Symbiosis, 9 (1990) 179–184
Balaban Publishers, Philadelphia/Rehovot



# What is the Role of Levansucrase in *Bacillus subtilis*?

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**Abstract**

*B. subtilis* possesses two metabolic pathways for sucrose and a complex constellation of regulators involved in the control of their expression. The constellation includes genes which are involved in the induction by sucrose of saccharolytic enzymes and the *deg* genes which have pleiotropic effects. The function of both these sets of regulators is beginning to be understood but the reasons of this complexity are unknown. A speculative interpretation is proposed.

## I. Introduction

The sucrose metabolism in *B. subtilis* is rather original because it involves two pathways and a very complex constellation of regulatory genes. Today, these genes have been cloned and one begins to understand how this constellation functions. In this paper, we would briefly present this system but also discuss the physiological significance of its complexity.

## II. Two Metabolic Pathways and two Induction Systems

The two pathways are shown in Fig. 1. The intracellular one, consisting of a PTS-dependent permease specific for sucrose ($II^{suc}$ encoded by *sacP*) and an intracellular sucrase (encoded by *sacA*) is fully induced in the presence of low concentrations of sucrose ($\geqslant 1$ mM) and repressed by glucose. Levansucrase (LS, encoded by *sacB*) is a secreted transfructosylase involved in the external pathway. LS is synthetized at its maximal level in the presence of high sucrose concentrations ($\geqslant 30$ mM) (Lepesant et al., 1976).

The regulatory elements involved in the induction of *sacB* were identified. *sacRt* is a sucrose-dependent conditional terminator located between the *sacB* promoter and

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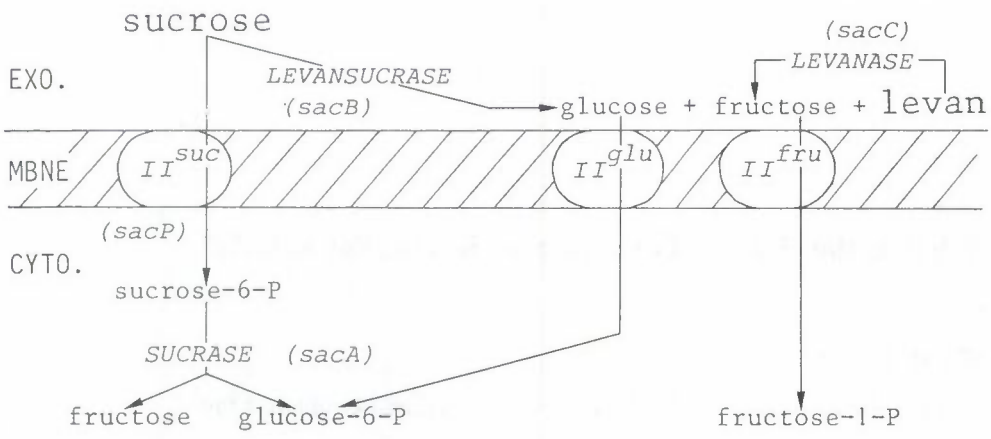


Figure 1. The sucrose metabolism in *B. subtilis*. MBNE: cell membrane. $II^{suc}$, $II^{glu}$, etc...: PTS-dependent permease specific for sucrose, glucose, etc... respectively. The extracellular levanase (*sacC*) is not inducible by sucrose.

the coding sequence (Shimotsu and Henner 1986). Both sucrose and the product of the *sacY* gene are required to prevent transcription termination at *sacRt* (Aymerich and Steinmetz 1987). *sacB* is constitutively expressed in mutants carrying a *sacX* null mutation: SacY and SacX would be respectively the *sacRt* specific antiterminator and the sucrose sensor controlling the antiterminator (Crutz et al., 1989). Various results indicated that an homologous but distinct regulatory system would control the sucrase induction by sucrose (Fig. 2) (Steinmetz et al., 1989, Débarbouillé: pers. com.).

## III. The DEG Genes

The *degS-U* locus (formerly *sacU*) was identified by pleiotropic mutations affecting the expression of LS and several degradative enzymes unrelated to the sucrose metabolism, including proteases (both intra- and extracellular species), amylase, etc... The *degS-U*(−) and the *degS-U*(Hy) alleles decreased and increased, respectively, the *sacB* expression. The *degS-U*(Hy) mutants are devoid of flagella, poorly transformable, and can sporulate in the presence of glucose, which inhibits sporulation in the wild type (Kunst et al., 1974). As shown recently by Kunst (1988) and Henner (1988) this locus contains two ORFs designated *degS* and *degU* which have similarities to two widespread families of proteins that mediate responses to environmental stimuli. (For a review on these systems see Bourret et al., 1989).

At least two genes unlinked to *degS-U*, *degQ* and *degR* are involved in this system. The overproduction of the small polypeptides encoded by these genes results in a phenotype similar to that of the *degS-U*(Hy) mutants (Yang et al., 1986; Nagami and Tanaka 1986).

