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Review article

Is Trehalose a Symbiotic Determinant in Symbioses Between Higher Plants and Microorganisms?

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Abstract

The common microbial metabolite trehalose is often found in the symbiotic organs of higher plants. Trehalose may be toxic for host cells unable to defend themselves by inducing trehalase activity. Trehalose may therefore be an early, broad-spectrum and/or unspecific agent preventing symbioses between susceptible plants and trehalose-producing microorganisms (bacteria and fungi).

Keywords: Angiosperms, symbiosis, trehalase, trehalose toxicity

1. Introduction

In a recent review Quispel (1988), states "The formation of a (symbiosis) is the consequence of a sequence of mutual interactions between host plant and microsymbiont. These interactions depend on the activities of many genes both on the bacterial and the plant side". Whilst Quispel goes on to discuss the molecular biology of the endophytic phase of nitrogen-fixing symbioses, I would like to go one step backward from the same starting point and consider if there may be some general properties of plants determining their interactions with microsymbionts. In particular this paper deals with the occurrence of the disaccharide trehalose in many symbiotic systems. My hypothesis can be reduced to the following:

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1. Whilst trehalose is a common sugar in insects, fungi and bacteria, it is uncommon in higher plants and indeed toxic for several plants.
2. Trehalose can be released from alive or dying cells of microorganisms and thus:
3. Only trehalose-tolerant plants can enter into close associations with trehalose-producing organisms.

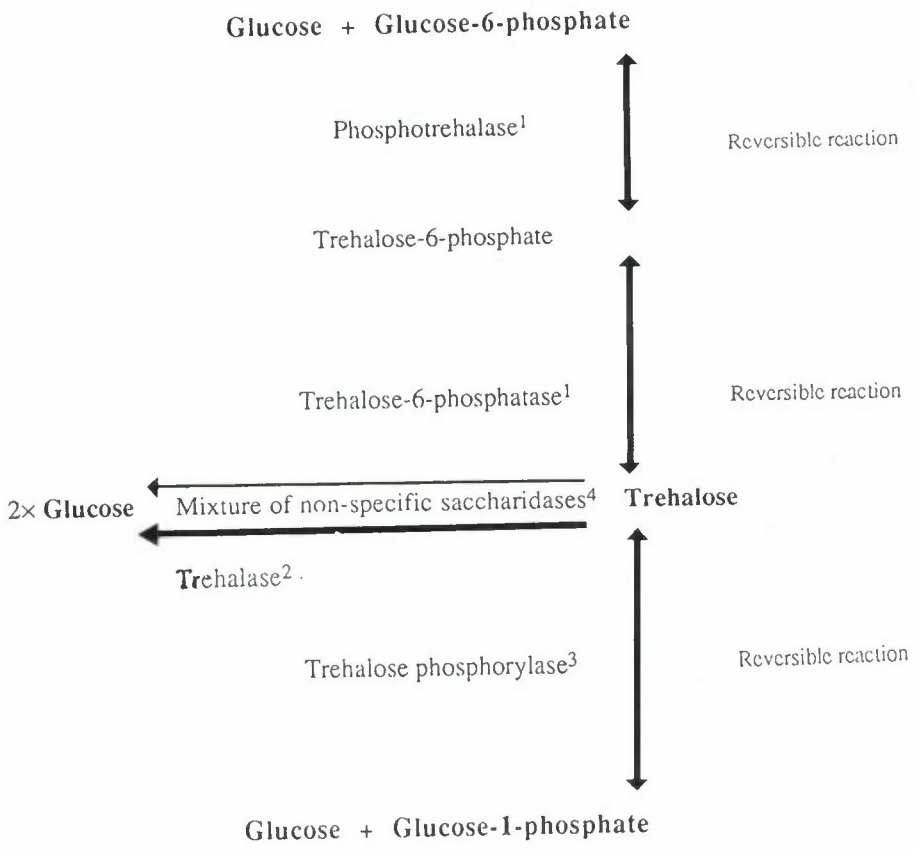
In this paper the scattered and sparse references to trehalose have been gathered together and reviewed in a symbiotic light.

