Instability of protease production in a rel\(^+\)/rel\(^-\)-pair of Bacillus licheniformis and associated morphological and physiological characteristics

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Abstract. A naturally occurring relaxed/protease-producing (A-type) versus stringent/not protease-producing (B-type) pair of an industrial Bacillus licheniformis has been characterized; either of the two types can convert into the other. Both types can sporulate, grow anaerobically, grow at 56\(^\circ\)C and reduce nitrate; morphologically, they can easily be distinguished by cell- and colony-shape. They differ in the ability to use 12 substrates, as determined in API-tests. The two types are remarkably different in their content of extrachromosomal elements (A-type: 2; B-type: 4); furthermore, they differ in their rel-status (A-type: relaxed; B-type: stringent). We propose that the differences in the ability of the two types to use different substrates probably are due to integration/extrusion of the extrachromosomal elements in and out of the chromosome, distorting or restoring a number of genes, together with induction of certain catabolic genes that are under control of the rel-system.

Introduction

The genus Bacillus is well-known for its production of extracellular enzymes of industrial importance, such as amylases, proteases and penicillinases (Aunstrup 1980; Priest 1984). In bacilli, mutations affecting the production and/or secretion of extracellular enzymes have been observed to have a pleiotropic effect. For instance, sacU\(^h\) mutants of Bacillus subtilis were shown to hyperproduce levansucrase with concomitant alterations in some cell-wall associated functions that led to morphological changes (Ayusawa et al. 1975; Chambert & Petit-Glatron 1984). A number of similar, pleiotropic effects was described for amyB- and pap mutations that caused overproduction of amylase and protease (Yoneda & Maruo 1975). Steinmetz et al. (1976) showed the unity of some of these mutations (i.e. sacU\(^h\), amyB and pap-9), thus somewhat limiting the number of loci attributed to pleiotropic effects. Apart from these
rather well-defined mutations, pleiotropic effects were also found as a consequence of storage and handling conditions (Nehete et al. 1985). Together with the observations on an essential role for the proton motive force in export of proteins (Muren & Randall 1985; Tai 1986), the regulation of protein transport is clearly associated with membrane-/cell-wall-functions, that, not surprising, will lead to morphological differences between producing cells and their non-producing derivatives.

While studying the production of an alkaline serine protease by an industrial *Bacillus licheniformis* strain, Frankena et al. (1985) noted the appearance of an aberrant phenotype. The new phenotype was designated as type B, the parent strain as type A. Some evidence was presented that, although different in a number of aspects, the A-type indeed arises from the B-type and vice versa. Here, we extend this evidence somewhat further and show what characterizes this naturally occurring relaxed/protease-producing versus stringent/not protease-producing pair of *Bacillus licheniformis*. A possible cellular background for the observed differences will be discussed.

**Methods**

*Organisms and growth conditions*

*Bacillus licheniformis* S1684 is a spore-forming, alkaline serine protease producing strain, supplied by Gist-brocades N.V. at Delft, The Netherlands; this strain is referred to as the A-type. The spontaneously occurring other type (Frankena et al. 1985) is referred to as the B-type. *Escherichia coli* CP 78 (*rel*⁺, *arg*H, *leu*, *his*, *thr*, *thia*) and *Escherichia coli* NF 859 (*relA*⁺, *relX*⁺, *spoT*⁺, *arg*, *met*) have been described elsewhere (Cashel 1969; resp. Gallant et al. 1977). *Bacillus licheniformis* was maintained on malt-extract peptone-agar plates (MP) and periodically transferred; fresh inocula were taken from spore-suspensions (stocked at −20°C in 30% glycerol) every four to five months. *Escherichia coli* strains were maintained on brain-heart infusion broth plates. In all experiments (except with radio-active labelling; see below), *Bacillus licheniformis* was grown in the minimal medium as described by Frankena et al. (1985), while *Escherichia coli*-strains were grown in the basal medium as described by van Verseveld et al. (1984), supplemented with 1 ml per liter of a trace element solution as described by Light & Garland (1971). For *Escherichia coli* CP 78, L-arg, L-leu, L-his, D and L-thr were added up to 100 mg per liter each, thiamine up to 50 mg per l. For *Escherichia coli* NF 859, 100 mg L-arg and 100 mg L-met were added per l. Growth proceeded at 37°C. Batch cultures and chemostats were run as described by Frankena et al. (1985), fed-batch cultures and recycling fermentor as described by van Verseveld et al.