MYCOLOGY ANSWERS

FUNGI NATURALLY FORM MANY DIVERSE BIOCHEMICAL PRODUCTS, SOME OF WHICH ARE NOW COMMERCIALLY IMPORTANT; HOW AND WHY DO THEY DO THIS?

Under natural environmental conditions, or in culture, growing fungi will take from their surroundings those nutrients that they can use easily as energy sources to produce materials such as proteins, lipids and nucleic acids, for continued growth and biomass production (primary metabolism). This allows them to proliferate and colonise new regions to obtain more nutrients. Primary metabolites are formed during the active growth of the fungus and these will accumulate in the surroundings if growth becomes restricted or when supplies of key nutrients are depleted. Some primary metabolites have commercial importance and large scale cultures are grown industrially with the specific purpose of obtaining large quantities of these fungal products including organic acids such as citric acid (used in food and soft drink manufacturing), ethanol (used in alcoholic drinks production), enzymes (e.g. pectinases, glucose isomerase and lipases used in food processing) and amino acids and vitamins (food supplements).

As nutrient depletion occurs in a particular environment, or in culture, growth of the fungus slows down and parts of the mycelium switch to using different biochemical pathways. This alteration in metabolism prevents the fungus from poisoning itself and maintains the biochemical machinery of the cells. Primary metabolites and intermediate compounds which have accumulated in the fungus are converted to different products (secondary metabolites) which are not normally made during active growth and are not essential for vegetative proliferation. Some of these secondary metabolites are complex molecules and are produced when the fungus is not actively growing; their formation may accompany differentiation and sporulation in the fungus. Some are very useful economically important products (e.g. antibiotics) but some are extremely harmful (mycotoxins) to man and animals.

The majority of fungi produce very similar primary metabolites. These contribute to the biosynthesis of the building blocks of fungal mycelium. Fungi are very versatile and can use a range of different sources of nutrients which are assimilated into the primary metabolic pathways at different points. When growth becomes restricted by some factor, products and intermediate compounds will then accumulate in the culture medium. This can be brought about by manipulation of the particular nutrients provided for growth or the provision of specific environmental conditions. It is this attribute which is exploited for the commercial production of useful primary and secondary fungal products.

For example, one of the intermediate compounds in primary metabolism is citric acid which is produced commercially in large quantities (several hundred thousand tons a year) using cultures of the fungus *Aspergillus niger*. On an industrial scale high levels of glucose are provided as substrate (often fed in as molasses) allowing the fungus to establish growth in a batch culture. The medium (pH in actively growing cultures is 5.0-6.0) is subsequently acidified to around pH 2.0 and this limits growth. The fungus then converts the sugar to citric acid but because growth is restricted it is not metabolised in the normal way and it accumulates in the medium. This production system is very sensitive to the balance of nutrients and the presence of trace metals in the culture. If levels of iron or manganese are too high in the medium other products may be formed and accumulate in place of citric acid, however careful control of the cultures will give rise to good yields.

Secondary metabolites are more species-specific and products are often unique to a particular species. For this reason it is likely that although a very large number of fungal secondary metabolites have now been identified many more will be described in future as the activities of more fungal species are investigated. By manipulation of the culture conditions it is possible for the production of a secondary metabolite to be prevented or a fungus may be induced to over-produce some of these compounds. The rate of use of sugar in primary metabolism may reflect the production of a primary metabolite but this is not so for secondary metabolite production. Most secondary products are formed at the end of active growth and are derived from primary products which were synthesised earlier.

The expression of the genes controlling secondary metabolism is controlled by nutrient supply (both the types and the quantity of nutrient) and growth rate. Nutrient limitation results in low growth rate which favours secondary metabolism. If easily used carbon sources (e.g. glucose) and nitrogen sources (e.g. amino acids) are provided then primary metabolism will continue or resume. Feeding compounds which are metabolised more slowly (e.g. starch or lactose) will lead to the formation of secondary products. In some instances, the presence of particular metals also influences secondary metabolism. Low growth rate (probably linked to nutrient supply) is most important for secondary metabolism to proceed. Eventually the formation of secondary metabolites ceases when the enzymes responsible for synthesis cease to function or when the levels of product build up and become self inhibitory (feed back inhibition).

Some commercially important secondary products including antibiotics (e.g. penicillin from *Penicillium chrysogenum*, cephalosporin from *Cephalosporium acremonium*, griseofulvin from *Penicillium griseofulvum*) and alkaloids (*Claviceps* spp.) are derived from filamentous fungi and used medicinally. Benzylpenicillin is produced by *P. chrysogenum,* grown on a mixture of glucose to support growth and lactose to support secondary metabolism, with growth factors and precursors of secondary metabolism (corn-steep liquor) and mineral salts, in large scale submerged culture. Alternatively a slow feed of glucose may be used to maintain a low growth rate. Penicillin is eventually extracted from the mycelium. Good understanding and careful manipulation of the cultural conditions is required because the synthetic process is sensitive to pH, temperature and the exact mix of nutrients present as metabolism proceeds. The use of mutation and selection has gradually led to an increase in the amounts now synthesised in industrial culture.

Secondary metabolites are not essential for growth but are produced naturally by many fungi. Many of the compounds produced have antifungal and antibacterial activity (e.g. antibiotics, mycotoxins) and may therefore impart a competitive advantage, acting as weapons for survival. The compounds have antimicrobial activity and producer organisms may well be sensitive to these. Most have mechanisms to prevent their own demise from the effects of the compounds they produce. In most cases the products are formed after active growth and by that time the mycelium is able to detoxify the compound or prevent entry of the antibiotic through the cell wall by a change in the permeability of the plasmamembrane.

Fungi are widely used for the production of commercially important products and are under constant investigation for other potentially useful products.

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