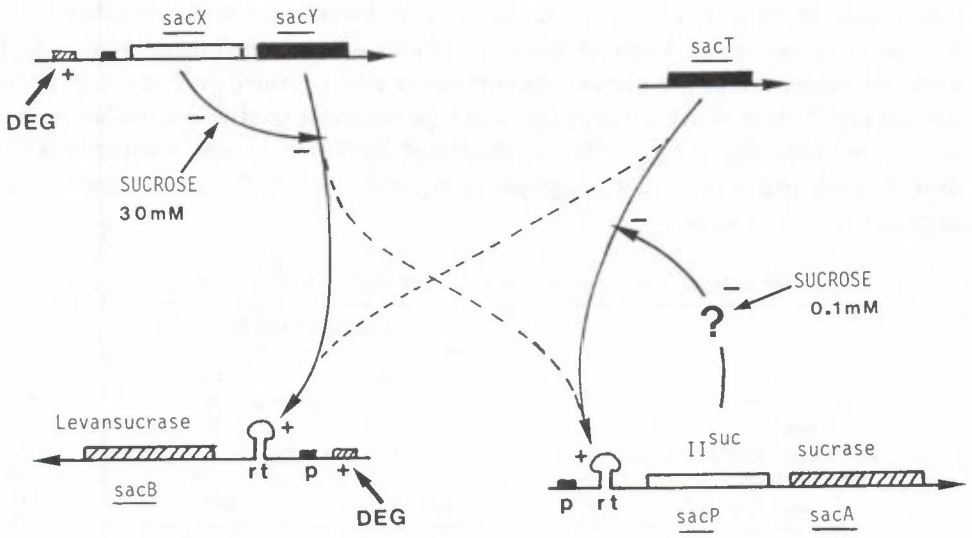


Figure 2. Regulation of *sacB* and *sacP-A*. The transcription of both *sacB* and *sacX-Y* is controlled by the Deg regulators (see text section III). Each antiterminator (SacY and SacT) is relatively specific for one regulatory terminator (rt), but a double cross-talk seems to exist (dotted lines). SacX likely inhibits SacY. The inhibition of SacT by SacP is an hypothesis suggested by analogy.

A target for the regulation by the *deg* genes is present upstream from the *sacB* promoter (Aymerich et al., 1986). By measure of the expression of a *sacY'-lacZ* transcriptional fusion in the wild type and a *degU*(Hy) mutant we recently showed that DegU also controlled the *sacY* transcription. It shows that DegU exerts a coordinate positive control on *sacB* at two levels (unpublished results; Fig. 2).

Henner (1988) underlined that the only link between the different Deg-dependent genes was that they encoded degradative enzymes. However, LS is both degradative and anabolic. On the other hand, the intracellular pathway of sucrose, which involves the products of the *sacP-A* operon (Fig. 1), is clearly degradative but not Deg-dependent. This operon and most of the Deg-dependent genes are catabolite repressed, but *sacB* is not (Lepesant et al., 1976). Levanase is clearly degradative but not Deg-dependent (Kunst et al., 1977). It seems therefore difficult to interpret the Deg regulatory system as an equivalent of the *E. coli* super-regulons involved in response to nutrient stresses.

## IV. What is the role of Levansucrase and of the DEG Regulators?

The role of levan is unknown. However, LS functions in the extracellular medium, with two important consequences: (i) the population which secretes LS competes for the benefit of LS products with other inhabitants of its niche; (ii) this population

cannot take levan with it but must use it in place. Therefore it could be valuable for *B. subtilis* to synthesize levan in some conditions and not in others, even if high sucrose concentrations are present. As previously noticed, the contribution of LS to sucrose metabolism is negligible in the wild type strain, at least under the laboratory conditions (Lepesant et al., 1976). As illustrated by Table 1, both sucrose and the *degU32* allele are required for a significant expression of *sacB*: sucrose alone is not sufficient to induce *sacB*.

Table 1. Saccharolytic activities in *deg*+ and *degU32* strains

| | Saccharolytic activity | |
|---|---|---|
| | Sucrase | LS |
| *deg*$^+$ | 0.3 | 0.03 |
| *deg*$^+$ plus 2% sucrose | 15 | 1 |
| *degU32* | 0.3 | 0.5 |
| *degU32* plus 2% sucrose | not determined | 100 |

We hypothesize that the Deg regulators recognize and transmit an additional inducer of *sacB*, required to synthesize levan when it is valuable for the cell. We would like to propose a scenario where levan plays a "structural role".

Extracellular polysaccharides (EPSs) play structural roles in various colonization or infection processes. Buccal *Streptococci* use sucrose to synthesize various EPSs allowing attachment to teeth (Rölla 1989). EPSs are involved in infection of plants by *Rhizobium* and *Agrobacterium* (Glazebrook and Walker 1989; Robertson et al., 1988). Most *Bacilli* are soil bacteria and preferential association were observed between some of these bacteria and plant rhizospheres. In Europe, LS producing strains of *B. polymyxa* and *B. circulans* are found in the rhizosphere of wheat and corn, respectively. The strains associated with wheat specifically prevent the growth of the wheat pathogen fungus, *Gaeumannomyces graminis* var. *tritici* (Berge and Heulin, personal communication). It was shown that the wheat root exudes sucrose (Hess et al., 1986). This costly process could be the price paid by the plant for the association. Regulatory systems such as the Deg system might be involved in the specificity of such an association.

This scenario is largely speculative and several others might be proposed. Our purpose was to remember that *B. subtilis* is a soil bacterium and to show one among various plausible roles which must be considered for the Deg regulators.

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