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Fungi in biotechnology. Past, present, future

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From multiple uses of fungi in early historic times, “early biotechnological processes” developed. Fungi were used here already e.g. for production of cheese, bread, wine, beer and other foodstuff.

Based on these processes, fungi today have become one of the most important group of organisms in modern technology, where food, fodder and various metabolites such as antibiotics, enzymes, steroids etc. are produced on an industrial scale.

An equally important role of fungi in biotechnology is also to be expected for the future where – in addition to existing uses – fungi will be increasingly used employing modern methods such as genetic engineering and will also new applications as in pollution control, biological control of pests, microbial leaching and even biotechnology.

Key words: Fungi, biotechnology, past, present, future

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Z četných použití hub v ranných historických dobách se vyvinuly „ranné biotechnologické metody“. Houby byly při nich už používány při výrobě sýrů, chleba, vína, piva a dalších potravin. Houby se dnes staly, na základě těchto metod, jednou z nejdůležitějších skupin organismů v moderních biotechnologiích, kdy potraviny, krmiva a různé metabolity jako antibiotika, enzymy, steroidy atd. se vyrábějí na průmyslovém základě. Rovnocenná role hub v biotechnologiích je očekávána i v budoucnu, kdy – kromě řady dosavadních použití – budou houby využívány za účasti moderních metod, jako je genetické inženýrství a naleznou využití při zvládnání znečištění prostředí, biologickém potlačování škůdců, mikrobiálním vyluhování a možná i v biotechnologiích v kosmickém prostoru.

INTRODUCTION AND DEFINITION

Fungi are becoming more and more important in our life. Therefore, as a start for the symposium on FILAMENTOUS FUNGI IN BIOTECHNOLOGY a short survey of the role of fungi in biotechnology in the past, in the present and in the foreseeable future shall be presented (see also Molitoris 1989, 1990a).

Biotechnology is a relatively new term meaning "the use of organisms for the production of biomass or metabolites on an industrial scale". BIOTECHNOLOGY involves and interconnects the fields of BIOLOGY (the organisms involved), ECONOMICS (costs), KINETICS (physical/chemical influences on biotechnological processes) and PROCESS MANAGEMENT (see Lafferty 1981).

FUNGI AND MAN

In their relation to man, fungi may be considered as FOE or as FRIEND (Table 1).

Tab. 1 Fungi and Man

FOE	FRIEND
Spoilage	Mineralization
Pathogen	Mykorrhiza
Allergen	Food and Fodder
Toxin production	Medicine
	Religious/mystical ceremonies
	Science and Research
	Biotechnology
	Biological control
	Pollution control

Examples for the negative action of fungi are their activities in causing SPOILAGE of natural substrates (dry-rot of wood in buildings by *Serpula lacrymans*) and man-made substrates (plastics by *Penicillium simplicissimum*), their action as PATHOGENS FOR PLANTS (corn-smut of maize, *Ustilago maydis*), for animals and man in the latter case not only as ALLERGEN but also causing often severe diseases (blastomycosis by *Blastomyces dermatitis*) or INTOXICATIONS (poisoning by consumption of *Amanita phalloides* poisoning).

In many respects, however, fungi have important and positive values in nature and for man: They are part of the microbiota in soil causing MINERALIZATION by degradation of organic material and as MYCORRHIZA they are important symbionts for many higher plants, especially trees. Coming to the topic of this review, they are used in the production of FOOD and FODDER; since ancient times they are used in MEDICINE (Molitoris 1978, 1994) and more recently they have become important tools in SCIENCE and RESEARCH and are used increasingly to fight increasing problems of our modern society in the BIOLOGICAL CONTROL OF PATHOGENS and in POLLUTION CONTROL.

FUNGI IN BIOTECHNOLOGY IN THE PAST

In the history of mankind, fungi have been used in various ways for a variety of purposes (Table 2).

Tab. 2 Fungi in biotechnology in the past

EARLY GENERAL USES OF FUNGI	EARLY BIOTECHNOLOGICAL PROCESSES
Food	Cheese
Poison	Bread
Medicine	Beer
Mystical/religious ceremonies	Wine
	Cultivated edible mushrooms

Early general uses of fungi

Food

Since earliest times, fungi have been widely used as FOOD as symbolized in the medieval fresco of the chapel of Plaincorault in France (1291 a.C.) where Adam and Eve in the famous temptation scene are seen eating from a "fungus-tree" (Wasson 1968).

Poison

Fungi were important also as POISON, either consumed accidentally or administered purposely like in the alleged poisoning of the Roman emperor Claudius by Agrippina (Ramsbottom 1972). They were also used for causing artificial abortion by the "wise women" in the middle ages which gave for this purpose the alkaloid-containing sclerotia of ergot (*Claviceps purpurea*), a fungal parasite of grasses.

Medicine

Ergot by the way, was used also as MEDICINE already in the middle ages in gynaecology and other areas, as also many other fungi have been used as medicinal plants (Molitoris 1978, 1994).

Mystical/religious ceremonies

Some fungi, in particular hallucinogenic ones, such as *Psilocybe cubensis* or *Amanita muscaria* have been used by many ethnic groups in mystical, long-forgotten religious or shamanic ceremonies, which often date back thousands of years as documented by the so-called "fungus stones" from Guatemala in Middle America (Heim, 1963).

"Early biotechnological processes" involving fungi

The use of fungi by early man led in some cases to processes which could well be termed "early biotechnological" processes by procedure importance, amount and value of the products.

