INDUSTRIAL MYCOLOGY

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Contents

1. Introduction
2. Product Range
2.1. Metabolites
2.2 Enzymes
2.3. Biomass
2.4. More recent and potential products
3. Solid State Fermentation
3.1 Products from Solid State Fermentation
3.1.1 Gibberellic acid – GA_3
3.1.2 Glucoamylase
4. Submerged Fermentation
4.1. Selected metabolites produced by Submerged Fermentation
4.1.1 Lovastatin
4.1.2 Red *Monascus* Pigments
4.1.3 Rennet (Chymosin) from *Mucor*
4.1.4 Quorn®
5. Other Developments of Industrial Mycology
5.1. Heterologous Proteins by Filamentous Fungi.
5.2 Flavoring Agents
5.3. Cheese Made with Fungi
5.4 Higher Fungi for Food Flavor and Medicine
6. Conclusions
Glossary
Bibliography
Biographical Sketch

Summary

Filamentous fungi are used by industry for manufacture of a large variety of useful products, all for the benefit of humankind. Examples of how some of these products are formed by an assortment of fungi and produced on a large scale are presented. The products include metabolites, enzymes and food. Fungal cells can grow at different environmental conditions. The chemical and physical conditions used for fungal propagation will have a great impact on the capability of these cells to accumulate the desired product(s).

Processes using solid state and submerged fermentations are described illustrated by a

few of the metabolites produced by industry. There are great economic benefits in the use of filamentous fungi and there is a great potential for these organisms to produce novel items.

Industrial processes using fungi are of great economical importance. The products are unique and there is usually no other economic way to manufacture these products. In this article only a few compounds will be described in greater detail, but it should be remembered that there are many more that are manufactured using filamentous fungi.

1. Introduction

Fungi have several distinct characteristics that make them different from other life forms. They grow in a filamentous branching mode, with apical growth, lateral branching and by heterotrophic nutrition [see also – *Mushroom Production*]. Their life cycle begins by germination of their resting structure or spore. Vegetative growth follows; where the biomass increase as the substrate is utilized and novel cells (hyphae) are formed. The fungal hyphae form a porous three-dimensional net that is known as mycelium. At a certain stage in the life cycle, usually when there is lack of nutrients, sporulation may occur, where morphologically distinct structures are produced that can detach from the mycelium. Spore formation is not found for all filamentous fungi, in those cases were spores are not found, it is usually due to incorrect nutritional conditions and when those are found, spores will be produced. Most filamentous fungi are found growing in their imperfect stage, where no sexual cycle is known, as for others a perfect stage (a sexual cycle) is found, also dependent on the right nutritional conditions The filamentous fungi play an important role in the biosphere, where their decomposing action of organic material, leads to the restoration of substrates like carbon, nitrogen, phosphorus and minerals to the biosphere. The filamentous fungi are utilized for many different purposes, in the food industry, for the manufacture of useful metabolites and in a variety of other processes (Table 1).

Food Applications	**Useful Products**	**Other Processes**
Baking	Alkaloids	Biobleaching/biopulping
Brewing	Antibiotics	Biological control agents
Cheese-making	Ethanol	Bioremediation of soils
Mushroom cultivation	Enzymes	Coal solubilisation
Oriental food fermentations	Gibberellins	Dyes/dye intermediates
Quorn myco-protein	Immunomodulators	Microencapsulation
	Organic acids	Mycorrhizal inoculants
	Polysaccharides	Steroid bioconversions
	Vitamins	Waste treatment

With permission. Fungal Biotechnology by P.F.Hamlyn, North West Fungus Group (NWFG) Newsletter, April 1997.

Table 1: Uses of filamentous fungi

Yeast, that in specific cases also can grow in a filamentous mode, are dealt with in other articles [see also – *Production of Alcoholic Beverages*]. The industrial production of both primary and secondary metabolites from filamentous fungi is well established [see

also – *Production of Organic Acids; – Production of* Antibiotics]. There are also articles describing different uses of filamentous fungi like Nutriceuticals from Mushrooms [*see Nutriceuticlas From Mushrooms*] and Mushroom Production *[see Mushrooms Production]*. In this article so called "micro fungi" are the main agents for which production are described.

As can be seen in Table 1, there is a large assortment of metabolites produced by filamentous fungi, including vitamins, polysaccharides, enzymes, immunosuppressive agents, hypercholesterolemic agents, pigments, antibiotics and organic acids. For each manufactured goods, a specific fungus and its growth conditions are characterized thoroughly. The determination of optimal product conditions start with the initial research finding, of a potentially commercial metabolite, followed by further research and development to a full scale industrial process (see also - *Process Optimization Strategies for Biotechnology Products*). For many cases, information of the actual industrial production process of a specific compound by filamentous fungi is confidential, and therefore it is not possible to acquire specific information for the process. In this article knowledge is presented that is available in the public domain. Information will be given for selected metabolites where more detailed information is available on their industrial production. There is a plethora of fungal products and in this article is described a few examples, considered to characterize some of the methods and products of industrial mycology.

