FUNGAL BIOTECHNOLOGY — THE FUNDAMENTAL PRINCIPLES

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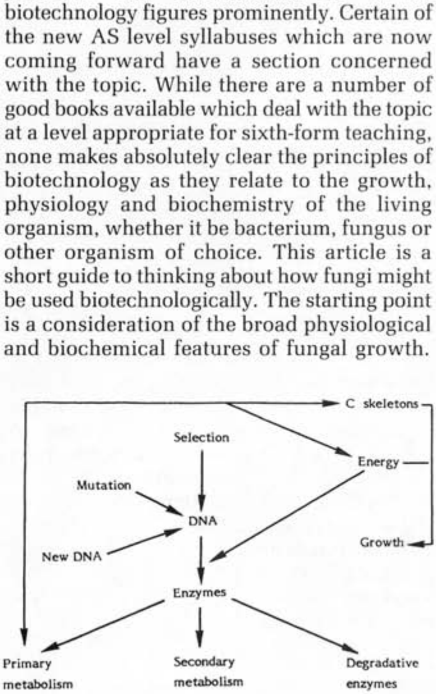
Sixth form biology students and their teachers are likely to have to grapple with biotechnology. Some are already doing so. The new A-level Biology syllabus of the Joint Matriculation Board has an option *Cells, their products and interactions* in which biotechnology figures prominently. Certain of the new AS level syllabuses which are now coming forward have a section concerned with the topic. While there are a number of good books available which deal with the topic at a level appropriate for sixth-form teaching, none makes absolutely clear the principles of biotechnology as they relate to the growth, physiology and biochemistry of the living organism, whether it be bacterium, fungus or other organism of choice. This article is a short guide to thinking about how fungi might be used biotechnologically. The starting point is a consideration of the broad physiological and biochemical features of fungal growth.



The growth and metabolism of a fungus — or indeed that of any micro-organism — can be represented diagramatically very simply as above. Growth itself requires the synthesis of the carbon skeletons of the molecules which compose the cell structure, such as wall, proteins, phospholipids. Those carbon skeletons are produced by catabolism of the carbon source in the growth medium of the fungus. Usually this carbon source is a carbohydrate, frequently glucose. The process of catabolism is brought about by the metabolic events of glycolysis or the citric acid cycle, by the formation and interconversion of amino acids and the like. The biochemical processes which bring about the synthesis of the compounds which go to make up the constituents of the normal growing cell or hyphae are known as *primary metabolism*. A good definition of primary metabolism can be based on that of Martin & Demain (1978), namely: Primary metabolism involves an interrelated series of enzyme mediated reactions which provide biosynthetic intermediates and energy and convert biosynthetic intermediates into essential macromolecules such as DNA, RNA, protein and polysaccharides.

One can see from the definition that primary metabolism provides not only the carbon skeletons but also the energy for growth. That energy will be used not only for biosynthetic reactions such as protein synthesis but also for accumulating compounds from the medium and for the excretion of hydrogen ions as part of those processes which are necessary for keeping a relatively constant internal pH. In the majority of fungi, accumulation of compounds or solutes from the external medium is necessary to produce a high internal osmotic pressure (low solute potential) such that turgor is generated, the necessary driving force for the growth of a walled-cell. Much of the energy for all these processes comes from the free energy of hydrolysis of the terminal phosphate group of ATP.

The process of fungal growth can be used biotechnologically. We speak of the generation of *biomass*. Cultivation of mushrooms is the classic instance. More recently the generation of biomass has become technologically more sophisticated and involves other fungi. Cultivation of mushrooms is a process which uses the reproductive or fruiting structure of the fungus. But the high-technology processes use the vegetative phase of moulds such as *Fusarium graminearum* which is the source of biomass produced by Rank Hovis McDougall from the carbohydrate-containing waste from the flour-milling industry. Such biomass tends to be called single cell protein. It needs to be remembered that fungi are selected for high-technology biomass production on the basis, amongst other properties, of having a high growth rate. This is thus, relatively speaking, compared with more conventional sources of protein, a much higher content of nucleic acids, principally RNA so necessary for protein synthesis.

