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SHORT COMMUNICATION

OXYGEN REQUIREMENTS FOR SECONDARY METABOLISM IN *TRICHODERMA VIRIDE*, AND THE EFFECT OF BARBITURATE

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Abstract—For maximum synthesis of gliotoxin and viridiol by *T. viride*, oxygen transfer rates markedly higher than that sufficient for full growth of the organism are required. The formation of gliotoxin and viridiol is increased by added phenobarbitone. The observations suggest the involvement of mixed function oxidase systems in both biosynthetic pathways.

THE NEED for particularly high aeration in the fermentation production of gliotoxin has been well-known for many years[1] but no quantitative data on this aspect of the fermentation (or indeed on any other) have been published. We found that in shake cultures the yield of secondary metabolites from *Trichoderma viride* varied with oxygen transfer within a range of oxygen transfer rates realizable with standard laboratory equipment, and present our observations here as having some general interest as well as relevance to further experimentation on this system.

RESULTS

Figure 1 shows the variation with oxygen transfer rates of the mycelial dry weight, gliotoxin, and viridiol levels at 68 hr, with an indication of the range of oxygen transfer rates which, in our experience, corresponds with 'normal' laboratory conditions. It is clear that mycelial growth, as measured by dry weight, is not enhanced at rates over 12 mM O_2/hr, but at this rate viridiol synthesis is only 80% of its maximum value and gliotoxin synthesis is only 40%. Maximum viridiol production requires 15 mM O_2/hr and maximum gliotoxin production requires 22 mM O_2/hr, with some deterioration at higher oxygen transfer rates.

The addition of 4 mM phenobarbitone to the cultures at 0, 18, or 42 hr had very little effect upon the dry weight, but caused marked increases in the production of viridiol and gliotoxin, e.g. for viridiol, 160–180% of controls (at 68 hr) and for gliotoxin, 150–190% of controls.

DISCUSSION

The structure of viridiol[2] is that of a triterpenoid which has undergone considerable oxidative modifications, and our recent study of gliotoxin biosynthesis[3] has shown that an

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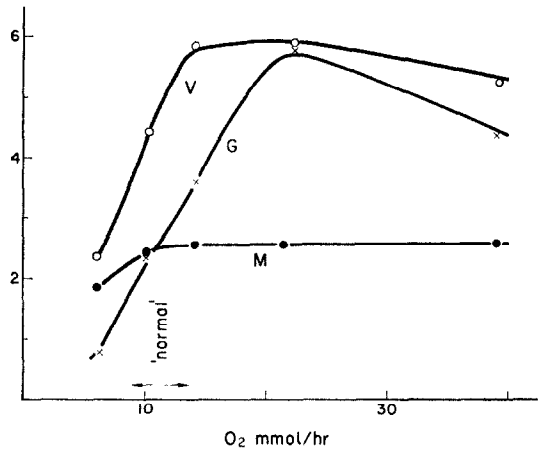

Fig. 1. Product yields at 68 hr for *T. viride* at various oxygen transfer rates.
M = Mycelial dry weight in g/500 ml. G = Gliotoxin in mg/100 ml. V = Viridiol in mg/100 ml.

early step in this sequence is probably the epoxidation of a phenylalanine derivative. Thus both products arise by pathways involving specific oxygenation reactions; more particularly, on general grounds, both pathways would be expected to involve enzyme systems of the mixed-function oxidase type. The higher oxygen transfer requirements for these two pathways, compared with the requirement for growth, might then be linked with either the formation or the operation of one or several components of such systems, which are otherwise rate-limiting.

The marked effect of barbiturate is at least consistent with this hypothesis; the most relevant comparison is the observation that in the formation of ergot alkaloids, the stimulatory effect of phenobarbitone, similar in magnitude to that reported here, is directly linked to the formation of higher concentrations of cytochrome P-450, which is a specific component in the microsomal mixed-function oxidase systems of the fungus.[4]

EXPERIMENTAL

Trichoderma viride strain NRRL-1828 (erroneously numbered NRRL-75 in a previous communication)[3] was grown from briefly homogenized vegetative inocula on a glucose–ammonia–peptone–salts medium.[5] Gliotoxin was estimated by titrimetric measurement of the gliotoxin-catalysed iodine–azide reaction;[6] viridiol[2] was estimated spectrophotometrically in chloroform extracts of culture media, evaporated and taken up in ethanol, using $\epsilon_{max} = 4700$ at 317 nm. A range of oxygen transfer rates was established by applying the sulphite method[7] to Erlenmeyer flasks of from 250 to 500 ml capacity, containing from 50 to 300 ml liquid, on a gyrotatory shaker at 60–300 rev/min, with a 1 in. or 2 in. throw; flasks were plugged with cotton wool at a standardized density (7 g/plug). Cultures were then grown under equivalent conditions and examined by removing duplicates at various times up to 96 hr: only the 68 hr data from one series of experiments (500 ml flasks, 300 rev/min) are presented here (Fig. 1) but our other data were entirely consistent with these.

Phenobarbitone (4·5 g) was dissolved in the minimum volume of N-NaOH and made up to 150 ml in water, 5 ml was added to each 150 ml of *T. viride* culture (giving 1·0 mg/ml final concentration) together with sufficient 2 N-H_2SO_4 to bring the final pH to *ca.* 3·5 as in the controls; additions were made at 0, 18, or 42 hr and the cultures analysed at 68 hr.

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