NEWS AND VIEWS/NUUS EN MENINGS

DegS and *degU* operon from *Bacillus brevis*: a combination that enhances the production of commercially valuable enzymes
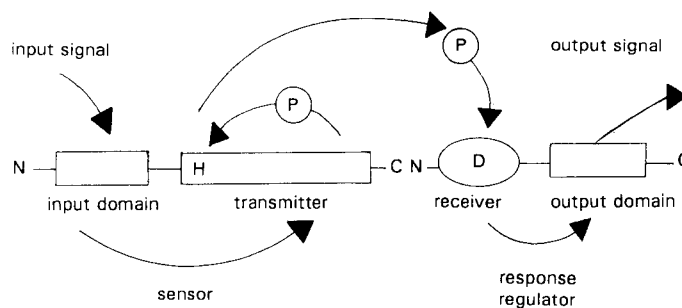
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A novel method has been developed for increasing the production of commercially valuable enzymes, such as proteases, β-glucanases, α-amylases and levansucrase. It is dependent on two genes cloned from Bacillus brevis, expressed on a multicopy vector and then transformed into a strain of B. subtilis. This work holds promise particularly for multi-enzyme industrial processes such as in the biotreatment of organic waste.

Bacillus as a soil bacterium has developed many regulatory mechanisms in order to adapt to environmental changes, as growth in the environment is very often limited by the availability of basic nutrients. Requirements for bacterial survival include a continuous monitoring of these changes and the flexibility to respond rapidly to a wide variety of stimuli. A common response to environmental changes is through positive control. This can involve the sensing of a

domain of the kinase to the second component, the response regulator, which is generally a transcriptional activator (Fig. 1).

The common mechanism underlying this signal transduction process involves a now-classic phosphotransfer reaction between the two proteins. The protein kinase is first autophosphorylated at a conserved histidine residue in an ATP-dependent reaction. In a second step, the phosphoryl group is transferred to an aspartate residue

any of the target genes and constitute an operon.[6] The operon encodes the DegS and DegU proteins, both of which display amino acid sequence homology with the sensor and effector proteins respectively, of the bacterial two-component regulatory system. The DegS modulator was postulated to control the DegU effector, which was thought to be a transcriptional activator. As with other modulator/effector pairs, the DegS and DegU proteins contain several conserved domains.

The *degS* and *degU* genes were initially defined by different classes of mutations, leading either to deficiency of degradative enzyme synthesis (designated *degS* or *degU* mutations) or to overproduction of degradative enzymes [designated *degS* (HY) or *degU*(HY) mutations]. The pleiotropic HY phenotype not only caused hyperproduction of degradative enzymes but also decreased genetic competence, caused the loss of flagella and of the ability to sporulate in the presence of glucose.

In a collaborative project between researchers at the CSIR and University of Cape Town, the *sacU* locus containing the *degS-degU* genes was cloned from an alkalophilic *B. brevis* isolate.[7] A *Bacillus* promoter probe vector was used and the genes were transformed and expressed in a rec⁻ *Bacillus* strain and selected for their ability to increase extracellular protease production. This was achieved by developing a sensitive plate screen whereby the hydrolysis of casein in the medium was intensified by the presence of starch, causing a definite halo around positive colonies. This proved sufficiently sensitive to discriminate between the normal levels of protease secreted by cells and the increased levels produced by hyperproducing strains. When cloned on a high copy number plasmid, the *degS-degU* operon was found to be relatively stable and to cause the hypersecretion of several extracellular enzymes of commercial interest, such as proteases, β-glucanases and α-amylase. The increases in enzyme levels varied between 4- and 36-fold, depending on the enzyme measured. In addition, this phenotype was found to occur even when the gene was expressed in a *degU*(HY) strain, which was not the case with the *B. subtilis deg* operon. From the literature it would seem that the only other *degS-degU* sequence to have been published was from *B. subtilis*.[3-5]

The *sacU* region from *B. brevis* was sequenced and the two open reading

Fig. 1. Schematic representation of the two component sensor - regulator mechanism. A signal is received by the input domain from the sensor protein. This signal is forwarded to the sensor -transmitter domain, which has autokinase activity. A histidine residue (H) from the transmitter domain receives a phosphate group (P), which is transferred to an aspartate residue (D) Situated in the receiver domain of the response regulator. The phosphorylation of the receiver domain regulates the activity of the output domain, which triggers the regulatory response via an output signal.[8]

signal and its transduction to the nuclear machinery in order to stimulate the transcription of the target genes. Since two regulatory proteins play a central role in this process they have been referred to as 'two component systems', although in many cases more than two regulatory proteins are involved.[1] Interaction of these proteins typically involves the first component, a histidine protein kinase, receiving an extracellular signal directly or indirectly, possibly through its amino-terminal domain, which often includes transmembrane sequences. This signal is then transduced via the carboxy-terminal

in the amino-terminal domain of the response regulator. Several members of a histidine protein kinase family also act as phosphatases, catalyzing dephosphorylation of the associated response regulator. Regulation may therefore take place by modulating either the kinase activity or the phosphatase activity of the protein.[2]