Some of the products of industrial mycology are made on a routine bases, however, the detailed technical details are not publicly available. The products described are a biased choice, where public information is available, containing more or less traditional compounds. Other trends will also be discussed, specifically the attempts to use filamentous fungi for the formation of heterlogous proteins for various applications and higher fungi for food and therapeutical purposes.

2. Product range

The variety of metabolites produced by filamentous fungi by industry is vast. In this article different categories of compounds are described with the intention to give a representative presentation.

One group are called "metabolites", by which is meant small molecular weight (> 1000 Dalton) compounds that can be either primary or secondary metabolites. The structural complexity, including different stereochemistry and also chirality, is usually the main reason why these "metabolites" are produced by biological methods and not by chemistry. This is of course related to the economy of production, since the chemical route would result in a much more expensive product.

The following products will be described in some more detail in this article.

2.1. Metabolites

The metabolite named gibberellic acid, a representative of the gibberellins, is used as plant growth stimulators, produced by growing the fungus *Giberella fujikuroi* on solid

substrates. Gibberelic acid stimulates both cell division and cell elongation, and hastens plant maturation and seed germination. It is applied to growing crops (field crops, small fruits, vines and tree fruits), ornamental and shade tress, and ornamental plants, shrubs and vines.

Another group of metabolites are the red pigments produced by *Monascus* species, traditionally achieved by cultivation of the fungus on rice grains or bread. It is a common process in South East Asia. Submerged fermentation methods have partially replaced the solid state fermentation process [see also – *Microbial Cell Culture***]** for production of the *Monascus* red pigments (monascorubramine and rubropunctamine) [see also – *Natural Food Colorants*].

Many of the most economically interesting metabolites are antibiotics *[see also – Production of Antibiotics]*. There are other metabolites with different therapeutic effects, like lovastatin (mevinolin), that belong to the group of statin drugs used for hypercholesterolemia. Statins have a large market, estimated at $15 billion/year and was first approved for clinical use by the FDA in 1987. The statins are manufactured by submerged fermentation of filamentous fungi.

2.2 Enzymes

Enzymes have many beneficial characteristics which make them ideal as catalysts in a large variety of reactions *[see also – Enzyme Production]*. Their main advantage is their activity at ambient conditions, compared to the high temperature and pressure required for many of the chemical reactions performed in industry. Their catalytic capability is substantial and the amounts required are many times orders of magnitude less material, than for chemical catalysis. Enzymes are versatile and are able to perform a multiplicity of reactions. Novel activities are looked for and they are also compatible with sustainable development. Enzymes are produced by all different cells and among them also filamentous fungi. Enzymes from filamentous fungi are produced by industry and also used by industry for many different purposes (Table 2).

Enzyme	Main Source
Asparaginase	*Aspergillus* spp. and *Penicillium* spp.
Amylase	*Aspergillus niger, Aspergillus. oryzae*
Catalase	*A. niger, Penicillium* spp.
Cellulase	*A. niger, Trichoderma reesei, T. viride, Penicillium finiculosum*
Dextranase	*Penicillium* spp.
ß-Glucanase	*A. niger, Penicillium emersonii, T. reesei, T. viride*
Glucoamylase	*A. niger, A. oryzae*
Glucose oxidase	*A. niger, Penicillium* spp.
Hemicellulase	*A. niger, A. oryzae, T. reesei, T. viride, P. emersonii*
Laccase	*Pyricularia oryzae*
Lipase	Several species including *A.niger, A. oryzae*
Pectinase	Several species including *A. niger, Rhizopus oryzae*
Protease	Several species including *A. niger, A. oryzae*

Rennet	*Mucor miehei, Endothia parasitica*
Tannase	*A.niger, A. oryzae*
Xylanase	*A. niger, T. reesei*

Table 2: Fungal enzymes of commercial importance

More and more enzymes are being introduced for novel purposes, and they are made by other types of cells too. It is not possible to give a detailed description of the manufacture of all the enzymes mentioned in Table 2. Therefore, in this article detailed information will be presented for the production of glucoamylase. The glucoamylase enzyme is a large volume product that is used by the sugar and starch industry to generate glucose. The glucose is used for different purposes, for example the conversion, by another enzyme, i.e. glucose isomerase, to high fructose syrup. Glucoamylase is considered one of the main industrial enzyme products and the solid state fermentation is described for the production of this enzyme (submerged fermentations are also used for the production of this enzyme in industry).