2. The Occurrence of Trehalose

Trehalose (alpha-D-Glucopyranosyl-(1-1)alpha-Glucopyranoside) was first isolated in 1832 (Wiggen, 1832) from Ergot, Since then it has been discovered in a wide variety of lower organisms including bacteria and cyanobacteria, red algae, liverworts, some lower vascular plants, fungi (including yeasts), insects, crustaceans, nematodes and anilids (reviewed by Elbein, 1974). In the vascular plants as a whole, trehalose is a rare sugar. It occurs in the pteridophyte lesser clubmosses, in the leaves of several eusporangiate ferns and in the ripening fruits of several members of the *Apiaceae* (Kandler and Hopf, 1980). Where trehalose occurs in vascular plants, it often exceeds sucrose in concentration and indeed appears to replace sucrose as translocated disaccharide (Lewis, 1984; Arnold, 1968). With the exception of the above, trehalose has not been reproducibly identified in angiosperms (Gussin, 1972; Brocklebank and Hendry, 1989). One report on trehalose in the cambial sap of beech (Oesch and Meyer, 1967, demented by Gussin, 1972) was probably due to mycorrhization (see section 3). In dramatic contrast to the situation in plants alone, trehalose is a very common sugar in plant symbioses (see section 3). The action of trehalose on higher plant cells is not well understood. Wagner et al. (1986) report that trehalose induces sucrose-sucrose fructosyltransferase in barley leaves, but no fructan synthesis took place, whereupon the cells rapidly died. Veluthambi et al. (1981) correlated the ability of plants to survive trehalose treatment with a largely inducible trehalase activity. Plants unable to raise enzyme levels sufficiently, blackened and died, predominantly at the sites of elongation growth. Although calli of some plants could grow on trehalose, to an extent proportional to the cellular trehalase, death rapidly occurred when trehalase inhibitors were included in the medium (Veluthambi et al., 1981).

3. The Occurrence of Trehalase (Trehalose-1-Glucohydrolase)

It should be briefly mentioned at this point that the addition of trehalose to a raw extract and measuring the glucose produced is not sufficient evidence for trehalase (EC 3.2.1.28) activity. As Fig. 1 shows, trehalose can be hydrolysed in a variety of ways. This has probably led to some confusion in the earlier literature, especially with respect to prokaryotes (e.g. Crabbe, 1969; Guilloux, 1971).



Occurrence: [1]Trehalose uptake in bacteria; [2]Plants, fungi, animals; [3]Euglena; [4]Detected in Frankia, a source of artifacts.

Figure 1. Pathways of trehalose hydrolysis

The status of trehalase in the bacterium *Escherichia coli* is "entirely unclear" (Boos et al., 1987). What is however clear, is that *E. coli* can take up trehalose, after phosphorylation, *via* a phosphotransferase-mediated transport system, followed by immediate hydrolysis to glucose and glucose 6 phosphate (Boos et al., 1987; Giaever et al., 1988). Largely similar situations are found in *Salmonella typhimurium* (Postma et al., 1986) and *Bacillus popilliae* (Bhumiratana et al., 1974). It is also clear that rhizobia can grow on (Glenn and Dilworth, 1981) and accumulate (Streeter, 1985) trehalose. The uptake mechanism in rhizobia is unknown. The accumulation of trehalose in the bacterial cytoplasm argues against the presence of trehalase there. Bacteroids contain no trehalase activity (Mellor, 1988). Using cultured *Frankia*, Lopez and Torrey (1985) reported on a soluble trehalase activity with pH optimum 5.0 and commented that an unresolved mixture of alpha glucosidases could also hydrolyse trehalose. There are also reports of trehalase from *Streptomyces hygroscopicus* (Hey-Herguson et al., 1973; Hey and Elbein, 1968) and *S. antibioticus* (Brana et al., 1986).

