STUDIES ON PROTEASE AND α-AMYLASE SYNTHESIS BY *BACILLUS AMYLOLIQUEFACIENS*

A THESIS

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SUMMARY

1. Harvested cells of *B. amyloliquefaciens* have the capacity to synthesize alkaline and neutral protease for approximately 30 and 60 min respectively in the presence of rifampicin.

2. The rifampicin-insensitive capacity for alkaline and neutral protease production can be modulated. In each case, incubation in high casamino acids medium for 75 min exhausts it and subsequent incubation in low casamino acids medium causes it to be recovered. A maximum capacity is reached in 75 min then rapidly decreases and for alkaline protease a further oscillation is observed upon continued incubation. If the second incubation is in high casamino acids medium the recovery of the capacity for rifampicin-insensitive alkaline and neutral protease production occurs at a lower rate.

3. Incubation of cells with a maximum capacity for rifampicin-insensitive alkaline protease production in the presence of rifampicin for 20 min results in alkaline protease synthesis representing approximately 24% of total protein synthesis.

4. Alkaline protease is synthesized *de novo* in the presence of rifampicin.

5. The modulation of the capacity for rifampicin-insensitive alkaline and neutral protease production can be explained by proposing two forms of each mRNA

according to the scheme described by O'Connor *et al.* (1978).

6. Cells have a rifampicin-insensitive capacity for α-amylase production which can be modulated.

7. Lysozyme-treated cells of *B. amyloliquefaciens* secrete active neutral protease and α-amylase, but no alkaline protease, active or inactive, is secreted.

8. A correlation exists between active neutral protease production by protoplasts and the secretion of inactive alkaline protease. Inhibition of the former results in the production of the latter, the molecular weight of which is identical to that of active extracellular alkaline protease.

9. Protoplasts have a rifampicin-insensitive capacity for alkaline protease (inactive) production.

10. Rapid-cooling of protoplasts to 4°C inhibits general protein and exo-enzyme synthesis to the same extent when subsequently measured at 30°C. The magnitude of this inhibition can be reduced by the presence of spermidine prior to rapid-cooling.

11. Polysomes extracted from protoplasts capable of exo-enzyme production direct the *in vitro* synthesis of a number of products. Characterisation of these products by immunoprecipitation with antisera raised against exo-enzymes results in non-specific precipitation.

12. Sepharose-2B column chromatography of protoplast lysate results in no separation of free and membrane-associated polysome fractions.

13. A procedure has been developed for the isolation of pure alkaline protease.

14. *B. amyloliquefaciens* produces three proteases: neutral, alkaline and a third minor species which is detected only by electrophoresis toward the cathode.