Another enzyme of interest is the milk clotting enzyme, rennin (chymosin, rennet), used in cheese production. The original source is the lining membrane of the fourth stomach of the calf. There are many alternative sources for rennin that have been developed and are also in use, since the supply of the fourth stomach of the calf is limited. One of the ways to produce rennin is by submerged fermentation of the filamentous fungi of the species *Mucor*.

2.3. Biomass

There are many foods produced with the aid of fungi, changing the texture and taste of the material used as substrate (see also – *Fermented Foods and Their Processing***]**. In one case the growth of a filamentous fungus biomass under submerged conditions is the source for a novel food. The fungal biomass has been named Quorn® to be described in this article.

2.4. More recent and potential products

Other fungal products already in production and with a great promise for the future are recombinant proteins **[**see also – *Industrial Recombinant Protein Production***]**, produced by filamentous fungi, as well as the potential for growth of higher fungi in liquid culture.

3. Solid State Fermentation

Solid State Fermentation (SSF) is defined as the growth of microorganisms on moist solid substrate **[**see also – *Microbial Cell Culture*]. The growth can be on natural substrates (termed Solid Substrate Fermentation) or on inert substrate used as solid support. In both cases enough water is present to maintain microbial growth and metabolism. There is no free-moving water and air is the continuous phase. SSF technology provides many new opportunities, as it allows for the use of agricultural waste products as fermentation substrates, without the need for extensive pretreatment

of the substrate.

SSF involves the heterogeneous interactions of microbial (fungal) biomass with moistened solid support. In SSF, the microorganisms can grow between the solid fragments, i.e., inside the matrix, or on the surface.

The microbial biomass inside the matrix and on the surface consumes substrates and secretes metabolites and enzymes. As there is no active transport in the solid mass, concentration gradients are the driving forces to supply the substrates and to remove the products. Gradients in the concentrations of substrates and products may also cause local differences in metabolic activity.

Similarly, gradients in the concentrations of inducers or repressors may affect expression of different genes. These gradients are the most typical differences between SSF and submerged fermentation (SmF) and it is assumed that the gradients contribute to the observed differences in gene expression, metabolism, product spectrum, and process efficiency between SSF and equivalent SmF processes.

The main disadvantage of SSF processes are that they are hard to reproduce compared to SmF processes. However, SSF compared to SmF is claimed to be simpler, requires lower capital, has superior productivity, reduced energy requirement, simpler fermentation media, no need for rigorous control of fermentation parameters, uses less water, produces lower volumes of wastewater and requires low cost for downstream processing. In the SSF process, the solid substrate not only supplies the nutrients to the culture, but also serves as an anchorage for the microbial cells.

Filamentous fungi are the most commonly used microorganisms in SSF. This is mainly due to their high potential to excrete hydrolytic enzymes, their relatively high tolerance to low water activities, and their morphology.

The filamentous fungi colonize the surface of the substrate and also penetrate into the substrate matrix in search for nutrients, in those cases where the solid support is not inert. SSF is ideal in cases where the purity of the product, for example in enzyme production, is of less importance.

In many cases the enzyme formulation is the fungal biomass, where the enzyme activity resides together with the substrate, after growth and production are terminated and used as is as the “enzyme preparation”.

3.1 Products from Solid State Fermentation

3.1.1 Gibberellic acid – GA_3

Gibberellins are diterpenes synthesized from acetyl CoA via the mevalonic acid pathway. The first gibberellin to be structurally characterized is referred to as GA_3 (Figure 1).

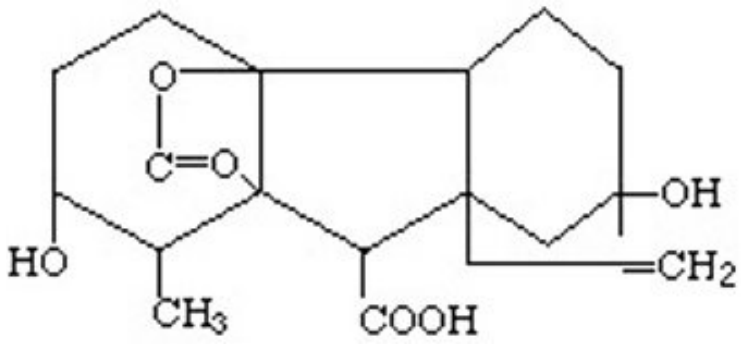

Figure 1: Chemical structure of gibberellic acid (GA_3)

The elongation of rice plants induced by cell free culture filtrates of a fungus (*Fusarium moniliforme*) was already demonstrated in 1926. Gibberelins function as plant growth regulators, influencing a range of developmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction and leaf and fruit senescence. GA_3 is used in agriculture to eliminate dormancy of seeds, in nurseries, for viticulture, in tea gardens, for induction of flowering and acceleration of germination in the brewing industry. The annual world production of GA_3 is estimated to exceed about 25 tons, with a market value of US $100 million. Improved strains are available that produce GA_3 and also the precursors GA_4 and GA_7, at concentrations of several grams per liter by specialized fermentation conditions.
The mechanism of action of the gibberellin GA_3 (the most studied gibberellin) is thought to be due to molecular rearrangements in the cell wall matrix of the plants that could promote wall extension.

