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Use of Fluorescamine to Estimate the Rate of Biosynthesis of Benzylpenicillin

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The fluorescamine reaction with 6-aminopenicillanic acid (6-APA) at pH 4 was investigated for its use in following the biosynthesis of benzylpenicillin. A number of amino acids and penicillin amine derivatives, which reacted with fluorescamine at pH 7–9, were unlikely to do so at pH 4, and were therefore unlikely to interfere with results that led to the biosynthesis of benzylpenicillin.

Biosynthesis was investigated by mixing 6-APA and phenylacetic acid (PAA) in the presence of a partly purified preparation of penicillin acylase at pH 5, and directly estimating the disappearance of the 6-APA. In this case the enzymic treatment at pH 5 of the raw ingredients rapidly reached equilibrium where the reactants were in greater concentration than the products. A pH of 5 is considered optimum for biosynthesis by this procedure.

Biosynthesis of benzylpenicillin was also followed in *Penicillium chrysogenum* Wis F3-64, grown in a corn steep liquor medium in a shaking flask fed with phenylacetic acid daily. A sample of the fermentation broth was treated with buffer at pH 7.8, followed by an active penicillin acylase solution for 1 hr at 37°C. The pH was then lowered to 4 by the addition of acetate buffer and the solution was then treated with fluorescamine. The resulting fluorescence was compared to that of a standard 6-APA solution treated in the same manner. In this case the biosynthesis of benzylpenicillin was found to increase over 6 days. Results were compared to a control broth to which the penicillin acylase was not added.

This technique is currently being applied to the analysis of the culture broths of *Pycnoporus cinnabarinus* (Jacq.: Fr.) P. Karst. (Coriolaceae, Polyporales, Higher Basidiomycetes) and other white-rot fungi.

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