In the filamentous fungi, trehalase has been reported from a wide variety of sources. These include: *Aspergillus niger* (Ng et al., 1974), *A. oryzae* (Horikoshi and Ikeda, 1966), *Aurobasidium pullulans* (Catley and Kelley, 1975), *Corprinus* (Rao and Niederpruem, 1969), *Humicola languginosa* (Prasad and Maheshwari, 1978), *Mucor rouxii* (van Laere and Slegers, 1987) and *Phycomyces blakesleeanus* (van Laere and Hendrix, 1983). The yeasts include *Schizosaccharomyces pombe* (Inove and Shimoda, 1981) and *Saccharomyces cereviceae* (Londesborough and Varimo, 1984). In bakers yeast, the best studied system, two trehalases are present. In the vacuole is a trehalase with an acid (4.0) pH optimum. The enzyme is highly glycosylated (86% of the molecular mass of 218 kDa is sugar) and is inhibited by acetate. The Km for trehalose is 4.7 mM. The second trehalase has a neutral pH optimum, a molecular mass of 86 KDa and is inhibited by Zn^{++} and EDTA. The Km of the cytoplasmic enzyme for trehalose is around 5 mM but it can be activated several-fold by cAMP-dependent phosphorylation (Dellamora-Ortiz et al., 1986).

Several membrane-associated trehalases have been reported from a range of animal sources as diverse as rabbit kidney (Sacktor, 1968), the labial glands of ants (Paulsen, 1971), bee (Lefebre and Huber, 1970), moth (Gussin and Wyatt, 1965), cockroach (Gilby et al., 1967) and shrimp (Hand and Carpenter, 1986). One early review is by Friedman (1966).

In the alga *Euglena gracilis*, trehalase is not present. Trehalose degradation is achieved by trehalose phosphorylase (Marechal and Belocopitow, 1972).

In the higher plants trehalase occupies a quixotic position insofar as where present, its substrate is absent (except in symbiotic organs, see section 3), a similar situation to that of chitinase (Boller, 1986). Trehalase activity has been reported from sugar cane (Glasziou and Gayler, 1969; Alexander, 1973; Fleischmacher et al., 1980). Trehalase is also an established pollen enzyme in *Lilium* (Gussin and McCormack, 1970), *Lycopersicon, Hermerocallis, Glatonia, Carmellia* and *Lathyrus* (Gussin et al., 1969). The pollen enzyme is similar to soya nodule enzyme (see later) with a broad pH optimum (5± 2), km for trehalose of about 1 mM and an optimum temperature of about 50°C. Veluthambi et al. (1981) report on trehalase activity in *Lemna, Nicotina, Datura, Daucus, Glycine, Zea, Raphanus* and *Quamoclit.* Trehalase activity was low in *Phaseolus* primary leaves and practically undetectable in *Cuscuta.*

4. Trehalose and Trehalase in Symbioses

Cyanobacterial symbioses

Although cyanobacterial symbioses are actually outside the scope of this article, and data are very sparse, they should briefly be considered here for the sake of completeness. Data on the mechanism of trehalose uptake and/or synthesis in cyanobacteria, as well as trehalose in symbiotic organs and trehalase in host organisms, are lacking. Trehalose is considered to be an osmoprotectant in many cyanobacteria (e.g. Reed et al., 1984). Table 1 summarizes data taken from a more thorough work (Mackay et al., 1984) and relates this to symbiotic properties of cyanobacteria. Care should be taken in interpreting these data since the strains assayed by Mackay et al. (1984) were isolated from

Table 1. Trehalose accumulation in cyanobacterial genera

Genus	Symbiotic properties	Trehalose
Anabaena	Exosymbiont (*Azolla*[1])	–
Calothrix	Microsymbiont (*Rhizosolena*[2]) association (*Sargassum*[3])	+
Nostoc	Endosymbiont (*Gunnera*[4]) Microsymbiont (cyanolichens[5]) Exosymbiont (cycads[6])	+
Chamasiphon	None	+
Dermocarpa	None	–
Gloeotheae	None	+
Synechococcus	None	–

Data rearranged from Mackay et al. (1984).
References: [1]Newton and Herman, 1979, [2]Pascher, 1929, [3]Carpenter and Fox, 1974, [4]Bonnett, 1989, [5]Rai, 1989, [6]Lindblad and Bergman, 1989.