The main producers are strains of *Gibberella fujikuroi* (the perfect stage of *Fusarium moniliforme*) from which the genes have been cloned **[***see also– Genetic Engineering of Fungal Cells]* and well characterized. Certain agricultural and horticultural applications specifically require GA_4 instead of the more traditional use of GA_3. The final two enzymes in the GA biosynthetic pathway leading to GA_3 in *G. fujikuroi* is of special biotechnological interest. The inactivity of these enzymes allow for construction of strains producing GA_4 and GA_7 as the main products of the *G. fujikuroi*, with the absence or low concentrations of GA_3. Such strains are of more interest for industry (see below).

Production of gibberellins by *Gibberella fujikuroi* is dependent on the quality and quantity of the carbon and nitrogen source, and is stimulated by a high carbon to nitrogen ratio. The initiation of gibberellins production is concomitant with the exhaustion of the nitrogen source. The SSF fermentation is recommended by many sources due to its advantages in use of agricultural waste and also with the claims for higher concentrations of gibberellins. With substrates such as corn grains, wheat bran and rice, the accumulation of GA_3 was reported to be 1.6 times higher than in submerged culture, based on equivalent carbohydrate content of the medium. A concentration of 3 g GA_3 per kg dry weight of the solid substrate was produced under sterile conditions in a 50 liter fermentor with solids as substrate.

The use of submerged fermentation is well described in the literature and there are several patents describing production conditions. The industrial process used for the

production of GA_3 is mainly based on submerged fermentation (SmF) techniques. The fermentation conditions are growth of the fungus at a temperature of 28-32^{o}C, at a pH of between 5 and 7 and time of 4 to 6 days. A typical culture medium contains about 0.4 g L^{-1} of nitrogen source and about 75 g L^{-1} of carbon source (usually a carbohydrate).

Despite the use of the advanced process technology, the yield of GA_3 reported for SmF is lower then SSF. The presence of the product in dilute form in SmF was also recognized as a major obstacle in economic manufacture of the product. This is due mainly to the consequent higher costs of downstream processing and also disposal of wastewater. The SSF technique is also at its advantage in countries with large agricultural residues.

Recently mutants have been developed producing a dissimilar mixture of gibberellins, with less GA_3 and more of GA_4 andGA_7. GA_3 primarily stimulates the growth of stem and leaves while GA_4 andGA_7 have their main effect on flowering and also cause fruit cells to elongate, with the result of enhancement of fruit development. Concentrations of over 1 g L^{-1} are reported to be produced; where at least 50 % is GA_4 and GA_7.

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Biographical Sketch

J. Stefan Rokem is a Senior Lecturer at the Department of Molecular Genetics and Biotechnology at the Hebrew University of Jerusalem, Israel. He earned his M.Sc. in Microbial Biochemistry and Fermentation Technology from the Imperial College of Science and Technology at the University of London, United Kingdom. He completed his Ph.D. studies in Applied Microbiology at the Hebrew University of Jerusalem, Israel with a thesis on "Production of Single Cell Protein". In addition to his teaching duties at the Hebrew University, Dr. Rokem serves as a scientific consultant to local biotechnology companies and is a reviewer of several journals and granting agencies. He has been on the executive committee of the International Organization of Biotechnology and Bioengineering for several years, serving as one of the representatives of the Middle East. Dr. Rokem has given several guest lectures and courses in Bioengineering and the use of Biotechnology for the improvement of the Environment in South and Central America. Recently Dr. Rokem developed a novel curriculum in Biotechnology for the Open University of Israel. During his academic career he has co-authored over 60 peer reviewed papers, written several book chapters, co edited one book and written a monograph on the use of Biotechnology to Improve the Environment. The main research interests of Dr. Rokem are the regulation of antibiotic production in *Actinobacteria*, production of organic acids with filamentous fungi and the use of Biotechnology to reach Sustainable Development, mainly novel methods for sewage treatment. He has spent sabbaticals at Yale University, New Haven, CT, USA; Royal Institute of Technology, Stockholm, Sweden; MIT, Cambridge, MA, USA and The Danish Technical University, Copenhagen, Denmark.