium				X
Proteus			X	X
Rothia	X			
Ruminococcus				X
Staphylococcus		X	X	
Streptococcus	X	X	X	X
Veillonella	X	X	X	

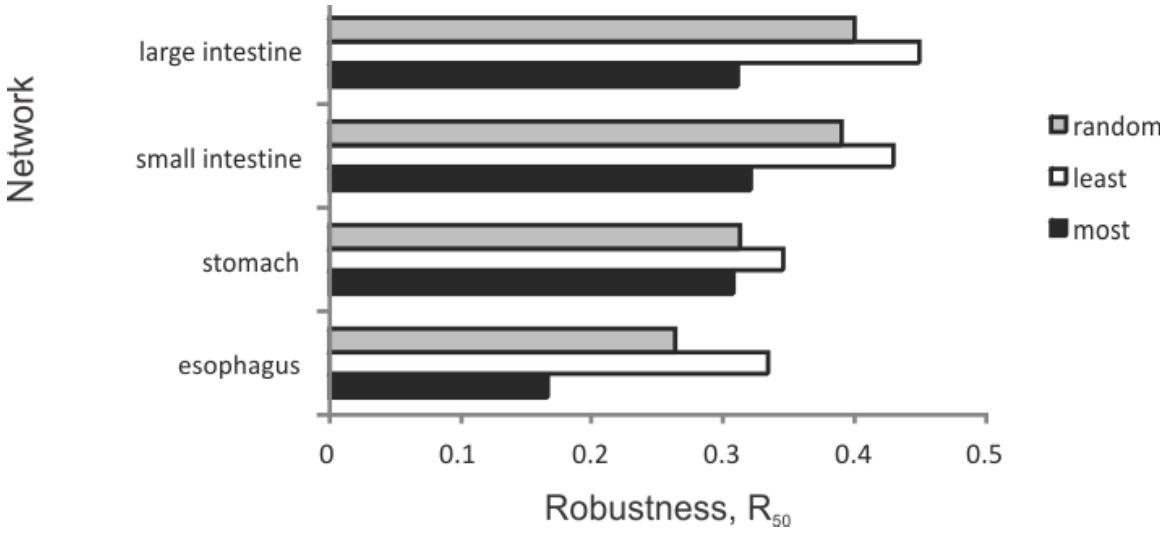

Figure 3.2. Robustness, R_{50} to node removal for most (black), least (white), and random (shaded) connected nodes for each of the composite gastrointestinal networks (large intestine, small intestine, stomach and esophagus).

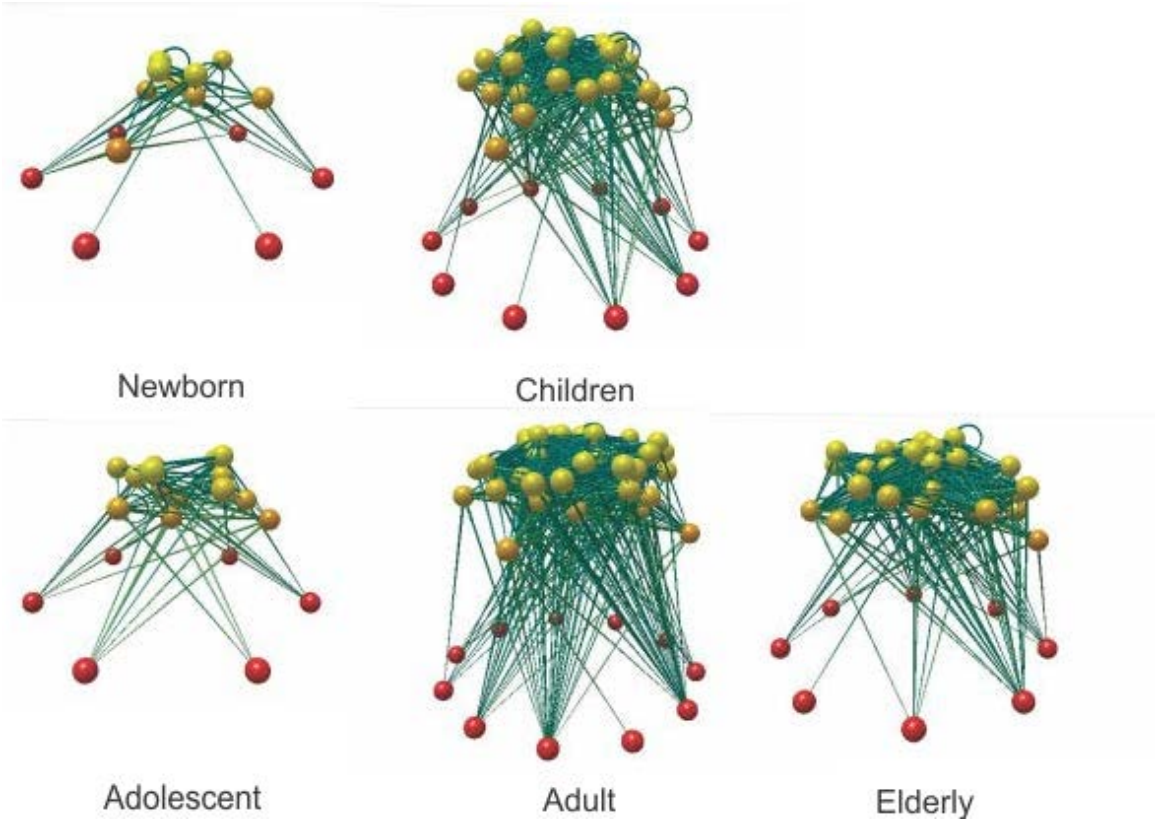

Figure 3.3. Visual representation of the composite 100% aggregated networks for five ontogenic life stages of the oral cavity (newborn, children, adolescent, adult, and elderly). Circles represent nodes, with red nodes representing source-specific nutrients, and orange and yellow nodes representing genera. Links are represented by the green/blue lines between the nodes. Images from Network 3D (software written by R. J. Williams).

Table 3.3. Structural properties for five ontogenic life stage networks of the oral cavity (newborn, child, adolescent, adult, elderly) of the human body. A) Composite networks, B) Consumer-resource networks, and C) Facilitative networks. The structural properties are number of nodes (NS), number of links (L), links/ nodes (L/NS), connectance (L/NS^2), self facilitation, path length, and clustering coefficient (CC).

A) Composite Networks (consumer-resource and facilitative interactions)					
	Newborn	Child	Adolescent	Adult	Elderly
Number of nodes	19	47	21	59	44
Number of links	84	504	105	957	593
Links/Node	4.42	10.72	5	16.22	13.48
Connectance	0.23	0.23	0.24	0.27	0.31
Self facilitation	0.26	0.26	0.19	0.25	0.2
Path length	1.71	1.74	1.67	1.63	1.52
Clustering coeficient	0.46	0.43	0.45	0.47	0.45
B) Consumer-resource networks					
	Newborn	Child	Adolescent	Adult	Elderly
Number of nodes	16	28	18	34	26
Number of links	24	45	24	64	48
Links/Node	1.50	1.61	1.33	1.88	1.85
Connectance	0.09	0.06	0.07	0.06	0.07
Self facilitation	0	0	0	0	0
Path length	2.37	2.83	2.26	2.76	2.49
Clustering coeficient	0	0	0	0	0
C) Facilitative networks					
	Newborn	Child	Adolescent	Adult	Elderly
Number of nodes	13	37	14	47	33
Number of links	59	429	80	848	479
Links/Node	4.53	11.59	5.71	18.04	14.52
Connectance	0.35	0.31	0.41	0.38	0.44
Self facilitation	0.38	0.32	0.29	0.32	0.27
Path length	1.49	1.52	1.33	1.4	1.28
Clustering coeficient	0.49	0.48	0.5	0.49	0.48

Table 3.4. List of genera in five ontogenic (newborn, child, adolescent, adult, and elderly) oral cavity networks; A) A-N, B) P-W.

A)

Genera	Newborn	Child	Adolescent	Adult	Elderly
Abiotrophia		X		X	
Acidaminococcus		X			X
Actinobacillus				X	
Actinomyces	X	X	X	X	X
Aeromonas					X
Aggregatibacter		X	X	X	
Atopobium				X	
Bacteroides		X	X		X
Bifidobacterium				X	
Campylobacter			X	X	X
Candida				X	X
Capnocytophaga	X	X	X	X	X
Cardiobacterium		X		X	X
Catonella		X		X	
Citrobacter					X
Corynebacterium		X		X	
Clostridium	X	X			X
Dialister		X		X	
Eikenella		X	X	X	
Enterobacter					X
Enterococcus		X		X	X
Escherichia		X			X
Eubacterium	X	X		X	X
Filifactor				X	
Fusobacterium	X	X	X	X	X
Gemella		X		X	X
Granulicatella		X		X	
Haemophilus		X		X	X
Kingella		X		X	X
Klebsiella		X			X
Lactobacillus	X	X	X	X	X
Lautropia				X	
Leptotrichia	X	X		X	X
Mitsuokella		X			
Megasphaera		X		X	
Neisseria		X		X	

B)

Genera	Newborn	Child	Adolescent	Adult	Elderly
Panteoa					X
Parvimonas					X
Peptostreptococcus	X	X	X	X	X
Porphyromonas	X	X		X	X
Prevotella	X	X	X	X	X
Propionibacterium				X	X
Proteus					X
Pseudomonas		X		X	X
Pseudoramibacter					X
Rothia				X	X
Selenomas	X	X	X	X	
Serratia				X	X
Shigella					X
Simonsiella				X	
Staphylococcus		X			X
Stomatococcus					X
Streptococcus	X	X	X	X	X
Synergistes					X
Tannerella		X	X	X	
Treponema		X		X	
Veillonella	X	X	X	X	X
Vibrio					X
Wolinella				X	X

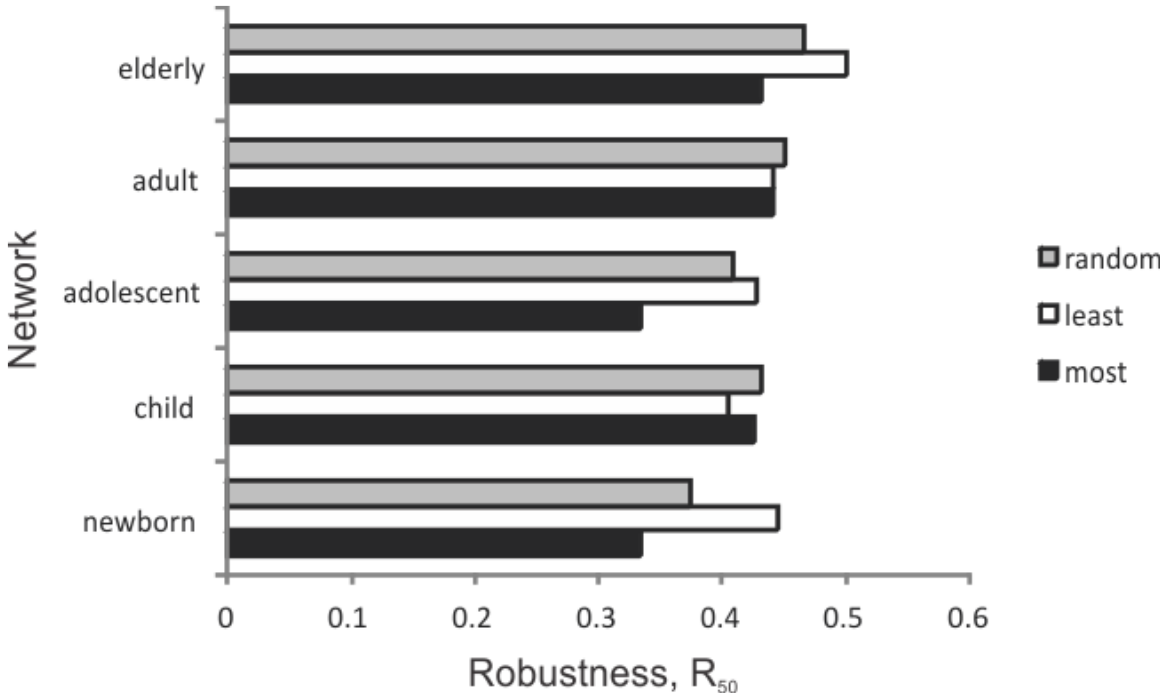

Figure 3.4. Robustness, R_{50} to node removal for most (black), least (white), and random (shaded) connected nodes for each of the composite oral cavity ontogenic networks (newborn, child, adolescent, adult, elderly).

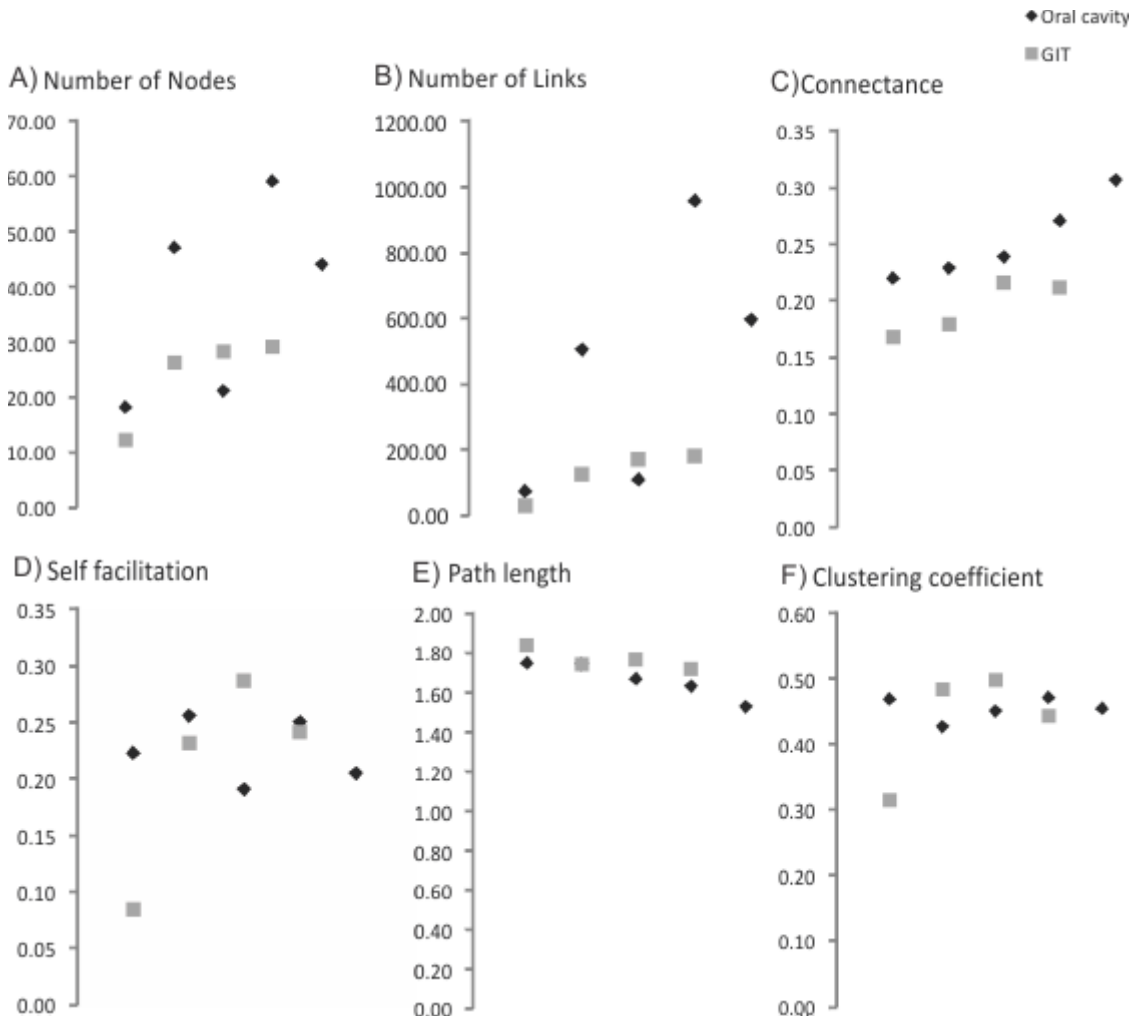

Figure 3.5. Differences in network topology between the ontogenic oral cavity composite networks (newborn, child, adolescent, adult and elderly), and the GIT regional pattern networks (esophagus, stomach, small intestine, and large intestine). Six topological properties are shown: A) Number of nodes, B) Number of links, C) Connectance, D) Self facilitation, E) Path length, and F) Clustering coefficient. The oral ontogenic networks are represented by a triangle (black), and the GIT regional pattern networks are represented by a square (shaded).

CHAPTER 4

A Meta-analysis of Probiotic Efficacy for Gastrointestinal Diseases

4.1 Abstract

Meta-analyses on the effects of probiotics on specific gastrointestinal diseases have generally shown positive effects on disease prevention and treatment; however, it is not clear whether different common gastrointestinal diseases are affected in similar manners by probiotics. I reviewed randomized controlled trials in humans that used a specified probiotic in the treatment or prevention of Pouchitis, Infectious diarrhea, Irritable Bowel Syndrome, *Helicobacter pylori*, *Clostridium difficile* Disease, Antibiotic-Associated Diarrhea, Traveler's Diarrhea, or Necrotizing Enterocolitis. Random effects models were used to evaluate efficacy as pooled relative risks across the eight diseases as well as across probiotic species, single vs. multiple species, patient ages, dosages, and length of treatment. Probiotics had a positive significant effect across all eight gastrointestinal diseases with a relative risk of 0.58 (95% (CI) 0.51-0.65). Six of the eight diseases: Pouchitis, Infectious diarrhea, Irritable Bowel Syndrome, *Helicobacter pylori*, *Clostridium difficile* Disease, and Antibiotic Associated Diarrhea, had positive significant effects. There were no significant differences in efficacy among disease groups. Of the 11 probiotic species and species mixtures, eight had positive significant effects whereas

Lactobacillus acidophilus, *Lactobacillus plantarum,* and *Bifidobacterium infantis* did not. Across all diseases and probiotic species, positive significant effects were observed for all age groups, single vs. multiple species, length of treatment, and doses except for the dose 1-9 x 10^{11}, 10^{12} CFU/day. Probiotics are generally beneficial in treatment and prevention of gastrointestinal diseases. Efficacy was not observed for Traveler's Diarrhea or Necrotizing Enterocolitis or for the probiotic species *L. acidophilus*, *L. plantarum*, and *B. infantis*.

4.2 Introduction

The efficacy of using probiotics in the prevention and treatment of gastrointestinal diseases has received considerable attention in recent years (McFarland, 2006; Sazawal et al., 2006; Tong et al., 2007; McFarland & Dublin, 2008; Hoveyda et al., 2009). In western civilization, there has been an increase in gut-related health problems, such as autoimmune and inflammatory diseases (Isolauri, 2001). Changes in the gut flora have emerged as a leading mechanism for the increased prevalence of certain gastrointestinal diseases (Isolauri, 2001; Sanderson & Walker, 1993; Carol et al., 1998). Due to improved hygiene and nutrition, the western human diet contains several thousand times less bacteria than pre-industrialized diets (Isolauri, 2001; Bengmark, 1998). This is partially due to the use of processed and sterile foods that contain artificial sweeteners and preservatives, rather than fresh fruits and vegetables (Soutar, et al., 1997), or foods containing important microbiota for anti-inflammatory processes (Sütas et al., 1996; Pessi et al., 1999). The functional composition of the gut flora also differs in species composition, the dominance of certain genera, and the diversity of microbiota. Therefore, probiotics are thought to be essential in boosting healthy microbiota communities in the GI tract.

Probiotics, which are products or preparations containing sufficient amounts of viable microorganisms to alter a host's microbiota communities (Johnston et al., 2006), are thought to exert beneficial effects by providing protective barriers, enhancing immune responses, and clearing pathogens in the gastrointestinal tract (McFarland, 2000; Qumar, et al., 2001; Elmer, 2001). Meta-analyses or clinical trials on the efficacy of probiotics have been conducted for a number of common gastrointestinal diseases including Irritable Bowel Syndrome (IBS) (Hoveyda et al., 2009), *Helicobacter pylori* infection (HPP) (Tong et al., 2007), Necrotizing Enterocolitis (NEC) (Deshpande et al., 2007), Pouchitis (Pouch) (Elahi et al., 2007), Antibiotic-Associated diarrhea (AAD) (Hawrelak, et al., 2005), *Clostridium difficile* Disease (CDD) (Dendukuri et al., 2005), Infectious diarrhea (ID) (Sazawal et al., 2006), and Traveller's diarrhea (TD) (Sazawal et al., 2006). These studies have shown that probiotics have significant effects on the prevention (e.g., Sazawal et al., 2006) and treatment (e.g., Dendukuri et al., 2005) of gastrointestinal disease. While numerous meta-analyses have been performed on the use of probiotics in the prevention and treatment of specific diseases (e.g., Tong et al., 2007; Hoveyda, et al., 2009; Sanderson & Walker, 1993), to my knowledge, a meta-analysis comparing the efficacy of probiotics across various diseases has not been conducted. Comparing the relative efficacy of different probiotic treatment across diseases is one potential way to determine common mechanisms of action of probiotics and isolate specific consequences of gastrointestinal disease that can be effectively treated with probiotics.

Here I report on a meta-analysis explicitly designed to determine whether probiotics are more or less effective in the prevention and treatment of eight different gastrointestinal diseases across 11 species or species mixtures of probiotics: *VSL#3*,

Lactobacillus rhamnosus GG (LGG), *Sacharomyces boulardii*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Clostridium butyricum*, *Enterococcus faecum*, *Lactobacillus plantarium*, *Bifidobacterium lactis*, and *Lactobacillus acidophilus* combined with *Bifidobacterium infantis*. We further assessed whether factors such as patient age, dose, length of treatment, and single vs. multiple probiotic species affect efficacy.

