THE UTILIZATION OF ε-CAPROLACTAM BY MOULDS

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Since the first announcement in the literature of the isolation of bacteria capable of utilizing ε-caprolactam as sole carbon and nitrogen source (Kato and Fukumura, 1962), several further reports have appeared from Japan and the USSR suggesting that the metabolic scheme for ε-caprolactam degradation involves initial hydrolysis to ε-amino caproic acid, followed by deamination to adipaldehydic acid, conversion to adipic acid and final oxidation in the tricarboxylic acid cycle (Fukumura, 1966, Naumova and Belov, 1967).

However, apart from work by Tosa and Chibata (1965), who isolated several micro-organisms growing in media containing glucose with ε-caprolactam serving merely as nitrogen source, there is no mention of the involvement of eukaryotic organisms in connection with ε-caprolactam breakdown. ε-caprolactam and its oligomers are intermediate products arising in substantial quantities in effluents from nylon manufacture. A fungus which would

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simultaneously degrade ε-caprolactam, reduce the polluting nature of the effluent and produce biomass is clearly attractive.

We therefore set out to investigate this possibility.

Numerous samples were taken from several locations within a synthetic fibres plant, and plated out on a variety of media (some incorporating actidione as yeast suppressant), of various pH values, but all containing ε-caprolactam as sole carbon and nitrogen source, and incubated at a range of temperatures.

A combination of low pH (pH 4) and actidione (25 µg/litre) was successful in suppressing yeasts and bacteria, with moulds growing only after a period of eight to ten weeks at an incubation temperature of 30°C. The latter organisms were therefore transferred to potato dextrose agar plates (on which growth was relatively quick), retransferred six times to ensure purity and finally replated onto ε-caprolactam - mineral salts agar. In addition, ε-amino caproic acid - mineral salts agar was also tested for each of these six isolates and this compound proved superior to ε-caprolactam in supporting growth

Finally, the two organisms which grew best were identified (see Acknowledgements) as follows.

The first, a septate fungus isolated from an ε-caprolactam discharge area at the plant, was found to be a strain of Penicillium lilacinum. The second, a non-septate organism isolated from a boiler room was a

strain of Byssochlamys fulva.

Further investigations were directed to the determination of the optimum combination of medium pH and ε-caprolactam concentration for growth. For both fungi this combination was an ε-caprolactam concentration of 10g/litre and a pH of 4.

Of the two identified organisms Penicillium lilacinum was selected for further study.

As the growth rate of this organism was impracticably low in ε-caprolactam media, batches were grown in Nutrient Broth (Oxoid Ltd.), centrifuged, washed aseptically and then transferred to sterile ε-caprolactam medium. Samples taken approximately six-hourly over a 48 hour period were subjected to uni-dimensional paper chromatography using n-butanol:acetic acid:water (4:1:2) as solvent and developed with a 0.3% ninhydrin solution in 75% ethanol.

Although several presumptive amino acids were detected, no spot corresponding to ε-amino caproic acid was ever seen even after considerable concentration of the samples.

In this connection the work of Noe and Nickerson (1957), who investigated the breakdown of the lower homologue α-pyrrolidone (γ-butyrolactam) by Pseudomonas aeruginosa is of interest here. They indicated by a series of adaptation and enzymic experiments that γ-amino butyric acid featured in the degradative sequence of α-pyrrolidone but failed to detect its presence. Tosa and Chibata (1965) obtained similar results for a number of micro-organisms utilizing both γ-butyrolactam and ε-caprolactam

and assumed that the ω-amino acids were further metabolized without accumulation.

From the observation that the growth of our isolates was more rapid on ε-amino caproic acid than on ε-caprolactam itself, it would appear that either ε-caprolactam uptake or its initial hydrolysis to ε-amino caproic acid is the rate limiting step in the metabolism of ε-caprolactam by these micro-organisms, with further degradation being relatively easy.

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