In *Bacillus subtilis*, the different two-component systems controlling postexponential-phase reponses appear to interconnect to a large extent to form a sensory transduction network. This network appears to be involved in a hierarchy of environmental signal responses, involving a choice between competence gene expression, degradative-enzyme production, and finally, sporulation.[1,2]

The expression of genes encoding extracellular enzymes in *B. subtilis* was found to be under control of the *sacU* region, which consists of two genes *degS* and *degU*.[3-5] These genes are unlinked to

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frames of the *degS-degU* operon were found to encode polypeptides which gave calculated molecular masses of 43.8 kDa and 27.0 kDa respectively. Comparison of the amino acid sequences with those of the *B. subtilis degS-degU* genes showed 74% and 84% similarity respectively.

The *B. brevis degS-degU* operon was found to be more efficient at producing the hypersecretion phenotype for extracellular degradative enzymes than the *B. subtilis* counterpart. Our achievement could lead to a number of industrial applications, specifically when it is desirable for more than one enzyme to be amplified within a system. Future work is being directed at exploring the feasibility of this method of enhanced enzyme production and to eluci-

dating the reasons for the efficient hypersecretion phenotype of the *B. brevis deg* operon.

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Desertification in developed countries: in search of the silver bullet

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'The question we should be asking is not why haven't we had more control over desertification, but how come we have had as much control over it as we have had'

Harold Dregne

Desertification, or dryland degradation, occurs in arid, semi-arid and dry sub-humid regions as a result of human mismanagement of agricultural and pastoral resources, coupled with the effects of climatic and other natural stresses on the environment. For at least the past five decades there have been commissions, committees, conventions and *ad hoc* discussion groups about desertification, both in developed and in developing countries, and on provincial, national and, recently, international scales. It has become a major concern of politicians and environmental scientists and has led to the expenditure of millions of dollars in attempts to return rangelands to more productive states. Successes are few and failures many, largely because symptoms, and not causes, are treated. Internationally, the problem is perceived as being extremely serious and at the 'Earth Summit', held in Rio de Janeiro, Brazil, in 1992, a separate United Nations Convention on Desertification was called for, aimed at curbing the degradation of arid lands worldwide.

Against this background, it is of interest to report on a symposium and workshop on desertification in developed countries, the first of two planned meetings on the theme, that took place in Tucson, Arizona, from 24 to 29 October 1994. The second of the two meetings, on desertification in developing countries, is scheduled for 1997, and very likely will take place at the

same venue. The present meeting addressed the question of controlling desertification, with the objectives of (1) identifying social, economic, political and institutional factors that have resulted in successful interventions, (2) assessing the extent of desertification, and (3) identifying specific techniques that have been tried and used to slow down or reverse various desertification processes.

The causes and control of desertification

Within this framework, divided among seven categories, 36 papers and 32 posters were presented to nearly 140 delegates from 14 countries. Few of the presentations provided convincing evidence that the control of desertification is economically feasible. Several of the papers catalogued the disastrous results of changes in land-use policies, such as the European Common Agricultural Policy, or emphasized the role of socio-economic changes in promoting and maintaining dryland degradation. Few papers presented the results of case studies where interventions have successfully reversed desertification, albeit at enormous cost. Overviews of desertification in the south-western United States, Mexico, Chile, Russia, South Africa and Australia provided useful insights into the speed of the desertification process.[1-7] What also emerged from these and other papers was that models of desertification are not general, and that the

pattern and process of desertification may be driven by different factors in different places. The pattern and process of desertification is poorly understood, and the knowledge base, in general, is insufficient to be able to address many of the key issues that promote and maintain dryland degradation.[8]

Although there is a strong link between poverty and desertification,[9] the loss of usable primary and secondary productivity from semi-arid and arid lands is not restricted to densely populated, poor countries. In the south-western US, the Homestead Act of 1862, which inadvertently allowed overstocking and overcultivation on public lands, played a major part, if not being directly responsible, for present-day dryland degradation. Public policies, including the grazing permit scheme, feed subsidy programme and other handouts to ranchers have maintained a non-sustainable livestock industry at enormous expense to government (that is, the taxpayer) in the south-western US and have allowed stocking rates to rise way above the carrying capacity of the rangelands.[1] Communal grazing land in the south-western US is similarly degraded, though the degradation is driven by different factors. In the 'four corners' area (the point at which Utah, Colorado, Arizona and New Mexico meet), there has been a steep rise in human

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