Probiotics have been used to prevent and treat a wide range of GIT diseases. The GIT diseases considered here can be grouped into those associated with diarrhea: AAD, CDD, ID, TD; verses those associated with destruction or inflammation of tissues in the stomach, large intestine, ileal reservoir, or bowel: NEC, Pouch, and HPP; verses abdominal pain, flatulence, and irregular bowel movements: IBS. Diarrhea is responsible for 4% of deaths worldwide (Sazawal et al., 2006) and is often associated with subsequent infections (McFarland, 2006). Diarrhea is caused by pathogenic bacteria or viruses (Bezkorovainy, 2001), and in the case of TD, amoebas and many other protozoan's (Sazawal et al., 2006) in either the small or large intestine. Impaired intestinal absorption is caused by diarrhea and can lead to malnutrition (Sazawal et al., 2006). The etiology of re-occurring and chronic inflammation in the gastrointestinal tract is not definitive (Santosa et al., 2006). Nevertheless, evidence suggests that an imbalance of intestinal bacteria may commence and perpetuate the inflammation that characterizes the gastrointestinal diseases related to chronic and re-occurring inflammation (Saarela et al., 2002; Sartor, 1995; Rath, 2003). Furthermore, pathogenic bacteria can invade tight junctions between epithelial cells and disturb the barrier function of the gut, resulting in translocation of pathogenic bacteria that leads to an inflammatory immune response

(Sakaguchi et al., 2002). IBS affects 11% to 14% of the North American population (Nobaek et al., 2000; Thompson, 1986; Mimura, 2004) and is the most common diagnosis across gastroenterological disorders. IBS etiology is still unclear but may include behavioural factors, genetic susceptibility, and stress (Verdu & Collins, 2004). Although the cause has still not been fully explained, the increase in flatulence is suspected to be a result of disturbed intestinal microbial population (Saarela et al., 2002; Lin, 2004).

Previous studies have shown probiotic efficacy in treating diarrhea-related, inflammation-related, and IBS symptoms (Hilton et al., 1997; Mimura et al., 2004; Halpern et al., 1996, consecutively). The primary active mechanisms of probiotics are modification of the gut microbiota (Isolauri, 2001), stabilization of the indigenous microbiota (Isolauri et al., 1994), reductions in the duration of retrovirus shedding (Saavedra et al., 1994), and a reduction in gut permeability which is caused by retrovirus infection (Isolauri et al., 1993). In diarrhea-related diseases, probiotics may induce a general immune response, in addition to increasing IgA antibodies against rotaviruses (Fric, 2002; Marteau, 2001a). In inflammatory diseases, probiotics are thought to decrease disease activity and promote remission (Hart et al., 2003). Reductions in inflammation are thought to occur by decreasing pathogenic bacterial growth through the enhancement of barrier functions, which prevent the invasion of tight junctions by lowering gut pH and by stimulating non-specific and specific immune responses (Hart et al., 2003). IBS has been correlated with a lower amount of *Lactobacilli* spp. and *Bifidobacterium* spp. colonies and an increase in anaerobic *Clostridium* spp. which has taken the place of anaerobic *Bifidobacterium* spp. and *Bacteriodes spp.* (Lin, 2004; Sen et

al., 2002). Therefore, there are links between humans consuming lactose and sucrose with an onset of IBS (Lin, 2004), which is thought to be caused by providing the pathogenic microbial population with a nutritional source (Lin, 2004). As a result, probiotics such as *L. plantarum* (Niedzielin et al., 2001) and *Enterococcus faecum* (Gade & Thorn, 1989) have been used to treat IBS because they compete for the same food source. In IBS probiotics are thought to modify and stabilize the indigenous microbiota (Isolauri et al., 2004). Not all these mechanisms of action will apply to all the GIT diseases considered here, thus by comparing probiotic efficacy across diseases it may be possible to assess the specific functional responses by which probiotics are operating.

The efficacy of probiotic treatment is also highly dependent on the genus, species, and even the strain of bacteria used (Van Neil, 2005). For example, not all lactic acid bacteria have probiotic effects (Vanderhoof, 2000). In the case of Traveler's diarrhea, *acidophilus* strain LB was found to be effective (Boulloche et al., 1994), whereas other strains of *Lactobacillus acidophilus* spp. were not (Katelaris et al., 1995). Also, different probiotics may confer different degrees of benefit depending on the condition. For example, McFarland (2006) found that 3 types of probiotics (*Saccharomyces boulardii, Lactobacillus rhamnosus GG* and probiotic mixtures) significantly reduced the development of AAD, while in the treatment of CDD only *Saccharomyces boulardii* was effective (Mcfarland, 2006).

Ontogenic changes in the composition of the gut microbiota might also affect efficacy of probiotics (Sanderson et al., 1993; Perin, 1997; Salminen et al., 1998; Simon & Gorbach, 1986). For example, in the colon of breast-fed infants prior to weaning, the fecal microbiota is dominated by *Bifidobacterium* spp., while in adults *Bifidobacterium*

spp. are only minor constituents (Wilson, 2008). Likewise, the colon of elderly individuals has decreased proportions of *Veillonella* spp. and *Bifidobacteria* spp., but increased proportions of *Clostridia* spp., *Lactobacilli* spp., and Enterobacteria spp. (Wilson, 2008). Ontogenic differences such as these suggest that efficacy of probiotic-use and potentially overall outcome may differ based on age. A number of studies have shown that probiotic efficacy can differ in infants, children, and adults (Bezkorovainy, 2001; Tannock, 1997; Benno & Mitsuoka, 1992; Ling et al., 1994). While the administration of probiotics to both infants and adults results in changes of the microbiota present in the feces and the metabolic activity of the microbiota (Bezkorovainy, 2001), a number of studies have shown greater differences between adults and children in the composition of their fecal microbiota communities than exist within a cohort (Tannock, 1997; Benno & Mitsuoka, 1992; Ling, 1994), suggesting strong ontogenic differences.

For acute diarrhea, higher doses of probiotics have been shown to be more effective than lower doses (Van Neil, 2002). Whether dose is an important determinant of efficacy, however, has not been considered for most GIT diseases. The question of appropriate dose is not generally considered when evaluating probiotic efficacy, generally due to the very high standard dosages, which range from 12 million to 3 billion (1.2×10^6 cfu or 3×10^{11} cfu). However, studies that have considered dose level have reported mixed effects (Floch, 2003). Van Neil (2002) has shown that dose is important in efficacy while Bezkorovainy (2001) has shown that dose does not affect treatment outcome, as long as it is over the sufficient amount (several billion).

Given the complex ecology of the gastrointestinal tract, probiotics containing multiple species have been predicted to be more effective than single species probiotics

(Saavedra, 2001). Multiple species probiotics might be more effective than single species probiotics if additive effects occur due to synergisms or facilitation amoung species (Timmerman et al., 2004). For example different strains of genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Bifidobacterium* can facilitate growth and metabolic activity in each other (Timmerman et al., 2004; Sodini et al., 2000). Few studies have directly tested whether efficacy differs between single and multiple strain probiotics (but see: Sodini et al., 2000; Pivetaeu et al., 2000; Looijesteijn et al., 2001). Floch (2001) has shown that in the treatment of Inflammatory Bowel Disease single and multiple species probiotics do not differ in efficacy.

The objectives of this meta-analysis were to: (i) determine the overall effect of probiotics on diseases of the gastrointestinal tract that have previously been shown to be affected by probiotics, (ii) determine whether certain diseases respond to probiotics more than others (iii) determine whether different species and species combinations differed in their overall effect size, and to (iv) determine whether efficacy differs based on dosage, length of treatment, and age group.

4.3 Method

4.3.1 Search strategy and study selection

I conducted a literature search for randomized controlled efficacy trials in humans for probiotics used in the prevention and treatment of gastrointestinal disease. I searched Pubmed, Medline, Google Scholar, Embase, Biological Abstracts and Science Direct from1970 to Jan 2011, using the following search terms: probiotics, probiotic, meta-analysis, *Helicobacter pylori* Diarrhea, Pouchitis, Antibiotic Associated Diarrhea, Irritable Bowel Syndrome, Travellers Diarrhea, *Clostridium difficle* Disease, Necrotising

Enterocolitis, Infectious Diarrhea, yogurt, *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus,* and gastrointestinal diseases. Searches were not restricted by language and secondary searches were conducted by reference lists, authors and reviews. Excluded trials included case reports or case series, trials of unspecified probiotics, trials on prebiotics, trials with inconsistent outcome measures, trials with no specific disease being studied, and trials on animals other than humans. Eligibility criteria included randomized controlled trials published in peer-reviewed journals, humans with gastrointestinal disease (AAD, CDD, HPP, IBS, ID, NE, Pouch, TD), and studies that compared probiotic therapy with placebo or no therapy. After excluding trials that did not fit the criteria, a total of 84 suitable trials were identified for analysis spanning 10,351 patients, 11 probiotic species or mixtures, and eight diseases. Of the 84 suitable trials that are analyzed in this meta-analysis, 79 have been cited in meta-analyses on their specific disease (McFarland, 2006; Sazawal et al., 2006; Tong et al., 2007; McFarland & Dublin, 2008; Hoveyda et al., 2009; Johnston et al., 2006; Deshpande et al., 2007; Elahi et al., 2007; Dendukuri et al., 2005) (Figure 4.1, Figure 4.2).

4.3.2 Outcome Assessment

The primary outcome assessed was the efficacy of treatment and prevention of GI disease with probiotics compared to the control. In this paper, I use prevention and treatment interchangeably when discussing the effects of probiotics across all diseases as for some diseases (i.e., CDD; Dendukuri et al., 2005) probiotics are effective in both prevention and treatment. For other diseases, probiotics only have efficacy in either prevention or treatment and this is noted in this discussion of specific diseases. For

example, probiotics are used in the prevention of diarrhea (Wenus et al., 2008) and in the treatment of IBS (McFarland & Dublin, 2008).

4.3.3 Data extraction and risk of bias

From each paper we extracted information related to disease, probiotic species, the dose amount, treatment length, age group, number of trials, number of patients receiving the probiotic or the control, and the number of patients that improved following probiotic/control. A few studies had multiple probiotic treatments with a common control group and were analyzed separately.

I searched the literature and assessed inclusion criteria and quality of trials. Each included study was assessed using a 5-point Jaded scale (Jaded et al., 1996) based on randomization, concealment of allocation, blinding of investigators, including outcome assessors, and completeness of follow-up. Inconsistencies were resolved by discussion with the authors. Weights for the meta-analysis are based on sample sizes.

4.3.4 Data synthesis and statistical analysis

A random effects meta-analysis was conducted with inverse variance weighting using the software MIX version 2.0 Pro (Bax, 2010). For each paper the relative risk ratio (RR), which is the ratio of the probability of the event occurring in the probiotic treatment versus the control group (Sistrom & Garvan), was calculated along with 95% confidence intervals, and summary statistics. Overall RR, heterogeneity (I^2), z-values, and p-values were computed across all studies and for each comparison. If significant heterogeneity (I^2) occurred ($p < 0.05$) studies were analyzed using a random effects model with a pooled relative risk. If the studies were not significant ($p > 0.05$) they were analyzed using a fixed effect model with a pooled relative risk. Effect sizes (RR values)

that were <1 favoured the probiotic while effect sizes that were >1 favoured the placebo. If the 95% confidence intervals of effect sizes do not overlap, the RR is considered significantly different. Publication bias was assessed by funnel plot asymmetry (Egger et al., 1997). Risk ratios were plotted against the standard error of the risk ratio of each study to identify asymmetry in the distribution of trials. Potential publication bias is suggested when there is a gap in the funnel plot. Begg's regression test was also used to assess potential publication bias (Begg & Mazumdar, 1994). The Failsafe N-Method defined as, "the number of new, unpublished, or un-retrieved non-significant or "null result" studies that would be required to exist to lower the significance of a meta-analysis to some specified level" (Egger & Davey, 1995) was also used for bias analysis.

Six different factors were included in the meta-analysis: the disease treated with probiotics (AAD, CDD, IBS, ID, TD, NEC, Pouch, and HPP), the type of probiotic used (VSL#3, LGG, S. *boulardii*, *B. infantis, L. acidophilus*, *L. casei*, *C. butyricum*, *E. faecum*, *L. plantarium*, *B. lactis* and L. *acidophilus* combined with *B. infantis*, the dose of the probiotic (1-9 x 10^{11}, 10^{12} CFU/day; 1-5.5 x 10^{6}, 10^{7}, 10^{8} CFU/day; 1-9 x 10^{9} CFU/day; 1-5 x 10^{10} CFU/day), the amount of time the probiotic was administered for (9-240 weeks, 5-8, 3-4, 1-2), the age group of the subjects receiving probiotics (infants (0-3yr), children (3≤18yr), adults(>18yr)) and single versus multiple species of probiotics.

4.4 Results

4.4.1 Overview of included studies

The literature search yielded 2,420 citations, of which 220 were screened and 80 were assessed for eligibility. Of these, 6 were excluded for various reasons (Figure 4.1),

leaving 74 studies that met the inclusion criteria. Therefore, 84 peer-reviewed trials were included in the meta-analysis. All trials included in this meta-analysis had a Jaded quality score of 3 or more, except for 4 of them which had a score of 2 due to unavailable information. The median number of patients per trial was 88.5 ranging from 15-756. In total, 10,351 subjects were included in the studies. Of the 84 trials, 31 (37%) found a significant reduction of GI diseases in the probiotic-treated patients compared with the control patients. 53 trials did not reject the null hypothesis of no difference in the incidence of GI disease for probiotic verses controls. The pooled estimate of efficacy of probiotics in prevention or treatment of disease yielded a relative risk of 0.58 (95% CI 0.51-0.65; $p<0.001$) and a heterogeneity (I^2) of 61.24% (95% CI 51-69; X^2 $p<0.001$) showing that across all diseases and probiotic species, probiotics were effective in the treatment and prevention of GI diseases (Figure 4.2).

4.4.2 Effect by disease

Within the eight diseases considered, Pouchitis (n= 4; RR= 0.17; 95% CI 0.10-0.30), AAD (n= 27; RR= 0.43; 95% CI 0.32-0.56), ID (n= 3; RR= 0.35; 95% CI 0.13-0.97), IBS (n=16; RR= 0.77; 95% CI 0.65-0.92), HPP (n= 13; RR= 0.70; 95% CI 0.54-0.91), and CDD (n= 6; RR= 0.60; 95% CI 0.41- 0.86) yielded significant effect sizes (Figure 3A). Significant effect sizes were not observed for probiotics for the diseases TD (n= 6; RR= 0.92; 95% CI 0.79-1.05) and NEC (n=9; RR= 0.54; 95% CI 0.23-1.24) (Figure 4.3A). Efficacy for Pouchitis was significantly greater than for TD, IBS, HPP, CDD, and AAD. When comparing the diseases that cause diarrhea to those that cause tissue damage/inflammation and to IBS, no significant effect was found (Figure 4.3A).

4.4.3 Effect by probiotic species

Across all diseases, eight probiotics had significant effect sizes including: VSL #3 which contains viable lyophilized bacteria of four species of *Lactobacillus* (*L. casei, L. plantarum, L. acidophilus*, and *L. delbrueckii* subsp. *bulgaricus*), three species of *Bifidobacterium* (*B. longum, B.breve*, and *B. infantis*), and one species of *Streptococcus salivarius* subsp. (n=3; RR= 0.17; 95% CI 0.09-0.33), *E. faecium* (n= 2; RR= 0.29; 95% CI 0.13-0.64), *C. butyricum* (n= 2; RR= 0.18; 95% CI 0.09-0.37), *L. acidophilus* combined with *B. infantis* (n= 3; RR= 0.37; 95% CI 0.17-0.83), *B. lactis* (n= 3; RR= 0.59; 95% CI 0.38-0.92), *LGG* (n= 14; RR= 0.54; 95% CI 0.39-0.75), *L. casei* (n= 3; RR= 0.42; 95% CI 0.24-0.76) and *S. boulardii* (n= 11; RR= 0.46; 95% CI 0.34-0.60) (Figure 3B). The other three probiotic species (*L. acidophilus, L. plantarum,* and *B. infantis*) did not have significant efficacy (Figure 4.3B). *S. boulardii* showed significantly higher efficacy than *L. plantarum* and *B. infantis*. *C. butyricum* had significantly higher efficacy from the species *L. plantarum*, *L. acidophilus*, *LGG*, *L. plantarum* and *B. infantis.* VSL #3 had significantly higher efficacy than the species *S. boulardii*, *B. infantis*, *L. plantarum*, *LGG*, *B. lactis*, and *L. acidophilus* (Figure 4.3B). As *L. acidophilus* is one of the most common probiotics we further considered whether differences in efficacy were observed based on particular strains. We found that when analyzed alone, *L. acidophilis* LB did show significant efficacy (RR= 0.40 95% CI 0.20-0.82) and *L. acidophilus* with no strain specified did not have a significant effect (RR= 1.17 95% CI 0.85-1.62).

4.4.4 Effects of age

Across all diseases and probiotic species, significant efficacy was observed for all of the age groups studied (infants (n=9; RR= 0.41; 95% CI 0.27-0.62, children (n= 14; RR= 0.36; 95% CI 0.24-0.55), and adults (n= 53; RR= 0.64; 95% CI 0.55-0.74) (Figure 4.4A). None of the age groups were significantly different from each other (Figure 4.4A).

4.4.5 Effects of dose

Across all diseases and probiotics species, significant efficacy was observed for three doses: 1-5 x 10^{10} CFU/day (n=20; RR= 0.51; 95% CI 0.39-0.65) , 1-5.5 x 10^{6}, 10^{7}, 10^{8} CFU/day (n=12; RR= 0.60; 95% CI 0.42-0.85), and 1-9 x 10^{9} CFU/day (n=25; RR= 0.61; 95% CI 0.49-0.75) (Figure 4B). One dose (1-9 x 10^{11}, 10^{12} CFU/day, n=7; RR= 0.73; 95% CI 0.46-1.15) did not have significant efficacy (Figure 4.4B). None of the dose groups were significantly different from each other (Figure 4.4B)

4.4.6 Effect of treatment length probiotic was administered

Subgroup analysis for length of treatment showed significant efficacy for all of the four groups; 1-2 weeks (n=30; RR= 0.53; 95% CI= 0.42-0.68), 3-4 weeks (n=21; RR= 0.78; 95% CI 0.68-0.89), 5-8 weeks (n=18; RR= 0.64; 95% CI= 0.51-0.82), and 9-240 weeks (n=7; RR= 0.27; 95% CI 0.14-0.54). The longest treatment period (9-240 weeks) had significantly higher efficacy than the 3-4 week treatment length group (Figure 4.4C).

4.4.7 Effects of single vs. multiple species

No significant difference between single and multiple species was observed (single species n= 51; RR= 0.73; 95% CI 0.68-0.79, multiple species n= 33; RR= 0.63; 95% CI 0.53-0.76) (Figure 4.4D).

4.4.8 Publication bias

The funnel plot had an asymmetrical distribution (Figure 4.5). The Egger regression test ($p>0.0001$) and the Begg rank correlation test ($p>0.0001$) showed significant evidence of publication bias. However, using the fail-safe N method, I estimated that a total of 3,657 missing studies that would bring the p-value greater than alpha, were required to overturn significance of the current results. The trim and fill method was used to correct for publication bias and yielded an overall effect size of 0.73 (95% CI 0.63-0.83), compared to the uncorrected overall effect size of 0.58 (95% CI 0.51-0.65).

4.5 Discussion

Across all 11 probiotic species and the eight different gastrointestinal diseases we found a significant effect of probiotics on prevention and treatment of gastrointestinal disease with a RR = 0.58 (95% CI 0.51-0.65). TD and NEC did not respond to probiotics and the species *L. acidophilus*, *L. plantarum,* and *B. infantis* showed no efficacy. Previous meta-analyses that focused on efficacy of probiotics in the prevention or treatment of specific diseases have reported similar results. For example Johnston et al. (2006) reported a significant effect size (RR= 0.43 95% CI 0.25-0.75) for AAD disease, McFarland & Dublin (2008) reported a significant effect size (RR= 0.78 95% CI 0.62-0.94) for IBS disease, and Elahi et al. (2007) reported a significant effect size (OR= 0.04 95% CI 0.01-0.14, $p< 0.0001$) for Pouchitis.

Pouchitis (RR= 0.17 95 % CI 0.10-0.30) had the greatest effect size of all the diseases analyzed and efficacy of probiotic treatment for Pouchitis was significantly different than TD, IBS, HPP, CDD, and AAD. Pouchitis occurs in 50% of patients with ulcerative colitis after undergoing ileal pouch anal anastomosis (IPAA) (Blumberg & Beck, 2002). Pouchitis is caused by inflammation of the ileal pouch that is caused

directly (toxins or invasions in the anal mucosa) or indirectly (changes in fatty acids and bile salts) (Kmiot et al., 1993). A previous meta-analysis on the prevention of Pouchitis in patients that have undergone IPAA surgery showed that probiotics have a positive effect on the prevention of Pouchitis (Elahi et al., 2007). Recent evidence proposes that bacteria play a primary pathogenic role in causing inflammation in patients with Pouchitis (Sandborn, 1994; Keighley, 1996; Nicholls & Banerjee, 1998). Ruseler-van Embden (1994) found that individuals with Pouchitis have fewer *Lactobacilli* and *Bifidobacterium.* Efficacy of probiotic treatment in Pouchitis was significantly higher than efficacy for TD, IBS, HPP, CDD, and AAD (Figure 4.3A). The high efficacy of probiotics we observed in the treatment of Pouchitis may be due to a number of factors related to trial design. For example, treatment of Pouchitis was limited to VSL #3 and LGG and the patients in Pouchitis trials were all adults.