a wide range of habitats and should not be presumed to be identical with symbiotic strains. It can be seen from Table 1 that, although some non-symbiotic genera accumulated trehalose, the greatest similarity can be observed between members of trehalose-accumulating genera and genera where members enter into close symbiotic associations (for review see Rao, 1989). One exception is *Anabaena*, but in this case the corresponding symbiosis i.e. with the water fern *Azolla* (Peters and Mayne, 1974) is not an endosymbiosis. The interesting question is, do the strains living in symbiosis with plants accumulate trehalose, as do the strains studied and can this trehalose then be released and diffuse to the host organism, as is proven for the symbioses (*Paramecium, Hydra*) with the green alga *Chlorella* (Pardy et al., 1989)? A further question is, then, does this trehalose induce a trehalase in the host?

The Actinorhiza

In the actinomycetes (Benson, 1988), isolates cannot grow on a wide range of hexoses, pentoses, disaccharides and trisaccharides (Stowers et al., 1986; Tisa et al., 1983) thus metabolic studies which tend to focus on C-sources for nitrogen fixation, have concentrated on organic acids (e.g. Akkermans et al., 1981). *Frankia*, however, accumulates glycogen as storage carbohydrate (Benson and Eveleigh, 1979) and also trehalose (Lopez et al., 1983, 1984). The amount of trehalose in *Frankia* is inversely correlated with nitrogen fixation (for similar results in the *Rhizobium* nodule system, see Streeter and Salminen, 1988) although Lopez and Torrey (1985) explain this as reduced trehalose synthesis during times of nitrogen fixation. Actinorhizal nodules contain large amounts of sucrose. Sucrose and fructose are able to sustain nitrogen fixation in intact nodule slices (Lopez and Torrey, 1985). The status of trehalose in nodules and of host trehalase, is unknown.

Rhizobial symbioses

In rhizobial soya nodules, sucrose and trehalose are the dominant carbohydrates. Amounts up to 2 mg trehalose per gram nodule fresh weight have been found (Streeter and Salminen, 1988). This trehalose is confined to the nodule (Streeter, 1981). This is also the case in nodules of *Phaseolus vulgaris, Pisum sativum, Archis hypogea, Medicago sativa, Trifolium repens, Lotus corniculatus* (Streeter, 1985) and *Sesbania rostrata* (unpublished observations). Thus amongst the Leguminaceae, members of the Papilionaceae and Caesalpiniaceae (Mimosaceae not tested) have trehalose-containing nodules. Amongst the Rhizobiaceae, trehalose accumulates in associations involving *Azorhizobium, Bradyrhizobium* and *Rhizobium (Agrobacterium* and

Phyllobacterium not tested). Carbon for trehalose in nodules is provided by plant photosynthate and enters the nodule as sucrose (Reibach and Streeter, 1983; Kouchi and Yoneyama, 1986). Trehalose synthesis takes place only in the bacteroids (Salminen and Streeter, 1986; Streeter, 1985). The major portion of this trehalose is, however, released and can be found again in the host cell cytoplasm (Streeter, 1987). The infected regions of nodules also contain large amounts of trehalase (Salminen and Streeter, 1986; Streeter, 1982). This trehalase is probably a host enzyme since it is found mostly in the host cell soluble fraction, whereas *Bradyrhizobium* bacteroids contain little (Salminen and Streeter, 1986) or no (Mellor, 1988; Hoelzle and Streeter, 1989) trehalase, although cultured *Rhizobium* and *Agrobacterium* spp. contain trehalose-splitting activities (Hoelzle and Streeter, 1989). It is also known that trehalase is induced in soya exposed to trehalose in the absence of rhizobia (Veluthambi et al., 1981). Interestingly, if legumes express trehalase in response to trehalose and the trehalose is only found in nodules, then trehalase may fulfill the definition of a nodulin (Legocki and Verma, 1980).