While primary metabolism is the source of carbon skeletons and energy for growth, primary metabolites may be of very considerable commercial and industrial importance. Here we are thinking about these relatively low-molecular weight compounds which are components of the metabolic pathways which lead to macromolecules. Ethanol or alcohol is the outstanding example. But it is unusual in that ethanol is produced by a fungus, namely *Saccharomyces cerevisiae*, growing relatively normally. But ethanol is exceptional; in other instances the metabolism of the fungus is closely regulated such that no primary metabolite increases to any great extent. In the case of primary metabolites other than ethanol, metabolism has to be *perturbed* to stimulate the production of the particular compound in sufficient quantity to make the process commercially viable. The classic case is the stimulation of the production of glycerol by yeast, under those conditions when the organism would normally be producing ethanol. Here acetaldehyde is trapped by the addition of sulphite to the medium. In normal metabolism, acetaldehyde has to be oxidised to ethanol to produce NAD^+ to allow glycolysis to proceed. In the presence of sulphite, the cell achieves the production of NAD^+ by the reduction of dihydroxyacetone to glycerol.

In a similar manner, other primary metabolites are produced by generating culture conditions which perturb metabolism. Thus citric acid production by *Aspergillus niger* depends on there being an absence in the medium of manganese which is required for normal metabolism. In the case of the production of amino acids by bacteria, metabolism is so to speak perturbed genetically by the use of mutants which have lost the regulatory control over the necessary metabolic pathway. Of course, although one speaks of ethanol production by *S. cerevisiae* as occurring under normal conditions, the fact that it occurs under low oxygen tensions may be considered as the equivalent of metabolic perturbation.

When a fungus is growing in a medium of finite volume, it will eventually reduce one of the nutrients in the medium to zero concentration. Thereafter growth in the sense of the production new cytoplasm will cease. So-called balanced growth will be impossible. Increase in dry mass may take place for a time but that is probably due to production of reserve material which contributes to the mass of the fungus but not to the functioning cytoplasm. In most media used to grow fungi, it is usually nitrogen and phosphorus which are exhausted first. When balanced growth finishes, the fungus may under-go differentiation. This may lead to readily observable change in morphology such as the production of spores. But new chemical compounds may be produced without readily identifiable change in morphology; these compounds are called secondary metabolites. However, it needs to be remembered that change in morphology will also mean a change in chemistry of the fungus. In either case therefore, there will be a change in biochemistry which is triggered by a change in the environment, namely its nutrient composition. New enzymes will be produced or come into action to generate the changed cell chemistry. The new biochemical pathways are different from those in the normal growing fungus and thus these new pathways make up what is known as *secondary metabolism*.

There is now a very long list of secondary metabolites produced by fungi. The most famous is, of course, penicillin produced by *Penicillium chrysogenum* but there are a considerable number of others also of marked commercial importance.

Since fungi are able to grow on what might otherwise be thought of as refractory material, it means that they are able to secrete enzymes to make that material soluble and thus absorbable. Mushrooms are an excellent example of this capability, producing enzymes to degrade the cellulose and lignin in the straw-based compost. Indeed basidiomycete fungi are the most effective living organisms at degrading wood and there are a number of research groups around the world examining how such fungi might best be used to remove wood waste, particularly from the paper-making industry. Essentially the aim is to convert the wood waste to ethanol which can be used as a fuel.

The enzymes produced from fungi can be used in isolation on commercially targeted operations. It is surprising how much food and drink involves the use of fungal enzymes. Thus $\propto$ -amylase produced by various *Aspergillus* species is used in bread-making, likewise pectinase also from various *Aspergillus* species is used in fruit processing. Mushrooms have been given as an example of fungi degrading the substrate on which they grow. The actual substrate degraded by a fungus may itself be a food and this is of course the case with cheese with *Penicillium roqueforti* being responsible for Roquefort, Stilton and the 'Blue' cheeses. Oriental food fermentations are the result of fungal activity, an example being soy sauce produced by *Aspergillus oryzae/sojae*.

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The above categorises the way in which fungi can be used biotechnologically. They can be used (i) to generate primary and secondary metabolites; (ii) to produce enzymes or (iii) as biomass. In all this, increased yield can be produced either by altering the conditions of growth in which case maximum yields by this means are quickly achieved or by altering the DNA of the fungus concerned and is a longer process but is very much the more powerful. Alteration of the DNA may be achieved by strain selection, breeding, mutation or the insertion of foreign DNA by genetic engineering. The first three procedures are the traditional ones used to improve plants and fungi of commercial importance; the fourth is only just making its impact on fungal biotechnology.

REFERENCES

MARTIN, J F & DEMAIN, A L (1978). Fungal development and metabolite formation. In *The Filamentous Fungi* Vol. III. *Developmental Mycology* (ed. J E Smith & D R Berry), pp 427-450. London: Edward Arnold.