AAD, ID, IBS, HPP, and CDD also had effect sizes that were significant with confidence intervals below one. AAD is present when an individual has three or more abnormally loose bowel movements over a twenty-four hour period following antibiotic use (D'Souza et al., 2002). HPP colonization is a common health problem, especially in developing countries (Cats et al., 2003; Tong et al., 2007), that causes chronic low-level inflammation in the stomach lining and duodenum leading to the development of gastric and duodenal ulcers, as well as stomach cancer (Olson & Maier, 2002). When treating HPP, patients are prescribed antibiotics which results in some individuals developing AAD. CDD, which is also associated with antibiotic use, occurs mostly in older adults, and usually only occurs in hospitalized patients (McFarland, 1998). Probiotics are thought to restore equilibrium in the gastrointestinal tract and protect against *C. difficile*

colonization. AAD, HPP colonization, and CDD are associated with antibiotic treatment (Tong et al., 2007; McFarland & Dublin, 2008). Probiotics are thought to be a useful treatment in these diseases as they occur in part from alterations of the intestinal microbiota (McFarland & Dublin, 2008). ID is a type of acute diarrhea that impairs intestinal absorption of nutrients and can lead to malnutrition (Sazawal et al., 2006). IBS leads to abdominal pain, bloating, diarrhea, constipation, and flatulence due to motor and sensory dysfunction of the gastrointestinal tract (McFarland & Dublin, 2008).

Our observation of significant efficacy for AAD, ID, IBS, HPP, and CDD support other recent meta-analyses on specific GIT diseases. McFarland (2006) showed that AAD is preventable by probiotics; McFarland & Dublin (2008) demonstrated that probiotics have a significant effect on the improvement of IBS, and Tong et al. (2007) suggested that probiotics could be effective in increasing eradication rates during anti-*H. pylori* therapy. Tong et al. (2007) showed that *H. pylori* eradication rates were 83.6% for patients with probiotics and 74.8% for patients without, and thus suggested that larger trials were needed to confirm a significant effect. Probiotics have also been shown to have significant efficacy for CDD (McFarland, 2006). Our result for ID represents the first meta-analysis of probiotic use in ID treatment as only single trials (e.g., Weizman et al., 2005) have previously been conducted.

Two of the GIT diseases considered here, TD and NEC, showed no significant response to probiotics. TD is a type of acute diarrhea that impairs intestinal absorption of nutrients and can lead to malnutrition (Sazawal et al., 2006). Traveller's diarrhea is typically caused by amoebae (Goodgame, 2003) and is treated with antibiotics that also lead to diarrhea. Our results support previous studies by Pozo-Olano et al. (1978) and

Katelaris et al. (1995) who both found probiotics to have no effect in people suffering with Traveller's diarrhea. In contrast, Hilton et al. (1997) showed that LGG can reduce the risk of developing diarrhea by 3.9% per day.

NEC was the only other gastrointestinal disease that did not show a significant effect for treatment with probiotics. NEC is a gastrointestinal disease that is a major issue in preterm (<28 weeks gestation) neonates and involves infection and inflammation that causes destruction of the bowel or part of the bowel (2007). NEC only affects 1% to 5% of neonatal intensive care unit (NICU) admissions, but it is common worldwide and is the most serious disorder among hospitalized preterm infants. A possible explanation is that NEC occurs mostly in infants and infants do not have their immune system or their microbial communities fully established (Wilson, 2008). Our results, based on ten studies, differ from those of Deshpande et al. (2007) who showed that probiotics significantly reduce the risk of NEC (RR= 0.36 95% CI 0.20-0.65) in preterm neonates, however they suggested that probiotics needed to be assessed in larger trails to determine their short and long term effects in the treatment of NEC. Our meta-analysis improves on their meta-analysis by adding three studies.

I initially hypothesized that probiotic use might be more efficacious in some broad types of GI diseases than in others due to the mechanisms of action of the disease. Specifically, there might be differences in efficacy related to diarrheal production versus inflammation or destruction of tissue, verses abdominal pain, flatulence and irregular bowel movements (IBS). I found no support for this hypothesis. AAD, CDD, ID, and TD are related to diarrhea and NEC, Pouch and HPP are related to inflammation/destruction of tissue. IBS is characterized by abdominal pain, increased flatulence and irregular

bowel movements. None of these groups differed significantly in probiotic efficacy and all disease showed significant effects except for NEC and TD, which are related to inflammation and diarrhea respectively.

Previous studies have focused on the effect of one to two species of probiotics (e.g., Cindoruk et al., 2007; Ruszcynski et al., 2008; Hawrelak et al., 2005) in the prevention of specific GI diseases. Of the 11 probiotics considered, VSL #3 (RR= 0.17 95% CI 0.09-0.33) and *C. butyricum* (RR= 0.18 95% CI 0.09-0.37) had the most significant effect sizes (Figure 4.3B). The high statistical efficacy for these probiotics could be due to the small number of patients analyzed compared to the other probiotics. For example, *C. butyricum* had 207 patients and VSL #3 had 116 patients which are small compared to LGG with 2782 patients. Higher efficacy for these species could also be related to their use in diseases that also showed high prevention/treatability with probiotics (e.g., AAD, HPP, Pouchitis), unlike species that are widely used across many different GI diseases, such as LGG, which is used in the prevention or treatment of TD, Pouchitis, CD, AAD, HPP, NEC, and IBS. LGG is used widely in clinical trials because of its beneficial effects on intestinal immunity (Pozo-Olano et al., 1978). Furthermore, LGG inhibits growth of *Esherichia coli*, *Streptococcus*, *C. difficile*, *Bacteriodes fragilis* and *Salmonella* by producing an antimicrobial substance (Gorbach, 1996). *S. boulardii*, *E. faecum, B. lactis, LGG, L. casei,* and *L. acidophilis* combined with *B. infantis* also showed significant efficacy in the treatment and prevention of GI disease. Our results support recent findings by McFarland et al. (1994), who showed that *S. boulardii* prevented AAD and by Orrhage et al. (1994), which showed that the combination of *L. acidophilus* and *Bifidobacterium* reduced the fecal counts of clostridia in CDD.

L. acidophilis, L. plantarum, and *B. infantis* did not have significant effect sizes, showing that they are not effective in the treatment of the GI diseases considered here. In this meta-analysis, all species of *L. acidophilus* were first analyzed together. This included strain LB, a common probiotic as well as unspecific strains. *L. acidophilus* (strain LB) is a heat-stabilized strain also known as LacteÂol Fort (Boulloche et al., 1994). In some previous studies, LacteÂol Fort (*L. acidophilus LB*) was effective in the treatment of acute diarrhea, reducing duration and severity (Boulloche et al., 1994; Bodilis, 1983) and in IBS (Halpern et al., 1996). In the treatment of HPP, inactive *L. acidophilus* showed an in vitro inhibitory effect on the attachment of *H. pylori* to gastric epithelial cell lines (Canducci et al., 2000). In other studies *L. acidophilus* has not had significant effects. For example, Katelaris et al., (1995) found no protection of TD with *L. acidophilus* and Witsell et al., (1995) found no effect of *L. acidophilus* on AAD. Our results suggest that when taken without other species, *L. acidophilus* is not significantly effective in preventing/treating GI disease (RR= 0.82 95% CI 0.47- 1.43). This result may be due to the strains *L. acidophilus LB* and *L. acidophilus* analyzed together and strain dependency could have an effect on the efficacy of GI disease. When analyzed alone, *L. acidophilis* LB did show significant efficacy (RR= 0.40 95% CI 0.20-0.82) and *L. acidophilus* with no strain specified did not have a significant effect (RR= 1.17 95% CI 0.85-1.62). Future studies should compare and report effects of different strains of *L. acidophilus* on GI diseases. Sazawal et al., (2006) found that prevention did not vary significantly for the probiotic species *S. boulardii*, *LGG*, *L. acidophilus*, or *L. bulgaricus*. In my meta-analysis *L. plantarum and B. infantis* also had no overall effect on GI disease. Similar negative results for *L. plantarum* have been previously found in the treatment of

IBS (Simren et al., 2006; Nobaek et al., 2000; Niedzielin et al., 2001). In contrast, *L. plantarum* has efficacy in the prevention of CDD (Wult et al., 2003). Additional studies across GI diseases need to be conducted to assess the specific diseases that respond to *L. plantarum*. We also found that *B. infantis* had no significant effect. There were very few trials available in the literature for this species (n= 3) (Wult et al., 2003) and additional studies should be done to test efficacy.

Probiotics may be given to patients as either single or multiple species. While some studies use one probiotic species e.g. *B. infantis* (Whorwell et al., 2006) others used multiple strains e.g. VSL #3 (Gionchetti et al., 2000; Gionchetti et al., 2003; Mimura et al., 2004). We found no significant difference between the efficacies of single or multiple species (Figure 4D). Instead, as discussed above, the particular strain used is key to efficacy. Since most studies only included the species of probiotic (e.g., *L. acidophilus)* used, it is critical for future studies to identify the exact probiotic strain.

It has been previously suggested that patient age may be a factor in probiotic efficacy (Sazawal et al., 2006; Tong et al., 2007; Hoveyda, et al., 2009). Efficacy may differ by age group due to factors such as the development of the GI and differences in hormones and immunology (Wilson, 2008). My results showed no difference in efficacy by age group with all age groups (infants, children, and adults) showing significant effect sizes with the use of probiotics for the prevention of GI disease (Figure 4.4A). Similar results have been reported by Tong et al. (2007) who showed that child and adult age group sub-analyses were both significant for HPP. Likewise, Sazawal et al., (2006) showed significant results for both children and adults for the prevention of acute diarrhea. A potential difference in the efficacy of probiotics in treating GI is an area

where additional studies are needed. Very few trials have been conducted on infants (n=9) or children (n=14) relative to adults (n=53). For example, Hoveyda et al., (2009) concluded that IBS was preventable for adults, but could not assess efficacy in children due to the lack of studies.

Another factor that has been previously considered in probiotic efficacy is dosage. Our results showed that three of the four dosage levels were significant in treating disease. Only the dose 1-9 x 10^{11}, 10^{12} CFU/day, which was the largest treatment dose, did not show a significant effect size. However, this result was likely due to the smaller sample size (n=7) relative to the sample sizes of the other doses (n= 20, 25, and 12), which contributed to a larger 95% CI. Whorwell et al. (2006) studied the probiotic *B. infantis* (strain 35624) at three different dosage strengths 10^{6}, 10^{8}, and 10^{10} and found 1 x 10^{10} CFU (for four weeks) was most effective. The dosages tested in the studies analyzed here all were well above the minimum in commercial preparations, which typically contain more than 1 billion bacterial units (Cremonini et al., 2002a). Correct dosage for specific diseases has been an area of some debate. For example, Bezkorovainy (2001) suggested that several billion organisms should be introduced into an organism since not all of the bacteria will reach target areas due to pH and salinity levels in the esophagus and stomach which can reduce colony size (Wilson, 2008). Our results suggest that dosage has relatively minor effects.

In the past, it has been suggested that the treatment length in which patients received the probiotic could be a factor in the treatment or prevention of disease and longer studies should be implemented (McFarland & Dublin, 2008; Hoveyda et al., 2009). To my knowledge, this is the only meta-analysis that has examined efficacy

according to the length of treatment. My results show no significant effect of treatment length on efficacy (Figure 4.4C). Taking probiotics for even a week is sufficient in preventing and treating GI disease.

In conclusion, our meta-analysis containing 74 studies, 84 trials and 10,351 patients shows that in general, probiotics are beneficial in treatment and prevention of GI diseases. The only GI diseases in which significant effect sizes were not observed were TD and NEC. This may be due to the low number of studies conducted on these diseases, or in the case of TD, the underlying mechanism of disease, which is often not bacterial. Of the 11 species or species mixtures only *L. acidophilus*, *L. plantarum* and *B. infantis* showed no efficacy however, for *L. acidophilus*, it was found that the strain LB was highly effective. No differences in efficacy were observed for age group or length of treatment or for single vs. multiple species. The highest dosage considered (1-9 x 10^{11}, 10^{12} CFU/day) did not show a significant effect size however, due to the small sample size, this result may be spurious. When choosing probiotics, the type of disease (treated/prevented) and probiotic species (strain) used are the most important factors to take into consideration.

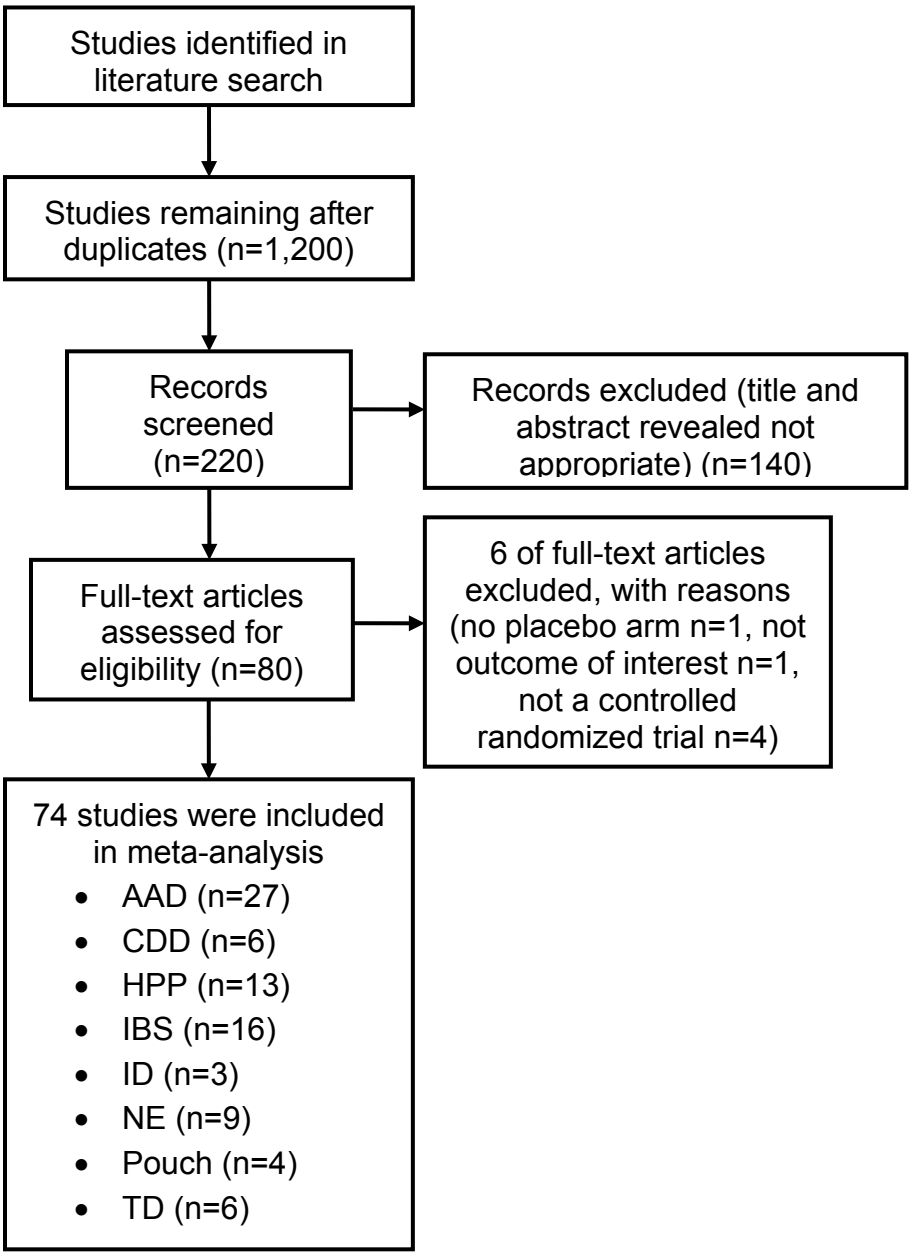

Figure 4.1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flow diagram showing an overview of the study selection process.

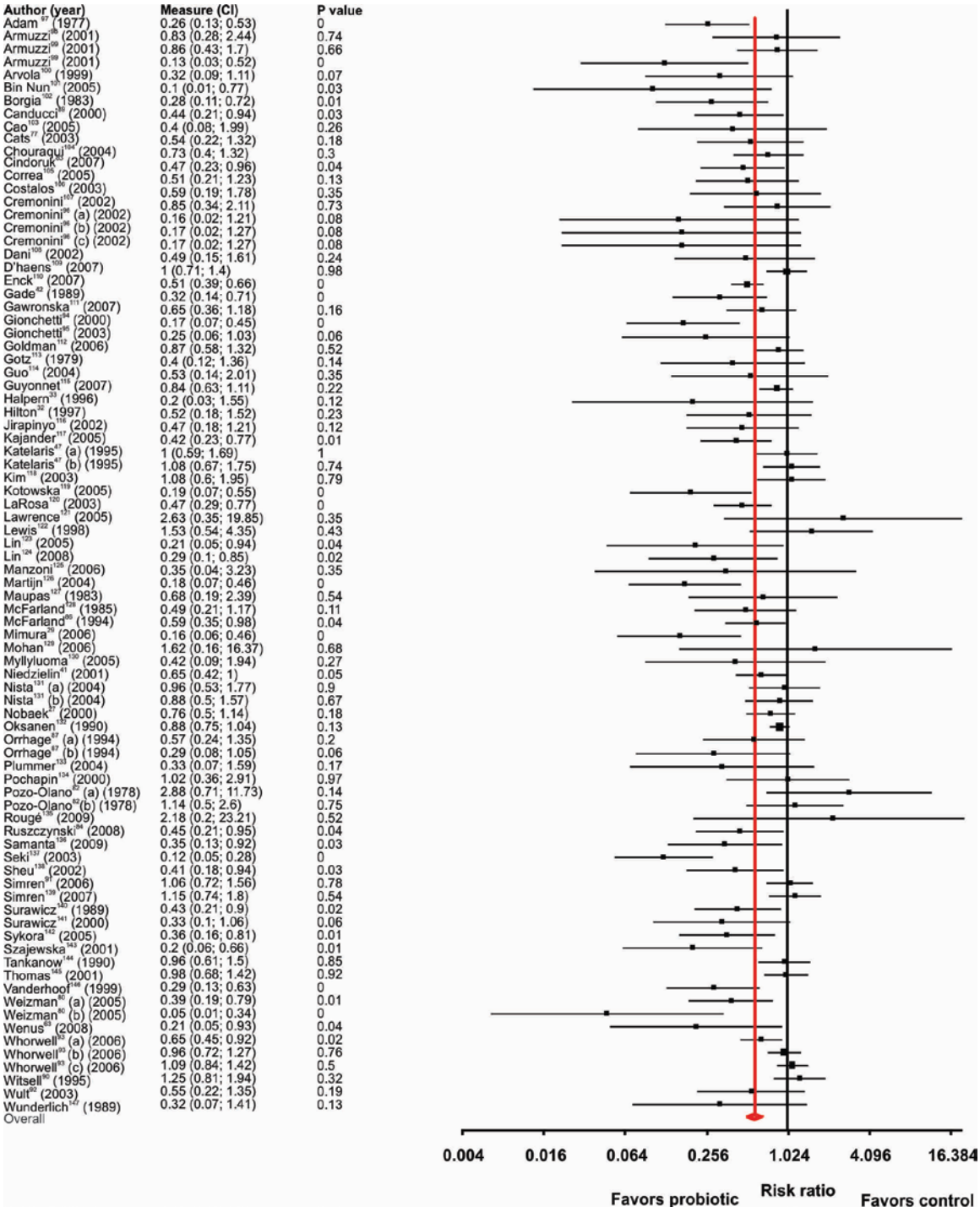

Figure 4.2. The effect size (risk ratio) for the overall effects of probiotics in the prevention and treatment of gastrointestinal (GI) diseases including the 95% confidence intervals. The author, date, measure (risk ratio (95% CI), and p value are shown. Larger data points have larger samples. Risk ratios below one favor the probiotic while risk ratios above one favor the placebo. The red line represents the mean relative risk for the 84 trials.