The Mycorrhiza

The mycorrhizal symbioses (Smith and Gianinazzi-Pearson, 1988) are the most common symbioses amongst vascular plants (Newman and Reddell, 1987). Excised ascomycete ectomycorrhizas have been shown to convert glucose and fructose into the "fungus specific" metabolites trehalose and mannitol (Lewis and Harley, 1965; Harley and Smith, 1983; Martin et al., 1985; Martin et al., 1988). Indeed mycorrhizas consume between 4% and 12% of total host photosynthate (see Harris et al., 1985). Trehalose can leak back to the plant host from ectomycorrhizas, as evidenced by Lewis and Harley (1965) and by Niederer et al. (1989), who also found trehalose concentrations in roots to be proportional to the degree of mycorrhization. Roots of soya (*Glycine max*) infected with the vesicular-arbuscular mycorrhiza fungus *Glomus mosseae* display raised levels of trehalose and trehalase (Dr. P. Wyss, University of Basel, personal communication). It is also likely in this case, that fungal trehalose can enter the plant tissue, due to the very intimate nature of this endosymbiosis, where cell-cell contact is much closer than in the ectomycorrhizas (Marx et al., 1982; Bonfante-Fasolo, 1987). Indeed, the vesicular-arbuscular mycorrhizae are often compared with rhizobial symbioses (e.g. Wyss et al., 1990).

Whereas the vesicular-arbuscular mycorrhizas (Order Endogonates) are obligate symbionts on the plant host, this situation is reversed in the mycotropic Orchids, where the plant partner is dependent on mycorrhizal fungi, mostly Basidiomycetes, for growth (at least in the early developmental stages,

Alexander and Hadley, 1985). In this system, carbon movement is from the fungal mycelium to the plant (Alexander and Hadley, 1985). Carbon sources are taken up by the fungal mycelium and converted into mannitol and trehalose. This carbon then reappears in the plant as glucose, fructose and sucrose (Hadley, 1984). The implication of this, that the plant (in this case *Goodyera repens*) possesses an active trehalase, has been indirectly confirmed by Ernst et al. (1971) who reported that non-symbiotic *Phalaenopsis* plants could develop on media containing trehalose as single carbon source.

Pathogenic interactions

The concept that microorganisms on or in plants leak trehalose into the host tissue is further supported by evidence involving pathogenesis. Keen and Williams (1969) found trehalose in tissue of *Brassica oleracea* infected with *Plasmodiophora brassicae*. Long and Cooke (1971) found a similar situation in leaves of *Senecio squalidus* infected with *Albugo tragopogonis*.

5. Is There a Role for Trehalose in Symbiosis?

The production of substances toxic to plants by microbes has been known for many years (Erdman et al., 1956; Wheeler and Luke, 1963). The best-known microbial toxin produced in symbiosis is rhizobitoxin, a substance inducing chlorosis in certain susceptible varieties of soybean (Owens and Wright, 1965a,b). The production of rhizobitoxin appears to play no role in promoting the symbiosis, since neither partner can be expected to benefit when the host is ill. We should not rule out that trehalose is a similar, almost incidental compound, whose biological relevance is simply in preventing stable interactions with susceptible (non-trehalase producing) hosts. On the other hand, it could also be speculated that trehalose plays a hitherto unsuspected role in symbioses. Before, however, considering a unique role for trehalose in symbioses, one should first consider roles postulated for trehalose in non-symbiotic systems. These are:

- an agent preventing phagolysosome fusion in host cells (Hohman et al., 1982)
- a reserve or storage form of reduced carbon (Sturgeion, 1985)
- a help in thermotolerance (Grba et al., 1975, 1979) in resistance to water stesss, e.g. dessication (Martin et al., 1986), osmoregulation (Mackay et al., 1984), or stabilization of biological structures (Crowe et al, 1984a,b,c).

