Applications and Systematics of *Bacillus* and Relatives

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PROGRAMME BOOK

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PARTIAL CHARACTERIZATION OF PLASMIDS FROM *GEOBACILLUS STEAROTHERMOPHILUS* STRAIN 3 AND THE OTHER RELATED STRAINS

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21 obligately thermophilic proteolytic endospore-forming strains were isolated from the oilfields in Lithuania. Six of them (unidentified strains 18, 19, 22, 32B and 32D, and *Geobacillus stearothermophilus* strain 3) were found to harbour large covalently closed circular DNA molecules. Plasmids from three of those strains were cleaved with 5 restriction endonucleases (*Eco*RI, *Bam*HI, *Hind*III, *Xho*I and *Bsp*68I). This restriction analysis showed sizes of these plasmids from strains 18, 19, and *G. stearothermophilus* strain 3 to be approximately 60 kb, 40 kb, and 42 kb, respectively. Restriction patterns of these extrachromosomal elements differed for all the five restriction enzymes. Attempts to cure plasmids from strain 18 and *Geobacillus stearothermophilus* strain 3 showed no observable influence on the phenotypic characteristics: sporulation and cell morphology for *G. stearothermophilus* strain 3, and the ability to produce antibacterial substances for strain 18. It was supposed that the plasmids of the thermophilic endospore-forming strains bear the genes essential for the cell survival. Spontaneous loss of the plasmid from strain 18 preserving it at 4 °C in cultures on slants and its possible vital function raised the possibility for the integration of this extrachromosomal element to the bacterial chromosome. As genotyping results suggested close relationships between the plasmid-bearing strains – ARDRA was performed with the 5 restriction enzymes (*Alu*I, *Hae*III, *Mse*I, *Rsa*I and *Taq*I) and the similar gel-electrophoretic patterns were obtained – and specific environment these strains were isolated from could afford ground for the subsequent horizontal transfer.

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METABOLISM OF ANTARCTIC *BREVIBACILLUS* ISOLATES

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Aerobic endospore-forming bacteria which grow optimally at pH 5.5 and 40 °C have been isolated from geothermal soils of Mount Melbourne and Mt Rittmann in northern Victoria Land, Antarctica. 16S rDNA sequencing, Amplified Ribosomal DNA Restriction Analysis, SDS-PAGE, and routine phenotypic tests indicate that they represent a new species of *Brevibacillus*. As they gave weak and inconsistent results in routine substrate utilization tests, metabolic studies were undertaken in order to identify their nutrient sources in their oligotrophic habitats. Tracing experiments required the development of a defined medium, a variation of *Bacillus fumarioli* agar, in which the yeast extract, due to its undefined nature, and $(NH_4)_2SO_4$ were substituted for L / DL amino-acids and a vitamin solution. The assimilation of different amino-acids was investigated, however since Biotype 100 assimilation tests demonstrated utilization of glutamate by these bacteria. an amino acid often available from microalgae growing in such habitats, glutamate was used in the initial studies. The elimination of non-essential amino-acids in the preparation of the defined medium showed that glutamate is not essential for growth. Scintillation counting demonstrated an initial increase in ^{14}C glutamate which was then followed by a decrease, suggesting that either decarboxylation or the export of labelled products had taken place. Deamination may also take place resulting in the production of ammonium. The presence of high affinity and low affinity uptake systems may also account for this decline in ^{14}C glutamate uptake. The growth of the organisms at pH 5.5 suggests that a system involved in pumping H^+ out of the organism could be involved in the uptake of particular amino-acids and vitamins as has been shown for other bacteria. The addition of NaCl and KCl resulted in inhibition of glutamate uptake, suggesting that a negative charge and possibly an osmotic effect may be involved in its uptake.