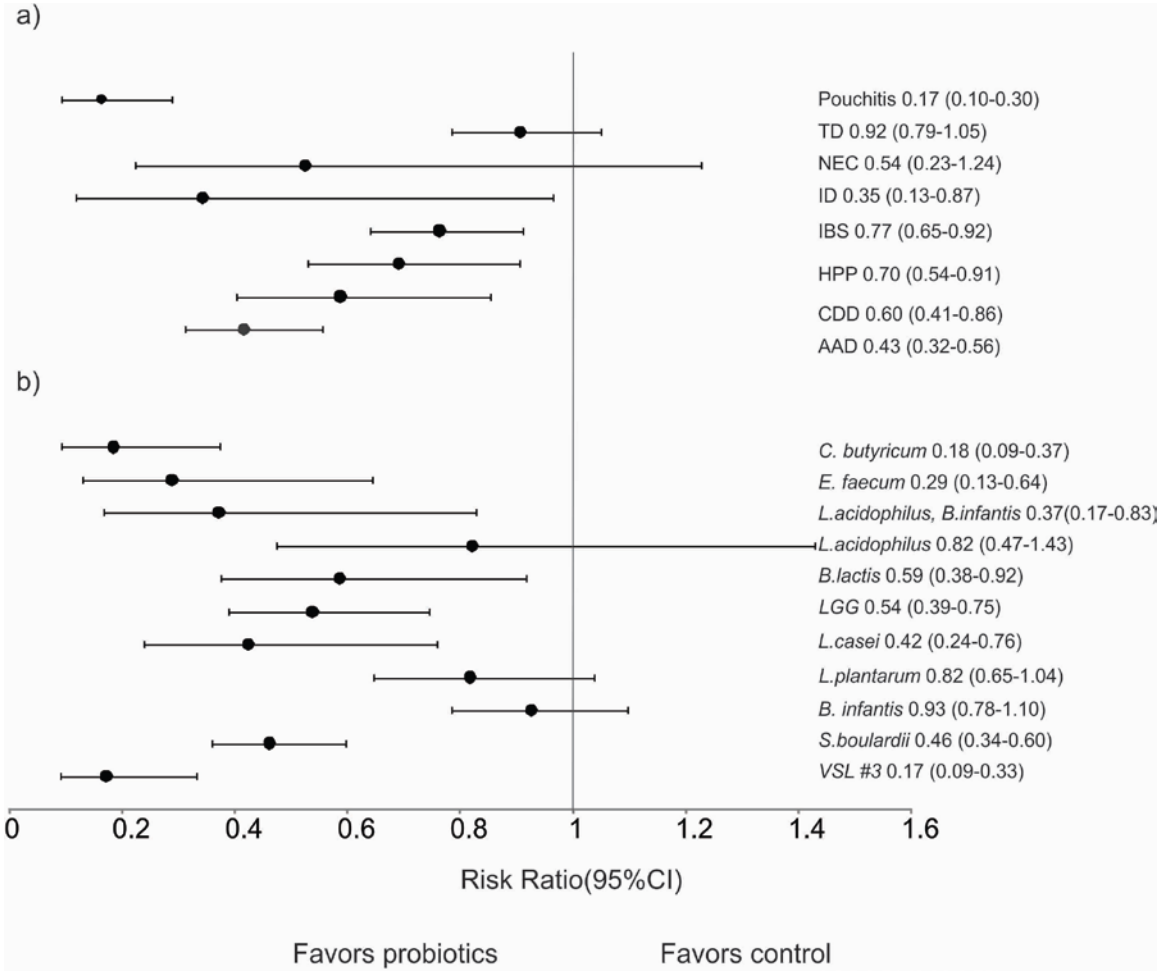

Figure 4.3. (A) The effect size (risk ratio) including the 95% confidence intervals for the total events of Antibiotic-associated diarrhea (AAD), *Clostridium difficile* disease (CDD), *Helicobacter pylori positive* (HPP), Irritable bowel syndrome (IBS), Infectious diarrhea (ID), Necrotizing Enterocolitis (NE), Traveler's diarrhea (TD), and Pouchitis during which probiotics were taken. (B) The effect size (relative risk) including 95% confidence intervals for the type of probiotic species that were used to treat and prevent gastrointestinal disease. Risk ratios below one favor the probiotic while risk ratios above one favor the placebo.

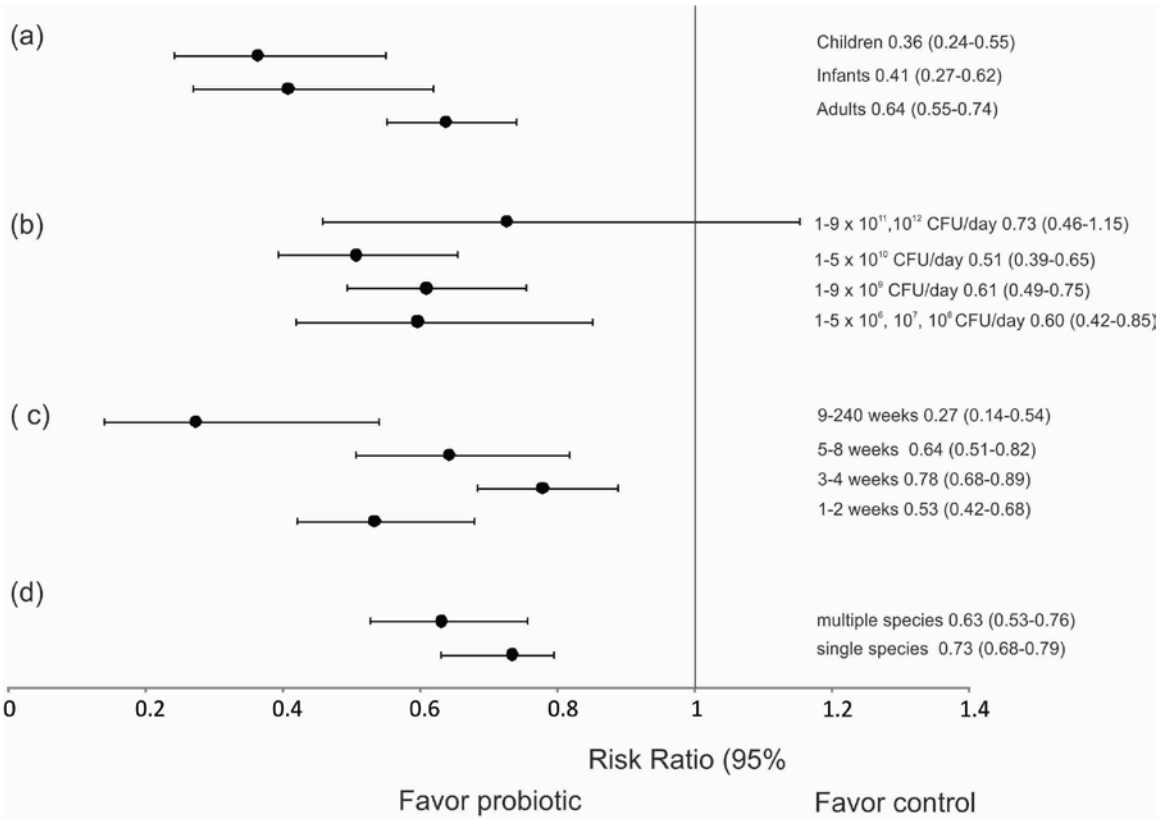

Figure 4.4. (A) The effect size (risk ratio) including the 95% confidence intervals for the age groups that had taken the probiotic vs. the controls. Age groups included were: adults (>18yr), children (3≤18yr) and infants (0-3yr). (B) The effect size (risk ratio) including the 95% confidence intervals for dose of probiotic. The doses that were included were: 1-9 x 10^{11}, 10^{12} CFU/day; 1-5.5 x 10^{6}, 10^{7}, 10^{8} CFU/day; 1-9 x 10^{9} CFU/day; 1-5 x 10^{10} CFU/day. (C) The effect size (risk ratio) including the 95% confidence intervals for treatment length. Treatment lengths that were included were: 1-2 weeks, 3-4 weeks, 5-8 weeks and 9-240 weeks. (D) The effect size (risk ratio) including the 95% confidence intervals for multiple or single species of probiotics. Probiotics that contain more than one species were considered multiple species, while probiotics only administered as one species were considered single species. Risk ratios below one favor the probiotic while risk ratios above one favor the placebo.

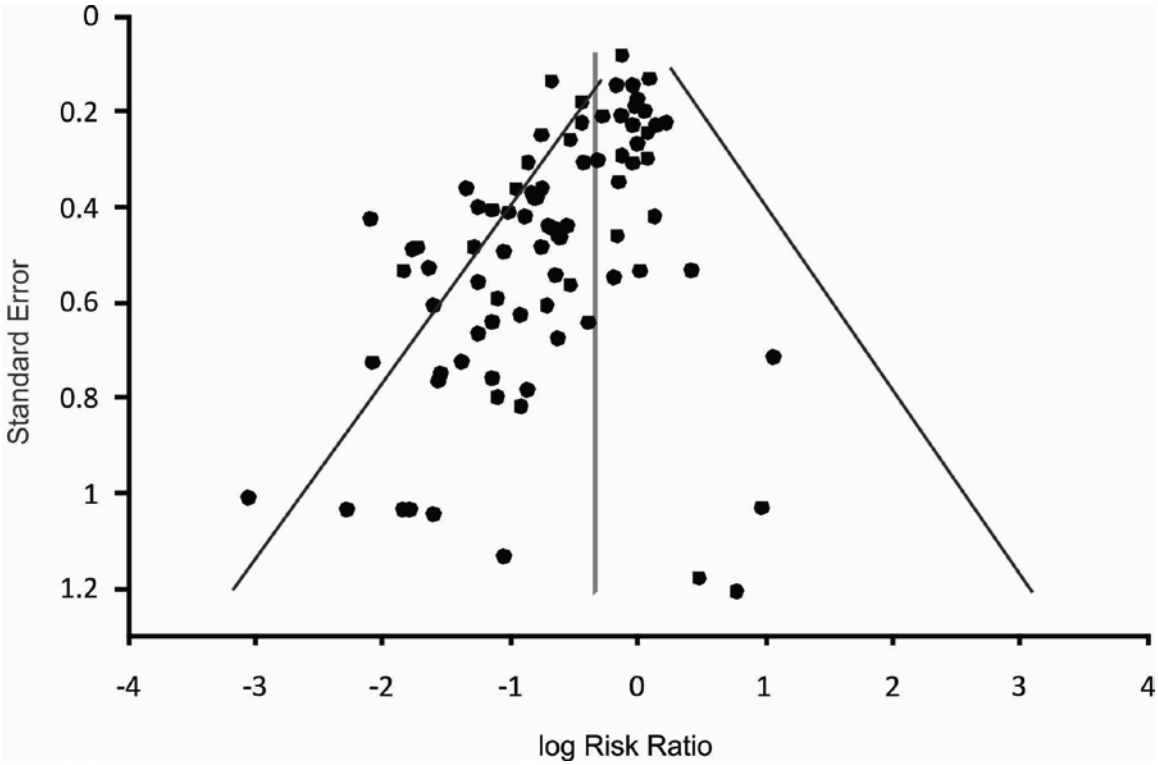

Figure 4.5. Funnel plot asymmetry used to determine publication bias. Log of the risk ratios were plotted against the standard error of the risk ratio of each study to identify asymmetry in the distribution of trials. Gaps in the funnel plot suggest potential publication bias. The synthesis estimate (outer lines) and the 0.01 limit are shown to distinguish asymmetry.

CHAPTER 5

Conclusion

Linking the human microbiome to human health is the goal of many specialists. The importance of human-associated microbiota in human health is increasingly being recognized as links have been made between the composition of human microbiomes and disease, obesity, and sexually transmitted infections. The goals of my research were too 1) assemble and compare network structure for five regions of the human body, 2) compare how robust these networks were to node removals, 3) determine whether there were significant changes in the topology of the oral cavity microbiomes through development and aging, 4) compare within-region spatial changes in the topology of gastrointestinal microbiomes, and 5) compare the efficacy of probiotics in the treatment and prevention of gastrointestinal disease. Here, I summarize the major results and conclusions of my findings.

5.1 Network structure and robustness of five regions of the human body

The first part of my research (Chapter 2) yielded three important results for which there are important theoretical and applied implications. First, to date the networks presented here represent the first to attempt to include two types of interactions: consumer-resource (negative) and facilitative (positive interactions) in energetic interaction networks. While the

importance of facilitative interactions in ecosystem networks has long been recognized, positive interactions between species have only recently been included in analyses of ecological networks. When positive interactions have been included, links between species have been limited to these interactions; thus to date ecological networks that include the full suite of interactions among species have not been assembled. One of the major consequences of this lack of inclusion of positive interactions in ecological networks is that the relative importance of facilitative vs. consumer-resource interaction is largely unknown. My results show that facilitative links are a very important component of organismal based energetic networks and dominate the networks of most human microbiomes. The high proportion of facilitative links has two potentially important consequences on network function, particularly with perturbations. First, a relatively diverse microbiome appears essential for function. Two-thirds of the links between microbiota in the composite networks were facilitative, suggesting that positive interactions among microbiota are more important, at least by proportion, than negative energetic links. Once a complete microbial community becomes established, the species rely on each other for nutrients (at least in terms of numbers) more than they rely on source-specific nutrients from the host. Second, loss of species in networks would affect both consumer-resource and facilitative interactions, although maybe not equivalently. The consequences of differences in connectivity for taxa based on consumer-resource vs. facilitative links in terms of secondary species loss in networks, with both types of interactions are unknown. For example, a genus can differ significantly in the number of links it has in a consumer-resource versus a facilitative network and thus removal of the most connected species in a consumer-resource versus a facilitative network could lead to very different outcomes.

I found that the structure of functional networks is very different from the structure of consumer-resource networks. The extremely high connectance and clustering coefficients suggest high redundancy for facilitative interactions, a redundancy that translated into increased robustness to node loss in the facilitative networks. It may be that facilitative links provide different and potentially even more essential nutrients to many microbiotas than are provided from host nutrients.

My research has also shown that robustness is strongly related to network topology (structure). Node richness and connectance were the primary drivers of robustness for microbiome networks. From an evolutionary perspective the structure of microbial communities is determined by the interplay between ecology and evolution. Consequently, the definitive determinant of robustness may be linked to environmental conditions in different regions and in particular aspects of homeostatic regulation that affect structure and function. For example, the oral cavity composite network was the most robust to node removal, which in a 'real world' context could be due to aspects of the environment that contribute to high species turnover, such as high rates of pathogen invasions.

5.2 Structure and robustness of ontogenic and regional networks

Understanding how human microbiomes change spatially within body regions and with ontogeny is important to understanding the functions of human-associated microbial communities. The second part of my research, Chapter 3, yielded two main results. First, there are some consistent changes in topology with age associated with increased complexity. Changes with region along the GIT are associated with increased node richness, links, and higher connectance consistent with the increased functional

importance of lower versus higher regions in terms of digestion. Node richness and connectance increased with ontogenic stage in the oral cavity and in regions further down the gastrointestinal tract.

Second, for the GIT regions, the small and large intestine were more robust on average to node removals, while in the oral ontogenic networks, the child, adult and elderly networks were more robust. While the significance of these trends is as yet unknown, they suggest that broad structural differences even at the level of genera can be observed through developmental stages and within sub-regions of specific systems.

5.3 Efficacy of probiotics on gastrointestinal disease

The third part of my research, Chapter 4, used meta-analysis to determine whether efficacy of probiotic-use differed across gastrointestinal diseases and different probiotics. Across eleven probiotic species and eight different gastrointestinal diseases I found a significant effect of probiotics on the prevention and treatment of gastrointestinal disease, except for the diseases Traveler's Diarrhea and Necrotizing Enterocolitis.
Three probiotic strains did not show efficacy towards gastrointestinal disease: *L. acidophilus*, *L. plantarum*, and *B. infantis*. This functional analysis of the role of probiotics on the treatment and prevention of gastrointestinal diseases showed that microbiota have widespread and strong effects on gastrointestinal health and may aid in determining the mechanisms by which probiotics act on the gastrointestinal tract.

5.4 Limitations

There are a number of factors that should be taken into account that may limit the applicability and interpretations of my results. A key limitation is that the microbiome networks presented here are resolved to the level of genera and not to the species level, which would be the ideal level of resolution. Resolving to species level is not possible at

this time due to limited pyrosequencing and lack of information on biochemistry for many species. Since the advancement of sequencing (pyrosequencing, metagenomic sequencing), many new species have been found in and on the human body, but their interactions in/on the body are unknown. New sequencing research must not only focus on finding new species but also on understanding the physiology and biology of these organisms. Although this is a limitation, by aggregating the nodes into genera that share 100% of their links the nodes represent functional groups of bacteria. In addition, analysis of nodes at the genera level minimizes bias due to uneven resolution and incomplete sampling effort.

The second potential limitation is that the nodes are binary and do not include information on relative abundance. Two issues are important to take into consideration here. First, species composition varies widely across humans based on a multitude of factors including age, sex, region etc. Relative abundances would likely differ even more across individuals than species composition thus assembling a 'realistic' relative abundance network for the human microbiome would be a major caricature of actual patterns. Second, data on relative abundance does not yet exist in the literature. Future pyrosequencing studies reporting abundance and prevalence will greatly improve the data situation for relative abundances.

Another limitation is that the links between the nodes identified in the different regions could have been described at an even finer level of regional detail than we have attempted here. For example, pyrosequencing in the gastrointestinal tract has shown that species composition differs depending on the area (e.g., esophagus, large intestine). Therefore, more highly resolved networks for sub-sets of the "regions" used here are

needed. This was performed in Chapter 3 with the spatial networks of the gastrointestinal networks but for the other regions (eye, skin, respiratory tract, oral cavity) not enough studies have been done to increase spatial resolution.

Lastly, models incorporating both consumer-resource and facilitative links have not yet been developed. Models that accurately predict the topological patterns in human microbiomes, similar to the niche model that has been developed for food-webs, are needed (Williams & Martinez, 2000; Dunne et al., 2009).

Even with these limitations, looking at regional human microbiomes from a network approach has a number of prospective benefits, even at this early stage of investigation. Mathematical network theory is ideal for studying the interactions between species in ecological networks, allowing us to understand both normal and disturbed microbial community functions, from the standpoint of systems biology (Foster et al., 2008).

5.5 Conclusion

It was my initial goal and true interest to conduct research that advanced both the theory of food web ecology and human health. The findings described in this thesis provide a strong empirical basis for the incorporation of facilitative interactions into food webs and have major implications for human health. Our bodies contain a diverse and complex microbial community that differs in structure regionally as well as with age. The consequences of these differences in structure are as yet unknown however, the use of a network perspective to view the structure of microbial communities has shown that differences in structure between regions can affect the response of our microbial communities to perturbations and thus may one day contribute to a systems view of the dynamics of human health and disease.

BIBLIOGRAPHY

Aas, J., Paster, B., Stokes, L., Olsen, I., & Dewhirsti, F. (2005). Defining the Normal Bacterial Flora of the Oral Cavity. *J Clin Microbiol, 43*, 5721-5732.

Abell, G., & McOrist, A. (2007). Assessment of the diversity and stability of faecal bacteria from healthy adults using molecular methods. *Microb Ecol Health Dis , 19*, 229-240.

Adam J., Barret C, Barret-Bellet A *et al*. (1977) Controlled double-blind clinical trials of Ultra-Levure: Multicenter study by 25 physicians in 388 cases. *Gaz. Med. Fr. , 184,* 072-8.

Al-Ahmad, A., Wunder, A., Auschill, T., Follo, M., Braun, G., Hellwig, E., et al. (2007). The in vivo dynamics of Streptococcus spp., Actinomyces naeslundii, Fusobacterium nucleatum, and Veillonella spp. in dental plaque biofilm as analysed by five-colour multiplex fluorescence in situ hybridization. *J Med Microbiol , 56*, 681-687.

Albert, R., & Barabási, A. (2002). Statistical mechanics of complex networks. *Rev. Mod. Phys. , 74*, 47–97.

Albert, R., Jeong, H., & A, B. (2000). Error and attack tolerance of complex networks. *Nature , 406*, 378-382.

Albietz, J., & Lenton, L. (2006). Effect of antibacterial honey on the ocular flora in tear deficiency and meibomian gland disease. *Cornea , 25*, 1012-1019.

Albiger, B., Dahlberg, S., Henriques-Normark, B., & Nomark, S. (2007). Role of innate immune system in host defence against bacterial infections: Focus on Toll-like receptors. *J Intern. Med. , 261*, 511-28.

Allesina, S., & Bodini, A. (2004). Who dominates whom in the ecosystem? Energy flow bottlenecks and cascading extinctions. *Journal of Theoretical Biology , 230* (3), 351-358.

Aly, R., & Maibach, H. (1977). Aerobic microbial flora of intertrigenous skin. *Appl Environ Microbiol , 33*, 97-100.

Andoh, A., Sakata, S., Koizumi, Y., Mitsuyama, K., Fujiyama, Y., & Benno, Y. (2007). Terminal restriction fragment lenght polymorphism analysis of the diversity of fecal mcrobiuota in patients with ulcerative colitis. *Inflamm Bowel Dis , 13*, 955-962.

Andrieux, C., Membre, J., Cayuela, C., & Antoine, J. (2002). Metabolic characteristics of the faecal microflora in humans from three age groups. *Scandinavian J Gastroenterol , 37*, 792-798.

Arantes, T., Cavalcanti, R., Diniz, M. d., Severo, M., Neto, J., & de Castro, C. (2006). Conjunctival bacterial flora and antibiotic resistance pattern in patients undergoing cataract surgery. *Arq Bras Oftalmol , 69*, 33-36.

Armuzz, A., Cremonini, F., Bartolozzi, F *et al*. (2001) The effect of oral administration of *Lactobacillus GG* on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy. *Aliment Pharmacol Ther, 15,* 163-9.

Armuzzi, A., Cremonini, F., Ojetti, V *et al*. (2001) Effect of *Lactobacillus GG* supplementation on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy: a pilot study. *Digestion, 63,* 1-7.

Arumugam, M., Raes, J., Pelletier, E., Paslier, D., Yamada, T., Mende, DR *et al.* (2011) Enterotypes of the human gut microbiome. Nature, 473, 174-180.

Arvola T, Laiho K, Torkkeli S *et al*. (1999) Prophylactic *Lactobacillus GG* reduces antibiotic associated diarrhea in children with respiratory infections: A randomized study. *Pediatrics*, 104, e64.

Ashbee, H. (2007). Update on the genus *Malassezia*. *Med Mycol , 45*, 287-303.

Ashely, E., Gallagher, J., & Wilson, R. (1988). The occurrence of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis*, *Bacteroides intermedius* and spirochaetes in the subgingival microflora of adolescents and their relationship with the amount of supragingival plaque and gingivitis. *Oral Microbiol Immunol , 3*, 77-82.

Baas-Becking, L. (1934). *Geobiologie of Inleiding Tot de Milieukunde.* Hague, The Netherlands: WP Van Stockum & Zoon.

Bäckhed, F., Ley, R., Sonnenburg, J., Peterson, D., & Gordon, J. (2005). Host-Bacterial Mutualism in the Human Intestine. *Science , 307* (5717), 1915-1920.

Bals, R., & Hiemstra, P. (2004). Innate immunity in the lung: How epithelial cells fight against respiratory pathogens. *Eur Respir J , 23*, 327-333.

Bax, L. (2010). MIX 2.0. Professional software for meta-analysis in Excel. Version (2.0.1.2). *BiostatXL,* http://www.meta-analysis-made-easy.com.

Begg CB, Mazumdar M. (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics, 50,* 1088-1101.