Considered in a symbiotic light, the first alternative, inhibition of phagolysosome fusion, seems attractive due to recent findings that bacteroids in the *Rhizobium*-legume symbiosis (and therefore presumably in other endosymbioses) inhabit a lytic compartment (for review see Mellor, 1989). Surely it must be an advantage for the endosymbiont to prevent host primary lysosomes from fusing with the compartment which it inhabits. The realization, however, that the microsymbiont inhabits a lytic compartment, is based upon the presence of lysosomal enzymes in the perisymbiontic space (the space between the microsymbiont outer membrane and the delimiting host membrane). Following the figures of Lin et al. (1988), infected cells account for about 40% of the total volume of a nodule. Assuming central vacuoles make up an average of 85% of the volume of uninfected and cortex cells, then the peribacteroid space makes up about 28% of the total lytic compartment of a nodule. Mellor et al. (1984) and Kinnback et al. (1987) reported that 12% of the total nodular alpha-mannosidase occurs in the peribacteroid space and that this is of the vacuolar type, isoenzyme II, which accounts for 48% of the total nodular alpha-mannosidase activity. These two sets of figures allow the amount of vacuolar enzyme in the peribacteroid space to be calculated as a percent of the total activity in an infected cell. This rough integration indicates that over 85% of the total isoenzyme II in an infected cell is located in the peribacteroid space. Thus it appears that the fusion between primary lysosomes and the compartment around the endosymbiont has not been significantly inhibited.

Trehalose is regarded as a reserve carbon source in microorganisms (e.g. Sturgeon, 1985), a view repeated so often that it is worth a closer examination. Wilkinson (1959) defined reserve carbon sources as substances which (a) accumulate in times of excess carbon, (b) are mobilized in times of carbon shortage, and (c) lead directly to energy production. Lille and Pringle (1980), working with yeast, showed that trehalose production starts only when the medium carbon is practically exhausted. Similar results have been published with *Bradyrhizobium* (Streeter, 1985). Grba et al. (1975, 1979) show further that, in yeast, trehalose production is dependent on incubation temperature, independently of carbon status. Hoelzle and Streeter (1990) show furthermore that trehalose production in *Rhizobium leguminosarum* is independent of carbon status but influenced by oxygen status. Barton et al. (1982) germinated spores of *Pichia pastoris* (which contain 23% dry weight trehalose) on glucose. Trehalose was rapidly lost whilst the medium glucose was used catabolically. A similar situation was observed by van Laere et al. (1987) with *Phycomyces blakesleeanus* and by van Laere and Slegers (1987) with *Mucor rouxii*, the trehalose was not used for metabolism but was rapidly degraded to glycerin and lost in the medium. The cellular localization of trehalose, in free-living

organisms and symbioses, is the cytoplasm, which is also at variance with the normal situation for reserve substances. Although trehalose accumulates in rhizobia, this accumulation varies between strains, but the strains able to accumulate large amounts are not the strains most persistent in soils (compare Streeter, 1985 with Keyser et al., 1984). Trehalose also represents a relatively small carbon pool in rhizobia in comparison to glycogen and poly-beta-hydroxy butyrate (Streeter, 1985). Since the growth rates of legume tissue cultures on trehalose are relatively poor in comparison to rates on sucrose (Veluthambi et al., 1981 and Mellor, unpublished observations), it appears that compelling arguments exist against a role for trehalose as simple carbon reserve for either partner, at least in the *Rhizobium*-legume symbiosis.

A direct role for trehalose in thermotolerance seems to be rather at odds with the physical conditions occurring in symbioses, where extreme temperatures are an exception rather than the rule.

Trehalose is especially common in the dormant stages of several organisms (e.g. Clegg and Filosa, 1961) or in organisms uncommonly resistant to storage and dessication. Trehalose is an osmoregulator in bacteria (Larson et al., 1987). Trehalose can stabilize membranes and proteins *in vivo* and *in vitro* (Crowe et al., 1984a,b,c, 1985). Glucose-6-phosphate dehydrogenase can be stabilized *in vivo* and *in vitro* by trehalose as can glutamate dehydrogenase and the restriction endonuclease EcoR1 *in vitro* (Hottiger, 1988). The question must therefore be posed: Do conditions of water stress occur in endosymbioses? This is an intriguing question since the concentration of biologically free water seems not to have been determined in any symbiosis. Thus the question of water stress remains open.

In conclusion, the information presently available (sections 1, 2 and 3) support the hypothesis outlined in the introduction, although clearly much more work remains to be done before this view can be confirmed or rejected. An active role for trehalose in symbioses is presently unknown. From the role for trehalose in nature generally, only the role of protectant for membranes, proteins or general biological structure, against water stress, stands a closer examination.

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