Beighton, D., Brailsford, S., Lynch, E., Chen, H., & Clark, D. (1999). The influence of specific foods and oral hygiene on the microflora of fissures and smooth surfaces of molar teeth: A 5-day study. *Caries Res , 33*, 349–56.

Beisswenger, C., & Bals, R. (2005). Functions of antimicrobial peptides in host defense and immunity. *Curr Protein Pept Sci , 6*, 255-264.

Belenguer, A., Duncan, S., Calder, A., Holtrop, G., Louis, P., Lobley, G., et al. (2006). Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. *Appl Environ Microbiol , 72*, 3593-3599.

Ben-Amor, K., Heilig, H., Smidt, H., Vaughan, E., Abee, T., & de Vos, W. (2005). Genetic diversity of viable, injured, and dead fecal bacteria assessed by fluorescence-activated cell cell sorting and 16S rRNA gene analysis. *Appl Environ Microbiol , 71*, 4679-4689.

Bengmark S. (1998) Ecological control of the gastrointestinal tract: the role of probiotic flora. *Gut, 42,* 2-7.

Benno Y, Mitsuoka T. (1992). Impact of *Bifidobacterium longum* on human fecal microflora. *Microbiol Immunol*, *36,* 683-94.

Berg, R. (1996). The indigenous gastrointestinal microflora. *Trends in Microbiology , 4* (11), 430-435.

Berg, R., & Savage, D. (1972). Immunological response to microorganisms indigenous to the gastrointestinal tract. *American Journal of Clinical Nutrition , 25*, 1364-1371.

Bernardini, G., Braconi, D., & Santucci, A. (2007). The analysis of Neisseria meningitidis proteomes: Reference maps and their applications. *Proteomics , 7*, 2933-2946.

Berry, M., Harris, A., Lumb, R., & Powell, K. (2002). Commensal ocular bacteria degrade mucins. *Br J Ophthalmol , 86*, 1412-1416.

Bezkorovainy A. (2001). Probiotics: determinants of survival and growth in the gut. *Am J Clin Nutr, 73*, 399S-405S.

Bik, E., Eckburg, P., Gill, S., Nelson, K., Purdom, E., Francois, F., et al. (2006). Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci USA , 103*, 732-737.

Bilen, H., Ates, O., Astam, N., Uslu, H., Akcay, G., & Baykal, O. (2007). Conjunctival flora in patients with type 1 or type 2 diabetes mellitus. *Adv Ther , 24*, 1028-1035.

Bin-Nun A, Bromiker R, Wilschanski M *et al*. (2005) Oral probiotics prevent necrotizing Enterocolitis in very low birth weight neonates. *J Pediatr, 147*, 192-96.

Blaser, M., & Atherton, J. (2004). Helicobacter pylori persistence: Biology and disease. *J Clin Invest , 113*, 321-333.

Blaut, M., & Clavel, T. (2007). Metabolic diversity of the intestinal microbiota: Implications for health and disease. *J Nutr , 137*, 751S-755S.

Blumberg D, Beck DE. (2002). Surgery for ulcerative colitis. *Gasroenterol Clin North Am*, *31*, 219-235.

Bodilis JY. (1983). Controlled clinical trial of Lacteol Fort compared with a placebo and reference drug in the treatment of acute diarrhea in the adult. *Med Actuelle*, *10,* 232-5.

Bohnhoff, M. a. (1962). Enhanced susceptibility to Salmonella infection in streptomycin-treated mice. *Journal of Infectious Disease , 111*, 117-127.

Bojar, R., & Holland, K. (2002). The human cutaneous microbiota and factors controlling colonization. *World J Microbiol Biotechnol , 18*, 889-903.

Boojink, C., Zoetendal, E., Kleerebezem, M., & de Vos, W. (2007). Microbial communities in the human small intestine : Coupling diversity to metagenomics. *Fut Microbiol , 2*, 285-295.

Borgia M, Sepe N, Brancato V *et al*. (1982) A controlled clinical study on *streptococcus faecium* preparation for the prevention of side reactions during long term antibiotic treatments. *Curr Ther Res, 31,* 265-71.

Boucher, R. (1999). Molecular insights into the physiology of the thin film of airway surface liquid. *J Physiol , 516*, 631-638.

Boulloche J, Mouterde O, Mallet E, *et al*. (1994). Management of acute diarrhoea in infants and young children: controlled study of the anti-diarrhoeal efficacy of killed *L. acidophilus* (LB Strain) versus a placebo and a reference drug (loperamide). *Ann Pediatr*, *41*, 457-63.

Bowden, G., Hardie, J., & Slack, G. (1975). Microbial variations in approximal dental plaque. *Caries Res , 9*, 253-277.

Breton, L., & Addicott, J. (1992). Density-Dependent Mutualism in an Aphid-Ant Interaction. *Ecology , 73* (6), 2175-2180.

Brook, I., & Gober, A. (2005). Recovery of potential pathogens and interfering bacteria in the nasopharynx of smokers and nonsmokers. *Chest , 127*, 2072-2075.

Brose, U., Berlow, E., & Martinez, N. (2005). Scaling up keystone effects from simple to complex ecological networks. *Ecology Letters , 8*, 1317–1325.

Brose, U., Jonsson, T., Berlow, E., Warren, P., Banasek-Richter, C., Bersier, L.-F., et al. (2006). Consumer-resource bodysize relationships in natural food webs. *Ecology , 57*, 2411–2417.

Brown, J., Georg, L., & Waters, L. (1969). Laboratory Identification of Rothia dentocariosa and Its Occurrence in Human Clinical Materials. *Appl Microbiol , 17*, 150-156.

Bruggemann, H., Henne, A., Hoster, F., Liesegang, H., Wiezer, A., Strittmatter, A., et al. (2004). The complete genome sequence of Propionibacterium acnes, a commensal of human skin. *Science , 305*, 671-673.

Bullard, B., Lipski, S., & LaFontaine, E. (2007). Regions important for the adhesion activity of Moraxella catarrhalis Hag. *BMC Microbiol , 7*, 65.

Campbell, N., Reece, J., Taylor, M., Simon, E., & Dickey, J. (2009). *Biology Concepts and Connections.* UK: Pearson: Benjamin Cummings.

Campos, M., Campos e Silva, L., Rehder, J., Lee, M., O'Brien, T., & McDonnell, P. (1994). Anaerobic flora of the conjunctival sac in patients with AIDS and with anophthalmia compared with normal eyes. *Acta Ophthalmol (Copenh) , 72*, 241-245.

Canducci, F., Armuzzi, A., Cremonini, F *et al*. (2000). A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. *Aliment Pharmacol Ther, 14,* 1625-9.

Cantin, A. (2001). Biology of respiratory epithelial cells: Role in defense against infections. *Pediatric Pulmonol , 26 (Suppl 23)*, 167-169.

Cao, YJ., Qu, CM., Yuan, Q *et al*. (2005). Control of intestinal flora alteration induced by eradication therapy of *Helicobacter pylori* infection in the elders. Chin J Gastroenterol Hepatol, 14, 195-9.

Caporaso, J., Lauber, C., Walters, W., Berg-Lyons, D., Lozupone, C., Turnbaugh, P., et al. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *PNAS , 108*, (Suppl 1) 4516-4522.

Capriotti, J., Pelletier, J., & Shah, M. (2009). Normal ocular flora in healthy eyes from a rural population. *Int Ophthalmol , 29*, 81–84.

Cardinale, B., Palmer, M., & Collins, S. (2002). Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature , 415*, 426-429.

Carol, M., Lambrechts, A., Van Gossum, A., Libin, M., Goldman, M., Mascart-Lemone, F., (1998) Spontaneous secretion of interferon-γ and leukin 4 by human intraepithelial and lamina propria gut lymphocytes. *Gut*, *42,* 643-9.

Carey, C., Kirk, J., Ojha, S., & Kostrzynska, M. (2007). Current and future uses of real-time polymerase chain reaction and microarrays in the study of intestinal microbiota, and probiotic use and effectiveness. *Can J Microbiol , 53*, 537-550.

Cats, A., Kuipers, EJ., Bosschaerts, AR., *et al.* (2003). Effect of frequent consumption of a *Lactobacillus casei*-containing milk drink in *Helicobacter pylori*-colonized subjects. *Aliment Pharmacol Ther*, *17*, 429-435.

Chaidaroon, W., Ausayakhun, S., Pruksakorn, S., Jewsakul, S., & Kanjanaratanakorn, K. (2006). Ocular bacterial flora in HIV-positive patients and their sensitivity to gentamicin. *Jpn J Ophthalmol , 50*, 62-80.

Chapin, F., Ravaleta, E., Eviner, V., Naylor, R., Vitousek, P., Reynolds, H., et al. (2000). Consequences of changing biodiversity. *Nature , 405*, 234-242.

Cheng, L., Moor, S., Kravchuk, O., Meyers, I., & Ho, C. (2007). Bacteria and salivary profile of adolescents with and without cleft lip and/or palate undergoing orthodontic treatment. *Australian Dental Journal , 52*, 315-321.

Chiller, K., Selkin, B., & Murakawa, G. (2001). Skin Microflora and Bacterial Infections of the Skin. *Journal of Investigative Dermatology Symposium Proceedings , 6*, 170-174.

Chisari, G., Cavallaro, G., Reibaldi, M., & Biondi, S. (2004). Presurgical antimicrobial prophylaxis: Effect on ocular flora in healthy patients. *Int J Clin Pharmacol Ther , 42*, 35-38.

Choi, C., Yin, C., Bakar, A., Sakewi, Z., Naing, N., Jamal, F., et al. (2006). Nasal Carriage of Staphylococcus aureus among healthy adults. *J Microbiol Immunol Infect , 39*, 458-464.

Chouraqui, JP., Van Egroo, LD., Fichot, MC. (2004). Acidified milk formula supplemented with *Bifidobacterium lactis*: impact on infant diarrhea in residential care settings. *J pediatr Gastroenterol Nutr, 38,* 288-92.

Cindoruk, M., Erkan, G., Karakan, T *et al*. (2007). Efficacy and Safety of *Saccharomyces boulardii* in the 14-day Triple Anti-*Helicobacter pylori* Therapy: A Prospective Randomized Placebo-Controlled Double Blind Study. *Helicobacter*, 12, 309-316.

Clarke, S., & Foster, S. (2006). Surface Adhesions of Staphylococcus aureus. *Adv Microb Physiol , 51*, 187-224.

Clavel, T., & Haller, D. (2007). Molecular interactions between bacteria, the epithelium, and the mucosal immune system in the uintestinal tract: Implications for chronic inflammation. *Curr Issues Intest Microbial , 8*, 25-43.

Cohen, J., Beaver, R., Cousins, S., DeAngelis, D., Goldwasser, L., Heong, K., et al. (1993). Improving food webs. *Ecology , 74*, 252–258.

Cole, A., Tahk, S., Oren, A., Yoshioka, D., Kim, Y., Park, A., et al. (2001). Determinants of Staphylococcus aureus nasal carriage. *Clin Diagn Lab Immunol , 8*, 1064-1069.

Correa, NB., Filho, P., Luciano, A *et al*. (2005). A randomized formula controlled trial of *Bifidobacterium lactis* and *Streptococcus thermophilus* for prevention of antibiotic – associated diarrhea in infants. *J Clin Gastroenterol, 39,* 385-9.

Corthesy, B. (2007). Roadtrip ticket for secretory IgA: Role in mucosal homeostasis? *J Immunol , 178*, 27-32.

Costalos, C., Skouteri, V., Gounaris, A *et al*. (2003). Enteral feeding of premature neonates with *Saccharomyces boulardii*. *Early Hum Dev, 74*, 89-96.

Costello, E., Lauber, C., Hamady, M., Fierer, N., Gordon, J., & Knight, R. (2009). Bacterial Community Variation in Human Body Habitats Across Space and Time. *Science , 326*, 1694-1697.

Courtney, H., Hasty, D., & Dale, J. (2002). Molecular mechanisms of adhesion, colonization, and invasion of group A streptococci. *Ann Med , 34*, 77-87.

Cremonini, F., Di Caro, S., Covino, M., et al. (2002a). Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study. *Am J Gastroenterol, 97,* 2744-9.

Cremonini, F., Di Caro, S., Nistta, EC *et al*. (2002b). Meta-analysis: The effect of probiotic administration on antibiotic-associated diarrhoea. *Aliment Pharmacol Ther, 16*, 1461-7.

Crespo-Erchiga, V., & Florencio, V. (2006). Malassezia yeasts and pityriasis versicolor. *Curr Opin Infect Dis , 19*, 139-147.

Cummings, J. (1998). Dietary carbohydrates and the colonic microflora. *Curr Opin Clin Nutr Metab Care , 1*, 409-414.

Dale, B., Tao, R., Kimball, J., & Jurevic, R. (2006). Oral antimicrobial peptides and biological control of caries. *BMC Oral Health , 6 (Suppl 1)*, S13.

Dani, C., Biadaioli, R., Bertini, G *et al*. (2002). Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm neonates. A prospective double-blind study. *Biol Neonate, 82,* 103-08.

Dash, M. (2001). *Fundamentals of Ecology.* New Delhi: Tata McGraw-Hill .

Dasilao Jr, J., Rossiter, A., & Yamaoka, K. (2002). Adaptive ontogenetic shape change in flyingfish Parexocoetus mento mento. *Fish Sci , 68*, 71-76.

De Lencastre, H., & Tomasz, A. (2002). From ecological reservoir to disease: The nasopharynx, day-care centers and drug-resistant clones of Streptococcus pneumoniae. *J Antimicrob Chemother , 50 (Suppl S2)*, 75-81.

Delgado, S., Suarez, A., & Mayo, B. (2006). Identification of dominant bacteria in feces and colonic mucosa from healthy Spanish adults by culturing and by 16S rDNA sequence analysis. *Dig Dis Sci , 51*, 744-751.

Dendukuri, N., Costa, V., McGregor, M *et al*. (2005) Probiotic therapy for the prevention and treatment of *Clostridium difficile*-associated diarrhea: a systematic review. *CMAJ*, *173(2)* doi:10.1503/cmaj.050350

Deshpande, G., Rao, S., Patole, S. (2007). Probiotics for prevention of necrotising Enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. *Lancet, 369,* 1614-1620.

Dethlefsen, L., McFall-Ngai, M., & Relman, D. (2007). An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature , 449*, 811-18.

D'haens, GR., Kovacs, G., Vergauwe, P *et al.* (2007). A randomized controlled trial of the probiotic combination Lactibiane (R) in irritable bowel syndrome, the Lactibiane(R) study group. *Gastroenterology, 132 Suppl 2*, A371.

Diaz, P., Chalmers, N., Rickard, A., Kong, C., Milburn, C., Palmer, R. J., et al. (2006). Molecular characterization of subject-specific oral microflora during initial colonization of enamel. *Appl Environ Microbiol , 72*, 2837-2848.

Diggle, M., & Clarke, S. (2006). Molecular methods for the detection and characterization of Neisseria meningitidis. *Expert Rev Mol Diagn , 6*, 79-87.

Donskey, C., Hujer, A., Das, S., Pultz, N., Bonomo, R., & Rice, L. (2003). Use of denaturing gradient gel electrophoresis for analysis of the stool microbiota of hospitalized patients. *J Microbial Methods , 54*, 249-256.

D'Souza, AL., Rajkumar, C., Cooke, J *et al*. (2002). Probiotics in the prevention of antibiotic associated diarrhea: meta-analysis. *BMJ*, *324*, 1361-6.

Duncan, M. (2005). Oral microbiology and genomics. *Periodontology 2000 , 38*, 63-71.

Duncan, S., Louis, P., & Flint, H. (2007). Cultivable bacterial diversity from the human colon. *Lett Appl Microbiol , 44*, 343-350.

Dunne, J. (2009). Food Webs. In R. Meyers, *Encyclopedia of Complexity and Systems Science* (pp. 3661-3682). New York : Springer.

Dunne, J. (2006). The network structure of food webs. In M. Pascual, & J. Dunne, *Ecological Networks: Linking Structure to Dynamics in Food Webs* (pp. 27-86). New York : Oxford University Press.

Dunne, J., & Williams, R. (2009). Cascading extinctions and commuunity collapse in model food webs. *Phil Trans R Soc B , 364*, 1711-1723.

Dunne, J., Brose, U., Williams, R., & Martinez, N. (2005). Modeling Food-Web Dynamics: Complexity-Stability Implications . In A. Belgrano, U. Scharler, & J. Dunne, *Aquatic Food Webs: An Ecosystem Approach* (pp. 39-41). New York: Oxford University Press.

Dunne, J., Williams, R., & Martinez, N. (2002a). Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology Letters , 5*, 558-567.

Dunne, J., Williams, R., & Martinez, N. (2002b). Food-web structure and network theory: The role of connectance and size. *PNAS , 99* (20), 12917-12922.

Dunne, J., Williams, R., & Martinez, N. (2004). Network structure and robustness of marine food webs. *Marine Ecology Progress Series , 273*, 291-302.

Dunne, J., Williams, R., Martinez, N., Wood, R., & Erwin, D. (2008). Compilation and network analyses of Cambrian food webs. *PLoS Biology , 6*, 693-708.

Eckburg, P., Bik, E., Bernstein, C., Purdom, E., Dethlefsen, L., Sargent, M., et al. (2005). Diversity of the Human Intestinal Microbial Flora. *Science , 308*, 1635-1638.

Edwards, A., Grossman, T., & Rudney, J. (2006). Fusobacterium nucleatum transports noninvasive Streptococcus cristatus into human epithelial cells. *Infect Immun* , 654-662.

Edwards, C., & Parrett, A. (2002). Intestinal flora during the first months of life: New perspectives. *Br J Nutr , 88 (Suppl 1)*, S11-S18.

Egert, M., de Graaf, A., Smidt, H., de Vos, W., & Venema, K. (2006). Beyond diversity: Functional microbiomics of the human colon. *Trends Microbiol , 14*, 86-91.

Egger, M., Davey, SG. (1995). Misleading meta-analysis. *BMJ, 311,* 753-4.

Egger, M., Davey, SG., Schneider M *et al.* (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, *315,* 629-634.

Elahi, B., Nikfar, S., Derakhshani, S *et al*. (2007). On the Benefit of Probiotics in the Management of Pouchitis in Patients Underwent Ileal Pouch Anal Anastomosis: A Meta-analysis of Controlled Clinical Trials. *Dig Dis Sci*, *53,* 1278-1284.

Elmer, GW. (2001). Probiotics: "Living Drugs." *Am J Health Syst Pharm, 58*, 1101-9.

Elsner, P. (2006). Antimicrobials and the skin physiological and pathological flora. *Curr Probl Dermatol , 33*, 35-41.

Elson, C., & Cong, Y. (2002). Understanding immune-microbial homeostasis in the intestine. *Immunol Res , 26*, 87-94.

Enck, P., Menke, G., Zimmermann, K *et al.* (2007). Effective probiotic therapy of the irritable bowel syndrome (IBS): A multi-center clinical trial with primary care physicians. *Gastroenterology, 132 Suppl 2,* A79.

Ermis, S., Aktepe, O., Inan, U., Ozturk, F., & Altindis, M. (2004). Effect of topical dexamethasone and ciprofloxacinon bacterial flora of healthy conjunctiva. *Eye , 18*, 249-252.

Erwin, A., & Smith, A. (2007). Nontypeable Haemophilus influenzae: Understanding virulence and commensal behavior. *Trends Microbiol , 15*, 355-362.

Evans, D., McNamara, N., & Fleiszig, S. (2007). Dissecting bacterial-host interactions at the ocular surface. *Ocul Surf , 5*, 213-227.

Fierer, N., Lauber, C., Zhou, N., McDonald, D., Costello, E., & Knight, R. (2010). Forensic identification using skin bacterial communities. *PNAS , 107*, 6477-6481.

Filippo, C., Cavalieri, D., Paola, M., Ramazzotti, M., Poullet, J., Massart, S., et al. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *PNAS , 107* (33), 14691-14696.

Finegold, S. (2004). Changes in taxonomy, anaerobes associated with humans. *Anaerobe , 10*, 309-312.

Finegold, S., Ingram-Drake, L., Gee, R., Reinhardt, J., Edel- stein, M., & MacDonald, K. (1987). Bowel flora changes in humans receiving cefixime (CL 284,635) or cefaclor. *Antimicrobial Agents and Chemotherapy , 31*, 443–446.

Flint, H., Duncan, S., Scott, K., & Louis, P. (2007). Interactions and competition within the microbial communityof the human colon: Links between diet and health. *Environ Microbiol , 9*, 1101-1111.

Floch, MH., Hong-Curtiss, J. (2001). Probiotics and functional foods in gastrointestinal disorders. *Curr Gastro Rep, 3,* 343-350.

Floch, MH. (2003). Probiotics, Irritable Bowel Syndrome, and Inflammatory Bowel Disease. *Curr Treat Options Gastroenterol, 6,* 283-288.

Foster, J., Krone, S., & Forney, L. (2008). Application of Ecological Network Theory to the Human Microbiome. *Interdisciplinary Perspectives on Infectious Diseases*, *2008* 1-6.

Francisco Guarner DrMDa, C. A., & Malagelada, J. (2003). Gut flora in health and disease. *Lancet , 361* (9356), 512-519.

Frandsen, E., Pedrazolli, V., & Kilian, M. (1991). Ecology of viridians streptococci in the oral cavity and pharynx. *Oral Microbiol Immunol , 6*, 129-33.

Frank, D., Spiegelman, G., Davis, W., Wagner, E., Lyons, E., & Pace, N. (2003). Culture-independant molecular analysis of microbial constituents of the healthy human outer ear. *J Clin Microbiol , 41*, 295-303.

Fredricks, D. (2001). Microbial ecology of human skin in health and disease. *J Investig Dermatol Symp Proc , 6*, 167-169.

Fric P. (2002). Probiotics in gastroenterology. *Z Gastroenterol*, *40,* 197-201.

Fujiwara, Y., Kazahaya, Y., Nakashima, M., Sato, M., & Otsuki, S. (1987). Behavioral sensitization to methamphetamine in the rat: an ontogenic study. *Psychopharmacology , 91*, 316-319.

Fung, K., Morris, C., & Duncan, M. (2002). Mass spectrometric techniques applied to the analysis of human tears: A focus on the peptide and protein constituents. *Adv Exp Med Biol , 506*, 601-605.

Funke, G., von Graevenitz, A., Clarridge III, J., & Bernard, K. (1997). Clinical microbiology of coryneform bacteria. *Clin Microbial Rev , 10*, 125-159.

Gao, Z., Tseng, C., Pei, Z., & Blaser, M. (2007). Molecular analysis of human forearm superficial skin bacterial biota. *Proc Natl Acad Sci USA , 104*, 2927-2932.

Gade J, Thorn P. (1989). Paraghurt for the patients with irritable bowel syndrome. A controlled clinical investigation from general practice. *Scand J Prim Health Care, 7,* 23-26.

Garcia-Rodriguez, J., & Fresnadillo Martinez, M. (2002). Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother , 50 (Suppl C)*, 59-73.

Gawronska A, Dziechciarz P, Horvath A *et al.* (2007). A randomized double-blind placebo-controlled trial of *Lactobacillus* GG for abdominal pain disorders in children. *Aliment Pharmacol Ther, 25(2)*, 177-84.

Ghannoum, M., Jurevic, R., Mukherjee, P., Cui, F., Sikaroodi, M., Naqvi, A., et al. (2010). Characterization of the Oral Fungal Microbiome (Mycobiome) in Healthy Individuals. *PLOS Pathogens , 6* (1), 1-8.

Gill, S., Pop, M., DeBoy, R., Eckburg, P., Turnbaugh, P., Samuel, B., et al. (2006). Metagenomic analysis of the human distal gut microbiome. *Science , 312*, 1355-1359.

Gilmore, M., & Ferretti, J. (2003). The thin line between gut commensal and pathogen. *Science , 299*, 1999-2002.

Gionchetti P, Rizzello F, Venturi A *et al.* (2000). Oral Bacteriotheraphy as Maintenance Treatment in Patients with Chronic Pouchitis: A Double-Blind, Placebo-Controlled Trial. *Gastroenterology, 119,* 305-309.

Gionchetti P, Amadini C, Rizzello F *et al.* (2003). Probiotics for the treatment of postoperative complications following intestinal surgery. *Best Pract Res Clin Gastroenterol, 17*, 821-831.

Glück, U., & Gebbers, J. (2003). Ingested probiotics reduce nasal colonization with pathogenic bacteria (Staphylococcus aureus, Streptococcus pneumoniae, and ß-hemolytic streptococci). *Am J Clin Nutr , 77*, 517-520.

Glück, U., & Gebbers, J. (2000). The Comet Assay of Nasal Epithelia: Measurement of DNA Damage for the Assessment of Genotoxic Air Pollution. *Laryngoscope , 110*, 426-428.

Go, M. (2002). Natural history and epidemiology of Helicobacterpylori infection. *Aliment Pharma Ther , 16 (Suppl 1)*, 3-15.

Goldman CG, Barrado DA, Balcarce N *et al*. (2006). Effect of a probiotic food as an adjuvant to triple therapy for eradication of *Helicobacter pylori* infection in children. *Nutrition, 22(10),* 984-988.

Golin, V., Mimica, I., & Mimica, L. (1998). Oropharynx microbiota among alcoholics and non-alcoholics. *Sao Paulo Medical Journal , 116* (3), 1727-33.

Gonzalez, A., Clemente, J., Shade, A., Metcalf, J., Song, S., Prithiviraj, B., et al. (2011). Our Microbial selves: what ecology can teach us. *EMBO , 12*, 1-10.

Goodgame R. (2003). Emerging causes of Traveler's Diarrhea: Cryptosporidium, Cyclospora, Isospora, and Microsporidia. *Current Infectious Disease Reports, 5,* 66-73.

Gorbach SI. (1996). The discovery of *Lactobacillus GG*. *Nutr Today, 31(6 suppl),* 2-4S.

Gordon, H., & Pesti, L. (1971). The gnotobiotic animal as a tool in the study of host-microbial relationships. *Bacteriological Reviews , 35*, 390-429.

Gordts, F., Abu Nasser, I., Clement, P., Pierard, D., & Kaufman, L. (1999). Bacteriology of the middle meatus in children. *Int J Ped Otorhinolaryngol , 48*, 163-167.

Gordts, F., Halewyck, S., Pierard, D., Kaufman, L., & Clement, P. (2000). Microbiology of the middle meatus: a comparison between normal adults and children. *J Laryngol Otol , 114* (3), 184-188.

Gostner, A., Blaut, M., Schaffer, V., Kozianowski, G., Theis, S., Klingeberg, M., et al. (2006). Effect of isomalt consumption on faecal microflora and colonic metabolsm in healthy volunteers. *Br J Nutr , 95*, 40-50.

Gotz V, Romankiewicz JA, Moss J *et al*. (1979). Prophylaxis against ampicillin-associated diarrhea with a *Lactobacillus* preparation. *Am J Hosp Pharm, 36*, 754-7.

Graham, J., Moore, J., Jiru, X., Moore, J., Goodall, E., Dooley, J., et al. (2007). Ocular Pathogen or Commensal: A PCR-Based Study of Surface Bacterial Flora in Normal and Dry Eyes. *Investigative Ophthalmology & Visual Science , 48* (12), 5616-5623.

Greene, E., & Voordouw, G. (2003). Analysis of environmental microbial communities by reverse sanple genome probing. *J Microbial Methods , 53*, 211-219.

Grice, E., Kong, H., G, R., Young, A., Program, N. C., Bouffard, G., et al. (2008). A diversity profile of the human skin microbiota. *Genome Research , 18*, 1043-1050.

Gritz, D., Scott, T., Sedo, S., Cevallos, A., Margolis, T., & Whitcher, J. (1997). Ocular Flora of Patients with AIDS Compared with Those of HIV-Negative Patients. *Cornea , 16*, 400-405.

Guarner, F., & Malagelada, J. (2003). Gut flora in health and disease. *Lancet , 361*, 512-519.

Gunnnarsson, R., Holm, S., & Soderstorm, M. (1998). The prevalence of potential pathogenic bacteria in nasopharynxgeal samples from healthy children and adults. *Scad J Prim Health Care , 16*, 13-17.

Guo JB, Yang PF, Wang MT *et al*. (2004). The application of *Clostridium* to the eradication of *Helicobacter pylori*. *Chin J Celiopathy, 4,* 163-5.

Gusberti, F., Mombelli, A., Lang, N., & Minder, C. (1990). Changes in subgingival microbiota during puberty. A 4-year longitudinal study. *J Clin Periodontol , 17*, 685-692.

Guyonnet D, Chassany O, Ducrotte P *et al.* (2007). Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicenter, randomized, double-blind, controlled trial. *Aliment Pharmacol Ther, 26(3),* 475-86.

Hall, S., & Raffaelli, D. (1991). Food-Web Patterns: Lessons from a Species-Rich Web. *J. Animal Ecol , 60*, 823-842.

Halpern GM, Prindiville T, Blankenburg M *et al.* (1996). Treatment of irritable bowel syndrome with Lacteol Fort: a randomized, double-blind, cross-over trial. *Am J Gastroenterol*, 91, 1579-1585.

Harbeck, R. (2000, December). *Definition: network*. Retrieved October 2011, from Search Networking: TechTarget: http://searchnetworking.techtarget.com/definition/network

Harder, J., Gläser, R., & Schröder, J. (2007). The role and potential therapeutical applications of antimicrobial proteins in infectious and inflammatory diseases. *Endocr Metab Immune Disord Drug Targets , 7*, 75-82.

Hart AL, Stagg AJ, Kamm MA. (2003). Use of Probiotics in the treatment of Inflammatory Bowel Disease. *J Clin Gastroenterol, 36(2),* 111-119.

Hartley, M., El-Maaytah, M., McKenzie, C., & Greenman, J. (1996). The tongue microbiota of low and malodorous individuals. *Microb Ecol Health Dis , 9*, 215-223.

Hawrelak JA, Whitten DL, Myers SP. (2005). Is *Lactobacillus rhamnosus GG* Effective in Preventing the Onset of Antibiotic-Associated Diarrhoea: A Systematic Review. *Digestion, 72*, 51-56.

Hayashi, H., Takahashi, R., Nishi, T., Sakamoto, M., & Benno, Y. (2005). Molecular analysis of jejunal, ileal, caecal and recto-sigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphism. *J Med Microbiol , 54*, 1093-1101.

Heffner, J., Brown, L., & Barbieri, C. (1997). Diagnostic value of tests that discriminate between exudative and transudative pleural effusions. *Chest , 111* (4), 970-980.

Hill, M. (1998). Composition and control of ileal contents. *Eur J Cancer Prev , 7 (Suppl 2)*, S75-8.

Hill, M., & Marsh, P. (1990). *Human Microbial Ecology.* Boca Raton, Florida: CRC Press, Inc.

Hilton E, Kolakowski P, Singer C *et al.* (1997). Efficacy of *Lactobacillus* GG as a diarrheal preventive in travelers. *J Travel Med, 4,* 41-43.

Hintao, J., Teanpaisan, R., Chongsuvivatwong, V., Ratarasan, C., & Dahlen, G. (2007). The microbial profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without type 2 diabetes mellitus. *Oral Microbiol Immunol , 22*, 175-181.

Hoefer, D., & Hammer, T. (2011). Antimicrobial Active Clothes Display No Adverse Effects on the Ecological Balance of the Healthy Human Skin Microflora. *ISRN Dermatology* , 1-8.

Hokama, T., Hirayama, K., & Takamine, F. (1999). Brief report. Isolation of respiratory bacterial pathogens from the throats of healthy infants fed by different methods. *J Trop Pediatr , 45* (3), 173-176.

Holt, J., Krieg, N., Sneath, P., Staley, J., & Williams, S. (1994). *Bergey's manual of determinative bacteriology. 9th Edition.* Williams & Wilkins.

Hong, P.-Y., Lee, B., Aw, M., Shek, L., Yap, G., Chua, K., et al. (2010). Comparative Analysis of Fecal Microbiota in Infants with and without Eczema. *Plos One , 5*, 1-10.

Hood, D. (2003). The genome sequence of Haemophilus influenza. *Methods Mol Med , 71*, 147-159.

Hooper, L., & Gordon, J. (2001). Commensal Host-Bacterial Relationships in the Gut. *Science , 292*, 1115-1118.

Hopwood, D., Farrar, M., Bojar, R., & Holland, K. (2005). Microbial colonization dynamics of the axillae of an individual over an extended period. *Acta Derm Venereol , 85*, 363-364.

Hori, R., Sato, M., Kohno, S., & Hoshino, E. (1999). Tongue Microflora in Edentulous Geriatric Denture-Wearers. *Microb Ecol Health D , 11*, 89-95.

Hoveyda N, Heneghan C, Mahtani KR et al. (2009) A systematic review and meta-analysis: probiotics in the treatment of irritable bowel syndrome. *BMC Gastroenterology, 9(15)* doi: 10:1186/1471-230X-9-15.

Hu, S., Loo, J., & Wong, D. (2007). Human saliva proteome analysis. *Ann N Y Acad Sci , 1098*, 323-319.

Ings, T., Montoya, J., Bascompte, J., Bluthgen, N., Brown, L., Dormann, C., et al. (2009). Ecological networks-beyond food webs. *Journal of Anuimal Ecology , 78*, 253-269.

Iskeleli, G., Bahar, H., Eroglu, E., Torun, M., & Ozkan, S. (2005). Microbial changes in conjunctival flora with 30-day continuous-wear silicone hydrogel contact lenses. *Eye & Contact Lens , 31*, 124-126.

Isolauri E. (2001)Probiotics in human disease. *Am J Clin Nutr, 73(suppl),* 1142S-6S.

Isolauri E, Hurre A, Salminens S, Impivaara O. (2004). The allergy epidemic extends beyond the past few decades. *Clinical and experimental allergy, 34(7),* 1007-1010.

Isolauri E, Kaila M, Arvola T, et al. (1993). Diet during rotavirus enteritis affects jejunal permeability to macromolecules in suckling rats. *Pediatr Res, 33,* 548-53.

Isolauri E, Kaila M, Mykkänen H, Ling WH, Salminen S. (1994). Oral bacteriotherapy for viral gastroenteritis. *Dig Dis Sci, 39,* 2595-600.

Jaded AR, Moore RA, Carroll D *et al*. (1996). Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Controlled Clin Trials*, *17*, 1-12.

Jeong, H., Mason, S., Barabasi, A., & Oltvai, Z. (2000). Lethality and centrality in protein networks. *Nature , 411*, 41-42.

Jirapinyo P, Thamonsiri N, Densupsoontorn N *et al*. (2002). Prevention of antibiotic-associated diarrhea in infants by probiotics. *J Med Assoc Thai, 85(suppl 2*), S739-42.

Johansson, M., Molin, G., Jeppsson, B., Nobaek, S., Ahrné, S., & Bengmark, S. (1993). Administration of different Lactobacillus strains in fermented oatmeal soup: in vivo colonization of human intestinal mucosa and effect on the indigenous flora. *Appl Environ Microbiol , 59* (1), 15-20.

Johnston BC, Supina AL, Vohra Sunita. (2006) Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized placebo-controlled trials. *CMAJ, 175(4),* 377-83.

Joly-Guillou, M. (2005). Clinical impact and pathogenicity of Actinetobacter. *Clin Microbiol Infect , 11*, 868-873.

Jordano, P. (1987). Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence, and coevolution. *American Naturalist , 129*, 657-677.

Jousimies-Somer, H., Savolainen, S., & Ylikoski, J. (1989). Pharyngeal carriage of Moraxella (Branhamellai catarrhalis) in healthy adults. *J Clin Microbiol , 27*, 2736-2743.

Jung, K., Brauner, A., Kuhn, I., Flock, J., & Mollby, R. (1998). Variation of coagulase-negative staphylococci in the skin flora of healthy individuals during one year. *Microbial Ecol Health Dis , 10*, 85-90.

Kajander K, Hatakka K, Poussa T *et al.* (2005). A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. *Aliment Pharmacol Ther, 22,* 387-394.

Karalusa, R., & Campagnaria, A. (2000). Moraxella catarrhalis: A review of an important human mucosal pathogen. *Microbes Infect , 2*, 547-559.

Katelaris PH, Salam I, Farthing MJ. (1995). Lactobacilli to prevent traveler's diarrhea? *N Engl J Med. 333,* 1046-49.

Kato, T., & Hayasaka, S. (1998). Methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase-negative staphylococci from conjunctivas of preoperative patients. *Jpn J Ophthalmol , 42*, 461-465.

Kazmierczak, A., Szarapinska-Kwaszewska, J., & Szewczyk, E. (2005). Opportunistic coryneform organisms -Residents of human skin. *Pol J Microbiol , 54*, 27-35.

Kazor, C., Mitchell, P., Lee, A., Stokes, Loesche, W., Dewhirst, F., et al. (2003). Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. *J Clin Microbiol , 41*, 558-563.

Kellner, J., McGeer, A., Cetron, M., Low, D., Butler, J., Matlow, A., et al. (1998). The use of Streptococcus pneumoniae nasopharyngeal isolates from healthy children to predict features of invasive disease. *Pediatr Infect Dis J , 17*, 279-286.

Keighley MR. (1996). Review article: the management of pouchitis. *Aliment Pharmacol Ther, 10*, 449-57.

Kim HJ, Camilleri M, McKinzie S *et al.* (2003). A randomized controlled trial of a probiotic, VSL#3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther, 17*, 895-904.

Kimura, K., McCartney, A., McConnell, M., & Tannock, G. (1997). Analysis of fecal populations of bifidobacteria and lactobacilli and investigation of the immunological responses of their human hosts to the predominant strains. *Applied and Environmental Microbiology , 63*, 3394-3398.

Kirkwood, B. (2007). Normal flora of the external eye. *Insight , 32*, 12-13.

Kloos, W., Musselwhite, M., & Zimmerman, R. (1975). A comparison of the distribution of Staphylococcus species on human and animal skin. In J. Jeljaszewicz, *Staphylococci and Staphyloccal Diseases. Proceedings of III International Symposium on Syaphylococci and Staphycoccal Infections* (pp. 967-973). Stuttgart, Germany: Fischer.

Kmiot WA, Youngs D, Tudor R *et al*. (1993). Mucosal morphology, cell proliferation and faecal bacteriology in acute Pouchitis. *Br J Surg*, *80,* 1445-1449.

Knight, P., Campbell, B., & Rhodes, J. (2008). Host-bacteria interaction in inflammatory bowel disease. *Br Med Bull , 88* (1), 95-113.

Koenig, J., Spor, A., Scalfone, N., Fricker, A., Stombaugh, J., Knight, R., et al. (2011). Succession of microbial consortia in the developing infant gut microbiome. *PNAS , 108*, (Suppl 1) 4578-4585.

Konno, M., Baba, S., Mikawa, H., Hara, K., Matsumoto, F., Kaga, K., et al. (2006). Study of upper respiratory tract bacterial flora: first report. Variations in upper respiratory tract bacterial flora in patients with acute upper respiratory tract infection and healthy subjects and variations by subject age. *Journal of Infection and Chemotherapy , 12* (2), 83-96.

Könönen, E., Jousimies-Somer, H., Bryk, A., Kilp, T., & Kilian, M. (2002). Establishment of streptococci in the upper respiratory tract: Longitudinal changes in the mouth and nasopharynx up to 2 years of age. *J Med Microbiol , 51*, 723-730.

Könönen, E., Kanervo, A., Takala, A., Asikainen, S., & Jousimies-Somer, H. (1999). Establishment of Oral Anaerobes during the First Year of Life. *J Dent Res , 78*, 1634-1639.

Kosko, B., & Burgess, J. (1998). Neural Networks and Fuzzy Systems. *J. Acoust. Soc. Am. , 103* (6), 3131-3131.

Kotowska M, Albrecht P, Szajewska H. (2005). *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea in children: A randomized double-blind placebo-controlled trial. *Aliment Pharmacol Ther, 21,* 583-90.

Kozlova, I., Vanthanouvong, V., Johannesson, M., & Roomans, G. (2006). Composition of airway surface liquid determined by X-ray microanalysis. *Ups J Med Sci , 111*, 137-153.

Kreth, J., Merritt, J., Shi, W., & Qi, F. (2005). Competition and coexistance between Streptococcus mutans and Streptococcus sanguinis in the dental biofilm. *J Bacteriol , 187*, 7193-7203.

Kumar, P., Griffen, A., Barton, J., Paster, B., Moeschberger, M., & Leys, E. (2002). Molecular analysis of bacterial species associated with childhood. *J Clin Microbiol , 40*, 1001-1009.

Kumar, P., Griffen, A., Moeschberger, M., & Leys, E. (2005). Indentification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. *J Clin Microbiol , 43*, 3944-3955.

Kurata, H., Awano, H., Yoshida, A., Ansai, T., & Takehara, T. (2008). The prevalence of periodontopathogenic bacteria in saliva is linked to periodontal health status and oral malodour. *J Med Microbiol , 57*, 636-642.

Langlands, S., Hopkins, M., Coleman, N., & Cummings, J. (2004). Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. *Gut , 53*, 1610-1616.

LaRosa M, Bottaro G, Gulino N *et al*. (2003). Prevention of antibiotic–associated diarrhea with *Lactobacillus* sporogens and fructo-oligosaccarides in children. A multi-centric double-blind vs placebo study. *Minerva Pediatr, 55(5),* 447-52.

Laval, C., de Andrade, A., Pimenta, F., de Andrade, J., de Oliveira, R., Silva, S., et al. (2006). Serotypes of carriage and invasive isolates of Streptococcus pneumoniae in Brazilian children in the era of pneumococcal vaccines. *Clin Microbiol Infect , 12*, 50-55.

Lay, C., Rigottier-Gois, L., Holmstrom, K., Rajilic, M., Vaughan, E., de Vos, W., et al. (2005). Colonic microbiota signatures across five northern European countries. *Appl Environ Microbiol , 71*, 4153-4155.

Lawrence , SJ., Korzenik, JR., Mundy, LM. (2005). Probiotics for recurrent *Clostridium difficile* disease. *J Med Microbiol, 54(pt 9),* 905-6.

Lazarevic, V., Whiteson, K., Huse, S., Hernandez, D., Farinelli, L., Østerås, M., et al. (2009). Metagenomic study of the oral microbiota by illumine high-throughput sequencing. *J Microbiol Meth , 79*, 266-271.

Le Vine, A., & Whitsett, J. (2001). Pulmonary collectins and innate host defense of the lung. *Microbes Infect , 3*, 161-166.

Ledder, R., Gilbert, P., Huws, S., Aarons, L., Ashley, M., Hul, I., et al. (2007). Molecular analysis of the subgingival microbiota in health and disease. *Appl Environ Microbiol , 73*, 516-23.

Lee, W. (2008). Bacteriial-Modulated Signaling Pathways in Gut Homeostasis. *Sci Signal. 2008 , 1* (21), pe24.

Lee, Y., Yim, S., Lim, S., Choe, Y., & Ahn, K. (2006). Quantitative investigation on the distribution of Malassezia species on healthy human skin in. *Mycoses , 49*, 405-410.

LePage, P., Seksik, P., Sutren, M., de la Cochetiere, M., Jian, R., Marteau, P., et al. (2005). Biodiversity of the mucosa-associated microbiota is stable along the distal digestive tract in healthy individuals and patients with IBD. *Inflamm Bowel Dis , 11*, 478-480.

Levy, R., Huang, E., Roling, D., Leyden, J., & Margolis, D. (2003). Effect of Antibiotics on the Oropharyngeal Flora in Patients With Acne. *Arch Dermatol , 139*, 467-471.

Ley, R., Peterson, D., & Gordon, J. (2006). Ecological and Evolutionary Forces Shaping Microbial Diversity in the Human Intestine. *Cell , 124* (4), 837-848.

Leyden, J., & McGinley, K. (1992). Coryneform bacteria. In W. Nobel, *The Skin Microflora and Microbial Skin Disease* (pp. 102-117). Cambridge, UK: Cambridge University Press

Lewis SJ, Potts LF, Barry RE. (1998). The lack of therapeutic effect of *Saccharomyces boulardii* in the prevention of antibiotic-related diarrhoea in elderly patients. *J Infect, 36,* 171-4.

Li, J., Helmerhorst, E., Leone, C., Troxler, R., Yaskel, T., Haffajee, A., et al. (2004). Identification of early microbial colonizers in human dental biofilm. *J Appl Microbiol , 97*, 1311-1318.

Li, M., Wang, B., Zhang, M., Rantalainen, M., Wang, S., Zhou, H., et al. (2008). Symbiotic gut microbes modulate human metabolic phenotypes. *PNAS , 105* (6), 2117-2122.

Lin HC. (2004). Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. *JAMA, 292,* 852-858.

Lin HC, Su BH, Chen Ac *et al*. (2005). Oral probiotics reduce the incidence and severity of necrotizing Enterocolitis in very low birth weight neonates. *Pediatrics, 115,* 1-4.

Lin HC, Hsu CH, Chen HL *et al.* (2008). Oral Probiotics Prevent Necrotizing Enterocolitis in Very Low Birth Weight Preterm Infants: A Multicenter, Randomized, Controlled Trial. *Pediatrics, 122,* 693-700.

Lina, G., Boutite, F., Tristan, A., Bes, M., Etienne, J., & Vandenesch, F. (2003). Bacterial competition for human nasal cavity colonization: role of staphylococcal agr alleles. *Appl Environ Microbiol , 69*, 18-23.

Liñares, J., Pallares, R., Alonso, T., Perez, J., Ayats, J., Gudiol, F., et al. (1992). Trends in antimicrobial resistance of clinical isolates of Streptococcus pneumoniae in Bellvitge Hospital, Barcelona, Spain (1979–1990). *Clin Infect Dis , 15* (1), 99-105.

Ling WH, Korpela R, Mykkanen H, Salminen S, Hanninen O. (1994). Lactobacillus strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. *J Nutr*, *124,* 18-23.

Locatelli, C., Kwitko, S., & Simonetti, A. (2003). Conjunctival endogenous microbiota in patients submitted to cataract surgery. *Braz J Microbiol , 34* (3), 203-208.

Looijesteijn PJ, Trapet L, de Vries E, Abee T, Hugenholtz J. (2001). Physiological function of exopolysaccharides produced by *Lactococcus lactis*. *Int J Food Microbiol*, *64,* 71-80.

Lopez, R. (2006). Pneumococcus: The sugar-coated bacteria. *Int Microbiol , 9*, 179-190.

Louis, P., Scott, K., Duncan, S., & Flint, H. (2007). Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol , 102*, 1197-1208.

Lucke, K., Miehlke, S., Jacobs, E., & Schuppler, M. (2006). Prevalence of Bacteriods and Provotella spp. in ulcerative colitis. *J Med Microbiol , 55*, 614-624.

Lux, T., Nuhn, M., Hakenbeck, R., & Reichmann, P. (2007). Diversity of bacteriocins and activity spectrumin Streptococcus pneumoniae. *J Bacteriol , 189* (21), 7741-7751.

MacFarlane, S., Furrie, E., Cummings, J., & Macfarlane, G. (2004). Chemotaxonomic analysis of bacterial populations colonizing the rectale mucosa in patients with ulcerative colitis. *Clin Infect Dis , 38*, 1690-1699.

MacFarlane, S., Furrie, E., MacFarlane, G., & Dillon, J. (2007). Microbial colonization of the upper gastrointestinal tract in patients with Barrett's esophagus. *Clin Infect Dis , 45*, 29-38.

Mack, D., Davies, A., Harris, L., Rohde, H., Horstkotte, M., & Knobloch, J. (2007). Microbial interactions in Staphylococcus epidermidis biofilms. *Anal Bioanal Chem , 387*, 399-408.

Mackie, R., Sghir, A., & Gaskins, H. (1999). Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr , 69* (5), 1035S-1045S.

Magalhaes, J., Tattoli, I., & Girardin, S. (2007). The intestinal epithelial barrier: How to distinguish between the microbial flora and pathogens. *Semin Immunol , 19*, 106-115.

Mager, D., Ximenez-Fyvie, L., Haffajee, A., & Socransky, S. (2003). Distribution of selected bacterial species on intraoral surfaces. *J Clin Periodontol , 30*, 644-654.

Mahowalda, M., Rey, F., Seedorf, H., Turnbaugh, P., Fulton, R., Wollam, A., et al. (2009). Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. *PNAS , 106* (14), 5859-5864.

Mai, L., Owl, M., & Kersting, M. (2005). Ontogeny. In L. Mai, M. Owl, & M. Kersting, *The Cambridge dictionary of human biology and evolution* (p. 648). Cambridge: Cambridge Press.

Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., et al. (2006). Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut , 55*, 205-211.

Manson, J., Rauch, M., & Gilmore, M. (2008). The Commensal Microbiology of the Gastrointestinal Tract. In G. Huffnagle, & M. Noverr, *GI Microbiota and Regulation of the Immune System* (pp. 15-28). Austin, Texas: Landes Bioscience and Springer Science+ Business Media .

Manzoni P, Mostert M, Leonessa ML *et al*. (2006). Oral supplementation with *Lactobacillus casei* subspecies rhamnosus prevents enteric colonisation by *Candida* species in preterm neonates: a randomised study. *Clin Infect Dis, 42,* 1735-42.

Marchesi, J., & Shanahan, F. (2007). The normal intestinal microbiota. *Curr Opin Infect Dis , 20*, 508-513.

Marples, M. (1965). *The Ecology of the Human Skin.* Springfield, IL: Charles C. Thomas, Bannerstone House.

Marsh, P. (2006). Dental plaque as a biofilm and a microbial community- implications for health and disease. *BMC Oral Health , 6*, S14.

Marsh, P. (2005). Dental plaque: Biological significance of a biofilm and community lifestyle. *J Clin Periodontol , 32 (Suppl 6)*, 7-15.

Marshall, R. (2004). Gingival defensins: Linking the innate and adaptive immune responses to dental plaque. *Periodontology2000 , 35*, 14-20.

Marteau, P., Pochart, P., Dore, J., Bera-Maillet, C., Bernalier, A., & Corthier, G. (2001a). Comparative Study of Bacterial Groups within the Human Cecal and Fecal Microbiota. *App Enviro Microbiol , 67* (10), 4939-4942.

Marteau, PR., de Vrese, M., Cellier, CJ., Schrezenmeir, J. (2001b). Protection from gastrointestinal disease with the use of probiotics. *Am J Clin Nutr, 73,* 430S-436S.

Martijn, PG., Schouten, WR., Leo, MCL *et al.* (2004). Delay of the first onset of pouchitis by oral intake of the probiotic strain *Lactobacillus rhamnosus* GG. *Dis Colon Rectum, 47*, 876-884.

Martinez, N. (1991). Artifacts or attributes? Effects of resolution on the Little Rock Lake food web. *Ecological Monographs , 61*, 367-392.

Maupas, JL., Champemont, P., Delforge, M. (1983). Treatment of irritable bowel syndrome. Double blind trial of *Saccharomyces boulardii*. *Medecine Chirurgie Digestives, 12,* 77-79.

Mazmanian, SK., Round, JL., Kasper, DL (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature 453*, 620-625.

McFarland, LV., Surawicz, CM., Greenberg, RN., *et al*. (1994). A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *JAMA*, *271,* 1913-8.

McFarland, LV., Surawicz, CM., Greenberg, RN *et al*. (1995). Prevention of beta-lactum-associated diarrhea by *Saccharomyces boulardii* compared with placebo. *Am J Gastroenterol, 90,* 439-48.

McFarland, LV. (1998). Epidemiology, risk factors and treatments for antibiotic-associated diarrhea [review]. *Dig Dis, 16,* 292-307.

McFarland, LV. (2000). Normal flora: Diversity and functions. *Microb Ecol Health Dis , 12*, 193-207.

McFarland, LV. (2006) Meta-Analysis of Probiotics for the prevention of Antibiotic Associated Diarrhea and the Treatment of *Clostridium difficile* Disease. *Am J Gastroenterol, 101*, 812-822.

McFarland, LV., Dublin, S. (2008) Meta-analysis of probiotics for the treatment of irritable bowel syndrome. *World J Gastroenterol*, *14(17),* 2650-2661.

Memmott, J., Waser, N., & Price, M. (2004). Tolerance of pollination networks to species extinctions. *Proc. R. Soc. Lond. B , 271*, 2605-2611.

Mimura T, Rizzello F, Helwig U *et al.* (2004). Once daily high dose probiotic therapy (VSL #3) for maintaining remission in recurrent or refactory Pouchitis. *Gut*, *53,* 108-114.

Mitchell, T. (2006). Streptococcus pneumoniae: Infection, inflammation and disease. *Adv Exp Med Biol , 582*, 111-124.

Mohan, R., Koebnick, C., Schildt, J *et al*. (2006). Effects of *Bifidobacterium lactis* Bb12 supplementation on intestinal microbiota of preterm neonates: a double placebo controlled, randomised study. *J Clin Microbial 2006, 44,* 4025-31.

Montoya, J., Pimm, S., & Sole´, R. (2006). Ecological networks and their fragility. *Nature , 442*, 259-264.

Morishita, N., & Sei, Y. (2006). Microreview of pityriasis versicolor and Malassezia species. *Mycopathologia , 162*, 373-376.

Mshvildadze, M., Neu, J., & Mai, V. (2008). Intestinal microbiota development in the premature neonate: establishment of a lasting commensal relationship? *Nutrition Reviews , 66* (11), 658-663.

Mueller, S., Saunier, K., Hanisch, C., Norin, E., Alm, L., Midvedt, T., et al. (2006). Differences in fecal microbiota in different European study populations related to age, gender, and country: A cross-sectional study. *Appl Environ Microbiol , 72*, 1027-1033.

Mukundan, D., Ecevit, Z., Patel, M., Marrs, C., & Gilsdorf, J. (2007). Pharyngeal colonization dynamics of Haemophilus influenza and Haemophilus haemolyticus in healthy adult carriers. *J Clin Microbiol , 45* (10), 3207-3217.

Myllyluoma, E., Veijola, L., Ahlroos, T *et al*. (2005). Probiotic supplementation improves tolerance to *Helicobacter pylori* eradication therapy- a placebo controlled, double-blind randomized pilot study. *Aliment Pharmacol Ther, 21*,1263-72.

Nakano, K., Miyamoto, E., Tamura, K., Nemoto, H., Fujita, K., Nomura, R., et al. (2008). Distribution of 10 periodontal bacterial species in children and adolescents over a 7-year period. *Oral Dis , 14*, 658-664.

Nasidze, I., Li, J., Quinque, D., Tang, K., & Stoneking, M. (2009). Global diversity in the human salivary microbiome. *Genome Research , 19*, 636-643.

Nemergut, D., & al, e. (2011). Global patterns in the biogeography of baterial taxa. *Environ Microbiol , 13*, 135-144.

Nicholls, RJ., Banerjee, AK. (1998). Pouchitis: risk factors, etiology, and treatment. *World J Surg, 22,* 347-51.

Niedzielin, K., Kordecki, H., Birkenfeld, B. (2001). A controlled, double-blind, randomized study on the efficacy of *Lactobacillus plantarum* 299V in patients with irritable bowel syndrome. *Eur J Gastroenterol Hepatol*, *13,* 1143-1147.

Nielsen, D., Moller, P., Rosenfeldt, V., Paerregaard, A., Michaelsen, K., & Jakobsen, M. (2003). Case study of the Distribution of Mucosa- Associated Bifidobacterium Species, Lactobacillus Species, and Other Lactic Acid Bacteria in the Human Colon. *Applied and Environmental Microbiology , 69* (12), 7545-7548.

NIH HMP Working Group, P. J., Garges, S., Giovanni, M., McInnes, P., Wang, L., & al., e. (2009). The NIH Human Microbiome Project. *Genome Res , 19*, 2317-2323.

Nista, EC., Candelli, M., Cremonini, F *et al*. (2004). *Bacillus clausii* therapy to reduce side effects of anti-*Helicobacter pylori* treatment: randomized, double blinded, placebo controlled trial. *Aliment Pharmacol Ther, 20,* 1181-8.

Nobaek, S., Johansson, ML., Molin, G *et al.* (2000). Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *Am J Gastroenterol, 95,* 1231-1238.

Noble, W. (1992). Staphylococci on the skin. In W. Nobel, *The Skin Microflora and Microbial Skin Disease* (pp. 135-152). Cambridge, UK: Cambridge University Press.

O' Hara, A., & Shanahan, F. (2007). Gut microbiota: Mining for therapeutic potential. *Clin Gastroenterol Hepatol , 5*, 274-284.

O'Hara, A., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO reports , 7*, 688-693.

Oksanen, PJ., Salminen, S., Saxelin, M *et al*. (1990). Prevention of travellers' diarrhoea by *Lactobacillus* GG. *Ann Med, 22,* 53-56.

Olesen, J., & Jordano, P. (2002). Geographic patterns in plant-pollinator mutualistic network. *Ecology , 83*, 2416–2424.

Olesen, M., Bascompte, J., Dupont, Y., & Jordano, P. (2007). The modularity of pollination networks. *PNAS , 104* (50), 19891-19896.

Olson, JW., Maier, RJ. (2002). Molecular hydrogen as an energy source for *Helicobacter pylori*. *Science, 298 (5599),* 1788–90.

O'Malley, M. (2007). The nineteenth century roots of everything is everywhere. *Nat Rev Microbiol , 5*, 647-651.

Ordovas, J., & Mooser, V. (2006). Metagenomics: the role of the microbiome in cardiovascular diseases. *Current Opinion in Lipidology , 17* (2), 157-161.

Orrhage, K., Brismar, B., Nord, CE. (1994). Effects of supplements of *Bifidobacterium longum* and *Lactobacillus acidophilus* on the intestinal microbiota during administration of clindamycin. *Microb Ecol Health Dis*, *7*, 17-25.

Paine, R. (1980). Food webs: linkage, interaction strength, and community infrastructure. *Journal of Animal Ecology , 49*, 667-685.

Pajecki, D., Zilberstein, B., Santos, M., Ubriaco, J., Quintanilha, A., Cecconello, I., et al. (2002). Megaesophagus microbiota: a qualitative and quantitative analysis. *J Gastrointest Surg , 6*, 723-729.

Palmer, R., Gordon, S., Cisar, J., & Kolenbrander, P. (2003). Coaggregation-mediated interactions of streptococci and actinomycesdetected in initial human dental plaque. *J Bacteriol , 185*, 3400-3409.

Paster, B., Russell, M., Alpagot, T., Lee, A., Boches, S., Galvin, J., et al. (2002). Bacterial Diversity in Necrotizing Ulcerative Periodontitis in HIV-Positive Subjects. *Annals of Periodontology , 7* (1), 8-16.

Patil, J., & Chopade, B. (2001). Studies on bioemulsifier production by Acinetobacter strains isolated from healthy human skin. *Journal of Applied Microbiology , 91*, 290-298.

Paulino, L., Tseng, C., Strober, B., & Blaser, M. (2006). Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. *J Clin Microbiol , 44*, 2933-2941.

Pei, Z., Bini, E., Yang, L., Zhou, M., Francois, F., & Balser, M. (2004). Bacterial biota in the human distal esophagus. *Proc Natl Acad Sci USA , 101*, 4250-4255.

Percival, R. (2009). Changes in Oral Microflora and Host Defences with Advanced Age. In S. Perceival, *In Microbiology and Aging: Clinical Manifestations.* (pp. 131-152). New York, USA: Springer Science+Business Media.

Perin, NM., Clandinin, MT., Thomson, ABR. (1997). Importance of milk and diet on the ontogeny and adaptation of the intestine. *J pediatr Gastroenterol Nutr, 24*, 419-25.

Perkins, R., Kundsin, R., Pratt, M., Abrahamsen, I., & Leibowitz, H. (1975). Bacteriology of normal and infected conjunctiva. *J CLin Microbiol , 1*, 147-149.

Perry, A., & Lambert, P. (2006). Propionibacterium acnes. *Lett Appl Microbiol , 42*, 185-188.

Pessi T, Sütas Y, Saxelin M, Kallionen H, Isolauri E. (1999) Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. *Appl Environ Microbiol, 65,* 4725-8.

Pivetaeu, P., Condon, S, Cogan, TM. (2000). Inability of diary propionibacteria to grow in milk from low inocula. *J Dairy Res*, *67,* 65-71.

Plummer S, Weaver MA, Harris JC *et al*. (2004). *Clostridium difficile* pilot study: Effects of probiotic supplementation on the incidence of *Clostridium difficile* diarrhoea. *Int Microbiol, 7,* 59-62.

Pochapin, M. (2000). The effect of probiotics on *Clostridium difficile* diarrhea. *Am J Gastroenterol, 95*, S11-13.

Pozo- Olano, JD., Warram, JH., Gomez, RG *et al.* (1978). Effect of a Lactobacillus preparation on traveller's diarrhea. *Gastroenterology*, *74,* 829-30.

Preza, D., Olsen, I., Willumsen, T., Boches, S., Cotton, S., Grinde, B., et al. (2009). Microarray analysis of the microflora of root caries in elderly. *Eur J Clin Microbiol Infect Dis , 28*, 509-517.

Qamar, A., Aboudola, S., Warny, M., et al. (2001) *Saccromyces boulardii* stimulates intestinal immunoglobulin A immune response to *Clostridium difficile* toxin A in mice. *Infect Immun, 69,* 2762-5.

Rajilić-Stojanović, M., Smidt, H., & De Vos, W. (2007). Diversity of the human gastrointestinal tract microbiota revisited. *Environmental Microbiology , 9* (9), 2125–2136.

Ramberg, P., Sekino, S., Uzel, N., Socransky, S., & Lindhe, J. (2003). Bacterial colonization during the novo plaque formation. *J Clin Periodontol , 30*, 990-995.

Randell, S., & Boucher, R. (2006). effective mucus clearance is essential for respiratory health. *Am J Respir Cell Mol Biol , 35*, 20-28.

Rasiah, I., Wong, L., Anderson, S., & Sissons, C. (2005). Variation in bacterial DGGE patterns from human saliva: over time, between individuals and in corresponding dental plaque microcosms. *Arch Oral Biol , 50*, 779-787.

Rasmussen, T., Kirkeby, L., Poulsen, K., Reinholdt, J., & Kilian, M. (2000). Resident aerobic microbiota of the adult human nasal cavity. *APMIS , 108*, 663-675.

Rath HC. (2003). The role of endogenous bacterial flora: bystander or the necessary prerequisite? *Eur J Gasrtroenterol Hepatol*, *15,* 615-620.

Robinson, C., Strafford, S., Rees, G., Brookes, S., Kirkham, J., Shore, R., et al. (2006). Plaque biofilms: The effect of chemical environment on natural human plaque biofilm architecture. *Arch Oral Biol , 51*, 1006-1014.

Roediger, W. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *GUT , 21* (9), 793-798.

Romanuk, T., Zhou, Y., Brose, U., Berlow, E., Williams, R., & Martinez, N. (2009). Predicting invasion success in complex ecological networks. *Philosophical Transactions of the Royal Society B , 364*, 1743-1754.

Roopnarine, P. (2006). Extinction cascades and catastrophe in ancient food webs. *Paleobiology , 32* (1), 1-19.

Roopnarine, P., Angielczyk, K., Wang, S., & Hertog, R. (2007). Trophic network models explain instability of Early Triassic terrestrial communities. *Proc. R. Soc. B , 274* (1622), 2077-2086.

Rose, M., & Voynow, J. (2006). Respiratory tract mucin genes and mucin glycoproteinsin health and disease. *Physiol Rev , 86*, 245-278.

Rosen, T. (2007). The propionibacterium acnes genome: From the labratory to the clinic. *J Drugs Dermatol , 6*, 582-586.

Rothenberg, R., Potterat, J., Woodhouse, D., Muth, S., Darrow, W., & Klovdahl, A. (1998a). Social network dynamics and HIV transmission. *AIDS , 12* (12), 1529-1536.

Rothenberg, R., Sterk, C., Toomey, K., Potterat, J., Johnson, D., Schrader, M., et al. (1998b). Using Social Network and Ethnographic Tools to Evaluate Syphilis Transmission. *Sexually Transmitted Diseases , 25* (3), 154–160.

Rougé C, Piloquet H, Butel MJ *et al.* (2009). Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double blind, placebo-controlled trial. *Am J Clin Nutr, 89(6),* 1828-1835.

Round, JL., O'Connell, RM., Mazmanian, SK. (2010). Coordination of tolerogenic immune responses by the commensal microbiota. *Journal of Autoimmunity, 34(3)*, 220-225.

Rubio, E. (2004). Climatic influence on conjunctival bacteria of patients undergoing cataract surgery. *Eye , 18*, 778-784.

Rudney, J., Chen, R., & Sedgewick, G. (2005). Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, and Tannerella forsythensis are components of a polymicrobial intracellular flora within human buccal cells. *J Dent Res , 84*, 59-63.

Ruhl, S., Sandburg, A., & Cisar, J. (2004). Salivary receptors for the proline-rich protein-binding and lectin-like adhesions of oral actinomyces and streptococci. *J Dent Res , 83*, 505-510.

Ruseler-van Embden, JG., Schouten, WR., van Lieshout, LM. (1994). Pouchitis: result of microbial imbalance? *Gut, 35*, 658-64.

Ruszczynski, M., Radzikowski, A., Szajewska, H. (2008). Clinical trial: effectiveness of *Lactobacillus rhamnosus* (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. *Aliment Pharmacol Ther*, *28,* 154-161.

Saarela, M., Lahteenmaki, L., Crittenden, R., Salminen, S., Mattila-Sandholm, T. (2002). Gut bacteria and health foods-the European perspective. *Int J Food Microbiol*, *78,* 99-117.

Saavedra, JM., Bauman, NA., Oung, I., Perman, JA., Yolken, RH. (1994). Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet, 344,* 1046-9.

Saavedra JM. (2001). Clinical applications of probiotic agents. *Am J Clin Nutr*, *73,* 1147S-1151S.

Saez Nieto, J., Marcos, C., & Vindel, A. (1998). Multicolonization of human nasopharynx due to Neisseria spp. *Int Microbiol , 1*, 59-63.

Sakaguchi, T., Kohler, H., Gu, X., McCormick, BA., Reinecker, HC. (2002). Shigella flexneri regulates tight junction-associated proteins in human intestinal epithelial cells. *Cell Microbiol*, *4,* 367-381.

Sakamoto, M., Takeuchi, Y., Umeda, M., Ishikawa, I., & Benno, Y. (2003). Application of terminal RFLP analysis to characterize oral bacterial flora in saliva of healthy subjects and patients with periodontitis. *J Med Microbiol , 52*, 79-89.

Sakamoto, M., Umeda, M., & Benno, Y. (2005). Molecular analysis of human oral microbiota. *J Periodont Res , 40*, 277-285.

Salminen, S., Bouley, C., Boutron-Ruault, MC., et al. (1998). Functional food science and gastrointestinal physiology and function. *Br J Nutr, 80(suppl),* S147-71.

Salminen, S., & Isolauri, E. (2006). Intestinal colonization, micrbiota, and probiotics. *J Pediatr , 149*, S115-S120.

Samanta, M., Sarkar, M., Ghosh, P *et al*. (2009). Prophylactic Probiotics for Prevention of Necrotizing Enterocolitis in Very Low Birth Weight Newborns. *J Trop Pediatr, 55(2),* 128-131.

Samaranayake, L. (2006). *Normal oral flora, the oral ecosystem and plaque biofilm. Essential microbiology for dentistry.* Philadelphia : Elsevier.

Sandborn, WJ. (1994). Pouchitis following ileal pouch-anal anastomosis: definition, pathogenesis, and treatment. *Gastroenterology, 107,* 1856-60.

Sanderson, IR., Walker, WA. (1993) Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterology, 104,* 622-39.

Santosa, S., Farnworth, E., Jones, P. (2006). Probiotics and Their Potential Health Claims. *Nutrition Reviews, 64(6),* 265-274.

Sartor, RB. (1995). Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. *Gastroenterol Clin North Am*, *24,* 475-507.

Sazawal, S., Hiremath, G., Dhingra, U, et al. (2006) Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. *Lancet Infect Dis, 6,* 374-82.

Savage, D. (1977). Microbial ecology of the gastrointestinal tract. *Ann Rev Med , 31*, 107-133.

Sen, S., Mullan, MM., Parker, TJ et al. (2002). Effect of Lactobacillus plantarum 299v on coloic fermentation and symptoms of irritable bowel syndrome. *Dig Dis Sci*, *47,* 2615-2620.

Seki, H., Shiohara, M., Matsumura, T *et al*. (2003). Prevention of antibiotic-associated diarrhea in children by *Clostridium butyricum* MIYAIRI. *Pediatr Int, 45*, 86-90.

Sheu, BS., Wu, JJ., Lo, CY *et al*. (2002). Impact of supplement with *Lactobacillus*- and *Bifidobacterium*- containing yogurt on triple therapy for *Helicobacter pylori* eradication. *Aliment Pharmacol Ther, 16,* 1669-75.

Simon, SL., Gorbach, SL. (1986). The human intestinal microflora. *Dig Dis Sci, 31(suppl),*147S-62S.

Simren, M., Syrous, A., Lindh, A *et al.* (2006). Effects of *Lactobacillus plantarum* 299v on symptoms and rectal sensitivity in patients with irritable bowel syndrome (IBS) - A randomized, double-blind controlled trial. *Gastroenterology, 130 Suppl 2*, A600.

Simren, M., Lindh, A., Sammelsson, L *et al.* (2007). Effect of yoghurt containing three probiotic bacteria in patients with irritable bowel syndrome(IBS)- A randomized, double-blind, controlled trial. *Gastroenterology, 132 Suppl 2*, A210.

Singer, T., Isenberg, S., & L, A. (1988). Conjunctival anaerobic and aerobic bacterial flora in paediatric versus adult subjects. *Br J Ophthalmol , 72*, 448-451.

Sistrom, CL., Garvan, CW. (2004). Proportions, odds, and risk. *Radiology*, *230(1),* 12–9.

Skinner, F., & Carr, J. (1974). *The Normal Microbial Flora of Man.* London, UK: Academic Press.

Slots, J. (1977). The predominant cultivable microflora of advanced periodontitis . *Scand J Dent Res , 85*, 114–121.

Snyder, L., Davies, J., Ryan, C., & Saunders, N. (2005). Comparitative overview of the genomic and genetic differences between the pathogenic Neisseria strains and species. *Plasmid , 54*, 191-208.

Sodini, I., Latrille, E., Corrieu, G. (2000). Identification of interacting mixed cultures of lactic acid bacteria by their exclusion from a model predicting the acidifying activity of non-interacting mixed cultures. *Appl Microbiol Biotechnol*, *54,* 715-718.

Solé, R., & Montoya, M. (2001). Complexity and fragility in ecological networks. *Proc. R. Soc. Lond. , 268* (1480), 2039-2045.

Soriano, F., & Rodriguez-Cerrato, V. (2002). Pharmacodynamic and kinetic basis for the selection of pneumococcal resistance in the upper respiratory tract. *J Antimicrob Chemother , 50 (Suppl 3)*, 51-58.

Soudakoff, P. (1954). Bacteriologic examination of the conjunctiva; a survey on 3,000 patients. *Am J Ophthalmol , 38* (3), 374-376.

Soutar, A., Seaton, A., Brown, K. (1997) Bronchial reactivity and dietary antioxidants. *Thorax, 52,* 166-70.

Srinivasan, R., Reddy, R., Rene, S., Kanungo, R., & Natarajan, M. (1999). Bacterial contamination of anterior chamber during IOL surgery. *Indian J Ophthalmol , 47*, 185-189.

Srinivasan, U., Dunne, J., Harte, J., & Martinez, N. (2007). Response of complex food webs to realistic extinction sequences. *Ecology , 88*, 671-682.

Staley, J., & Konopka, A. (1985). Measurement of in Situ Activities of Nonphotosynthetic Microorganisms in Aquatic and Terrestrial Habitats. *Annual Review of Microbiology , 39*, 321-346.

Stapleton, F., Willcox, M., Fleming, C., Hickson, S., Sweeney, D., & Holden, B. (1995). Changes to the ocular biota with time in extended- and daily-wear disposable contact lens use. *Infect. Immun. , 63* (11), 4501-4505.

Stjernquist-Desatnik, A., & Holst, E. (1999). Tonsillar microbial flora: Comparison of recurrent tonsillitis and normal tonsils. *Acta Oto-Laryngol (Stockholm) , 119*, 102-106.

Strogatz, S. (2001). Exploring complex networks. *Nature , 410*, 268-276.

Suau, A., Bonnet, R., Sutren, M., & Godon, J. (1999). Direct Analysis of Genes Encoding 16S rRNA from Complex Communities Reveals Many Novel Molecular Species within the Human Gut. *Applied and Environmental Microbiology , 65* (11), 4799-4807.

Sugita, T., Suto, H., Unno, T., Tsuboi, R., Ogawa, H., Shinoda, T., et al. (2001). Molecular analysis of Malassezia microflora on the skin of atopic dermatitis patients and healthy subjects. *J Clin Microbiol , 39*, 3486-3490.

Sullivana, A., Törnblomb, H., Lindbergb, G., Hammarlundb, B., Palmgrena, A., Einarssonb, C., et al. (2003). The micro-flora of the small bowel in health and disease. *Anaerobe , 9* (1), 11-14.

Suntharalingam, P., & Cvitkovitch, D. (2005). Quorum sensing in streptococcal biofilm formation. *Trends Microbiol , 13*, 3-6.

Surawicz, CM., Elmer, GW., Speelman, P *et al*. (1989). Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardii*- A prospective-study. *Gastroenterology, 90,* 981-8.

Surawicz, CM., McFarland, LV., Greenberg, RN *et al*. (2000). The search for a better treatment of the recurrent *Clostridium difficile* disease: Use of high-dose vancomycin combined with *Saccharomyces boulardii*. *Clin Infect Dis, 31*:1012-7.

Sütas, Y., Soppi, E., Korhonen, H., et al. (1996) Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolysed with *Lactobacillus* GG-derived enzymes. *J Allergy Clin Immunol, 98,* 216-24.

Suzuki, N., Yoshida, A., & Nakano, Y. (2005). Quantitative analysis of multi-species oral biofilms by TaqMan real-time PCR. *Clin Medicine Res , 3*, 176-185.

Sykora, J., Valeckova, K., Amlerova, J *et al*. (2005). Effects of a specially designed fermented milk product containing probiotic *Lactobacillus casei* DN-114 001 an the eradication of *H. pylori* in children: a prospective randomized double-blind study. *J Clin Gastroenterol, 39,* 692-8.

Szajewska H, Kotowska M, Mrukowicz JZ *et al*. (2001). Efficacy of *Lactobacillus GG* in prevention of nosocomial diarrhea in infants. *J Pediatr 138*:361-5.

Tagg, R., & Dierksen, K. (2003). Bacterial replacement therapy: adapting 'germ warfare' to infection prevention. *Trends in Biotechnology , 21* (5), 217-223.

Tankanow, RM., Ross, MB., Ertel, IJ *et al*. (1990). A double-blind placebo-controlled study of the efficacy of Latinex in the prophylaxis of amoxicillin-induced diarrhea. *DICP, 24,* 382-4.

Tanner, A., Paster, B., Lu, S., Kanasi, E., Kent, R., Jr. Van Duke, T., et al. (2006). Subgingival and tongue microbiota during early periodontitis. *J Dent Res , 85*, 318-323.

Tannock, GW. (2002). The bifidobacterial and Lactobacillus microflora of humans. *Clin Rev Allergy Immunol , 22*, 231-253.

Tannock, GW. (1997). Probiotic properties of lactic-acid bacteria: plenty of scope for fundamental R & D. *Trends Biotechnol*, *15,* 270-4.

Tannock, GW. (1999). *Medical Importance ot the Normal Microflora.* Dordrecht: Kluwer Academic Publishers.

Tauch, A., Kaiser, O., Hain, T., Goesmann, A., Weisshaar, B., Albersmeier, A., et al. (2005). Complete genome sequence and analysis of the multiresistant nosocomial pathogen Corynebacterium jeikeium K411, a lipid-requiring bacterium of the human skin flora. *J Bacteriol , 187*, 4671-4682.

Taylor, D., Daulby, A., Grimshaw, S., James, G., Mercer, J., & Vaziri, S. (2003). Characterization of the microflora of the human axilla. *International Journal of Cosmetic Science , 25* (3), 137-145.

Theilade, E., Fejerskov, O., Karring, T., & Theilade, J. (1982). Predominant cultivable micro-flora of human dental fissure plaque. *Infect Immun , 36*, 977-982.

Thomas, MR., Litin, SC., Osmon, DR *et al*. (2001). Lack of effect of *Lactobacillus* GG on antibiotic-associated diarrhea: A randomized, placebo-controlled trial. *Mayo Clin Proc, 76,* 883-9.

Thompson-Chagoyan, O., Maldonado, J., & Gil, A. (2007). Colonization and impact of disease and other factors on intestinal microbiota. *Dig Dis Sci , 52*, 2069-2077.

Thompson, WG. (1986). Irritable bowel syndrome: prevalence, prognosis and consequences. *CMAJ*, 134, 111-113.

Timmerman, HM., Koning, CJM., Mulder, L., Rombouts, FM., Beynen, AC. (2004). Monostrain, multistrain and multispecies probiotics- A comparison of functionality and efficacy. *International J Food Nutr*, *96*, 219-233.

Tlaskalová-Hogenová, H., Štˇepánková, R., Hudcovic, T., Tuˇcková, L., Cukrowska, B., Lodinová-Žádn´ıková, R., et al. (2004). Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunol Lett , 93*, 97-108.

Tong, JL., Ran, ZH., Shen, J., et al. (2007) Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during *Helicobacter pylori* eradication therapy. *Aliment Pharmacol Ther, 25(2),* 155-68.

Travis, S., Singh, P., & Welsh, M. (2001). Anti-microbial peptides and proteins in the innate defense of the airwayu surface. *Curr Opin Immunol , 13*, 89-95.

Trotter, C., Gay, N., & Edmunds, W. (2006). The natural history of meningococcal carriage and disease. *Epidemoil Infect , 134*, 556-566.

Tuohy, K., Pinart-Gilberga, M., Jones, M., Hoyles, L., McCartney, A., & Gibson, G. (2007). Survivability of a probiotic Lactobacillus casei in the gastrointestinal tract of healthy human volunteers and its impact on the faecal microflora. *J Appl Microbiol , 102*, 1026-1032.

Turnbaugh, P., Hamady, M., Yatsunenko, T., Cantarel, B., Duncan, A., Ley, R., et al. (2009). A core gut microbiome in obese and lean twins. *Nature , 457* (22), 480-484.

Turnbaugh, P., Ley, R., Hamady, M., Fraser-Liggett, C., Knight, R., & Gordon, J. (2007). The Human Microbiome Project. *Nature , 449* (7164), 804.

Ueta, M., Iida, T., Sakamoto, M., Sotozono, C., Takahashi, J., Kojima, K., et al. (2007). Polyclonality of Staphylococcus epidermis residing on the healthy ocular surface. *J Med Microbiol* (56), 77-82.

van Belkum, A. (2006). Staphylococcal colonization and infection: Homeostasis verses disbalance of human (innate) immunity and bacterial virulence. *Curr Opin Infect Dis , 19*, 339-344.

Vanderhoof, JA., Whitney, DB., Antonson, DL., et al. (1999). *Lactobacillus* GG in the prevention of anti-biotic associated diarrhea in children. *J Pediatr, 135,* 564-8.

Vanderhoof, JA. (2000). Probiotics and intestinal inflammatory disorders in infants and children. *J Pediatr Gastroenterol Nutr, 30(suppl 2),* S34-S38.

Van Neil, CW., Feudtner, C., Garrison, MM., Christakis, DA. (2002). Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics, 109,* 678-684.

Van Neil, C. (2005). Probiotics Not Just for Treatment Anymore. *Pediatrics*, *115*, 174-177.

Vazquez, A., Flammini, A., Maritan, A., & Vespignani, A. (2003). Global protein function prediction in protein-protein interaction networks. *Nature Biotech , 21*, 697-700.

Verdu, EF, Collins, SM. (2004). Microbial-gut interactions in health and disease: irritable bowel syndrome. *Best Pract Res Clin Gastroenterol*, *18,* 315-321.

Verduin, C., Hol, C. F., van Dijk, H., & van Belkum, A. (2002). Moraxella catarrhalis: From emerging to established pathogen. *Clin Microbiol Rev , 15*, 125-144.

Villadsen, J., Neilsen, J., & Linden, G. (2011). Biochemical reaction networks. In J. Villadsen, J. Neilsen, & G. Linden, *Bioreaction Engineering Principles: Third Edition* (pp. 151-212). New York: Springer .

von Graevenitz, A., Pünter-Streit, V., Riegel, P., & Funke, G. (1998). Coryneform bacteria in throat cultures of healthy individuals. *J Clin Microbiol , 1998*, 2087-2088.

von Graevenitz, A., Schumacher, U., & Bernauer, W. (2001). The corynebacterial flora of the normal human conjunctiva is lipophilic. *Curr Microbiol , 42*, 372-374.

Wang, H., Wang, Y., Chen, J., Zhan, Z., Li, Y., & Xu, J. (2007). Oral yeast flora and its ITS sequence diversity amoung a large cohort of medicine students in Hainan, China. *Mycopathologia , 164*, 65-72.

Wang, M., Ahrne, S., Jeppsson, B., & Molin, G. (2005). Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. *FEMS Microbial Ecol , 54*, 219-231.

Wang, X., Heazlewood, S., Krause, D., & Florin, T. (2003). Molecular characterization of the microbial species that colonize human ileal and colonic mucosa by using 16S rDNA sequence analysis. *J Appl Microbiol , 95*, 508-520.

Waterland, R., & Jirtle, R. (2004). Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition , 20*, 63-68.

Watts, D. (2002). A simple model of global cascades on random networks. *Proc Nat Acad Sci USA , 99*, 5766-5771.

Watts, D., & Strogatz, S. (1998). Collective dynamics of small world networks. *Nature , 393*, 440-442.

Webster, G. (2007). Skin microecology: The old and the new. *Arch Dermatol , 143*, 105-106.

Weizman, Z., Asli, G., Alsheikh, A. (2005). Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. *Pediatrics*, *115,* 5-9.

Wenus, C., Goll, R., Loken, EB *et al*. (2008). Prevention of antibiotic-associated diarrhea by a fermented probiotic milk drink. *Eur J of Clin Nutr*, *62,* 299-301.

Wertheim, H., Melles, D., Vos, M., van Leeuwen, W., van Belkum, A., Verbrugh, H., et al. (2005). The role of nasal carriage in Staphylococcus aureus infections. *Lancet Infect Dis , 5*, 751-762.

Whorwell, PJ., Altringer, L., Morel, J *et al.* (2006). Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. Am *J Gastroenterol, 101,* 1581-1590.

Williams, R., & Martinez, N. (2008). Success and its limits among structural models of complex food webs. *Journal of Animal Ecology , 77*, 512-519.

Williams, R., Berlow, E., JA, D., Barabási, A., & ND, M. (2002). Two degrees of separation in complex food webs. *Proceedings of the National Academy of Sciences USA , 99*, 12913-12916.

Willliams, R., & Martinez, N. (2000). Simple rules yield complex food webs. *Nature , 404*, 180-183.

Wilson, M. (2008). *Bacteriology of Humans: An Ecological Perspective.* Oxford, UK: Blackwell Publishing.

Wilson, M. (2005). *Microbial Inhabitants of Humans; Their Ecology and Role in Health and Disease.* Cambridge, UK: Cambridge University Press.

Witsell, DL., Garrett, G., Yarbrough, WG *et al*. (1995). Effect of *Lactobacillus acidophilus* on antibiotic-associated gastrointestinal morbidity: A prospective randomized trial. *J Otolarynogol, 24,* 231-3.

Woodmansey, E. (2007). Intestinal bacteria and ageing. *J Appl Microbiol , 102*, 1178-1186.

Wult, M., Hagslatt, ML., Odenholt, I. (2003). *Lactobacillus plantarum* 299v for the treatment of recurrent *Clostridium difficile*-associated diarrhoea: A double-blind, placebo-controlled trial. *Scand J Infect Dis, 2003, 35*:365-7.

Wunderlich, PF., Braun, L., Furmagalli, I *et al*. (1989). Double-blind report on the efficacy of lactic acid-producing *Enterococcus* SF68 in the prevention of antibiotic-associated diarrhoea and in the treatment of acute diarrhoea. *J Int Med Res, 17,* 333-8.

Ximenez-Fyvie, L., Haffajee, A., & Socransky, S. (2000). Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. *J Clin Periodontol* , 648-657.

Xu, J., Mahowald, M., Ley, R., Lozupone, C., Hamady, M., Martens, E., et al. (2007). Evolution of symbiotic bacteria in the distal human intestine. *Plos Biol , 5*, e156.

Yamauchi, Y., Minoda, H., Yokoi, K., Maruyama, K., Kumakura, S., Usui, M., et al. (2005). Conjunctival Flora in Patients with Human Immunodeficiency Virus Infection. *Ocular Immunology and Inflammation , 13* (4), 301-304.

Ylikoski, J., Savolainen, S., & Jousimies-Somer, H. (1989). The bacteriology of acute maxillary sinusitis. *ORL J Otorhinolaryngol Relat Spec , 51*, 50-55.

Zhao, H., Jumblatt, J., Wood, T., & Jumblatt, M. (2001). Quantification of MUC5AC protein in human tears. *Cornea , 20*, 873-877.

Zilberstein, B., Quintanilha, A., Santos, M., Pajecki, D., Moura, E., Alves, P., et al. (2007). Digestive tract microbiota in healthy volunteers. *Clinics , 62*, 47-54.

Zoetendal, E., Ben-Amor, K., Harmsen, H., Schut, F., Akkermans, A., & de Vos, W. (2002). Quantification of Uncultured Ruminococcus obeum-Like Bacteria in Human Fecal Samples by Fluorescent In Situ Hybridization and Flow Cytometry Using 16S rRNA-Targeted Probes. *Appl Environ Microbiol , 68* (9), 4225-4232.

Zoetendal, E., Rajilic-Stojanovic, M., & de Vos, W. (2008). High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. *Gut , 57*, 16.5-1615.

Zoetendal, E., Rajilić-Stojanović, M., & de Vos, W. (2008). High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. *Gut , 57*, 1605-1615.

Zoetendal, E., Von Wright, A., Vilpponen-Salmela, T., Ben-Amor, K., Akkermans, A., & de Vos, W. (2002). Mucosa-Associated Bacteria in the Human Gastrointestinal Tract are Uniformly Distributed along the Colon and Differ from the Community Recoved from Feces. *App Environ Microbiol , 68* (7), 3401-3407.