Cheese

One of the earliest examples would be the production of milk products such as CHEESE. When man turned from gatherer and hunter to be nomad and later farmer, herds of domesticated cattle gave meat, milk and other goods. Milk is one of the most nourishing but also perishable natural goods, which by action of microorganisms either can be destroyed but also can become a stable, nutritious and delicious food like cheese. One of the earliest documents for the use of milk is an almost 4000 year-old relief in the Sumerian town of Ur showing the whole procedure from getting the milk until making butter and/or cheese.

Bread

Another early example would be the preparation of BREAD from ground cereals where yeasts under appropriate conditions (water, temperature) are responsible for the leavening of the dough by their carbon dioxide production. Early bread-making has been shown in an old Egyptian relief dating back about 4500 years.

Already in ancient times BEER and WINE, two alcoholic beverages, were produced in large amounts and played an important role as recreational drinks, but also as necessary food. Here fungi (yeasts) convert the sugar of cereals and grapes into carbon dioxide and alcohol.

Wine

For making WINE particularly the Greeks, Jews and Romans were known in ancient times. This is often documented in sculptures, paintings and written documents as in the Holy Bible (the miracle of the wedding of Kanaan).

Beer

Also the history of beer-making goes back a very long time as is illustrated by the famous relief from ancient Babylon, showing two persons drinking beer with straws from an amphora. The explanation for this unusual way of drinking beer is of course that filtration of beer in ancient times was an unknown technique. Therefore the fresh beer was cleared first by sedimentation of the particles and then the supernatant beer was drunk with straws. It might be also interesting to know that the old Babylonians knew already about 20 different kinds of beer.

BEER and WINE became more and more important as food and even as medicine (every Roman soldier had to drink daily 2 liters of wine for his health – happy times!). No wonder that some of the oldest food laws were concerned with these liquids. The German “purity law for beer” of 1516 exactly regulated ingredients, production and marketing of beer and punished those not obeying it. This law is still in effect in Germany.

Cultivated edible mushrooms

Cultivation of edible mushrooms most probably started more than thousand years ago in the Far East with mushrooms such as *Lentinus edodes* (“Shii-take”).

In middle Europe the first reports are from the Middle ages about cultivation of the edible mushroom *Agaricus bisporus* in caves in France.

FUNGI IN BIOTECHNOLOGY IN THE PRESENT

From the impressive list given in table 3, just a few items will be discussed.

Tab. 3 Fungi in biotechnology in the present

FOOD	PRODUCTION OF FOOD AND FODDER
Mushrooms	Edible Asiatic fermentation products
Fungal biomass	Alcoholic beverages
	Milk products

METABOLIC PRODUCTS		
Alcohol	Organic acids	Vitamins
Steroids	Amino Acids	Hormones
Antibiotics	Nucleic acids	Ergot alkaloid
Enzymes		

RESEARCH	OTHER
Genetics	Biological control
Metabolism	Pollution control
	Recycling

Food

Fungi as food presently play an important role in our economy by amount and value.

Edible mushrooms

Edible fungi for human consumption are cultivated nowadays in highly mechanized, automatized and even computerized enterprises world-wide. This production is constantly increasing by rising demand, more efficient procedures, but with decreasing prices. Advantages of the cultivation of edible mushrooms are cheap substrates – even waste products such as straw – no need to use arable land, excellent flavour, high vitamin and mineral content, and in some cases even positive health effects such as in *Lentinus edodes*.

World-wide, the production of cultivated edible mushrooms reaches now more than 4 million tons per year, with the cultivated mushroom, *Agaricus bisporus*, leading, followed by the oyster mushroom, *Pleurotus ostreatus*, the Shiitake mushroom, *Lentinus edodes*, and others as shown in table 4.

Tab. 4 World production of cultivated mushrooms in 1991 (from Chang 1993)

Species	(common name)	Production (tons x 1000)
<i>Agaricus bisporus</i> and other spp.	(cultivated mushroom)	1,590
<i>Pleurotus ostreatus</i> and other spp.	(oyster mushroom)	917
<i>Lentinus edodes</i>	(Shii-take)	526
<i>Auricularia auricula-judae</i>	(jew's ear mushroom)	465
<i>Volvariella volvacea</i>	(straw mushroom, Chinese mushroom)	253
Others		531
Total		3,742

Biomass

In contrast to this, fungal biomass production from submerged cultivation on originally cheap hydrocarbons (single cell protein = SCP's), which was intended for fighting hunger in third-world-countries, did not fulfil the expectations because of rising costs of the substrates and for food preferences in those countries for which the product originally was meant for.

The only exception is QUORN, a British fermentation product from the Deuteromycete *Fusarium graminearum* which fulfils strict foodstuff requirements, has good nutritional values and seems to be produced at compatible prices.

Production of food and fodder

Presently about 25% of our food is already produced by biotechnological processes and fungi play an important part in it.

Alcoholic beverages

In the western world WINE and BEER are leading in importance and also this country is famous for these products. So one of the great typical beers, the "Pilsener" with its typical taste, originates from the Czech town of Pilsen, where it was originally brewed.

The production of WINE, the other major alcoholic drink, is still increasing. By new and improved methods and new breeds of grapes, new areas such as California and Chile in America, South Africa and Australia, in recent decades started to grow and sell quality wine with increasing success.

Milk products

CHEESE is still one of the most important fungal products from milk. A number of these cheeses are quite typical for certain areas and are even named for them, e.g. "Roquefort" (caves of Roquefort, France), "Edamer" (town in Holland), "Emmentaler" (valley in Switzerland), "Danish Blue" or "Bavarian Blue" and last not least the "Camembert" from which the producing fungus, *Penicillium*

camemberti, got its name. More recently, also other fungal products from milk, such as Kefir, Yoghurt and others, gain more and more importance.

Metabolic products

One of the main areas where fungi are used today in biotechnology, is in the production of certain metabolites for various purposes as given in table 3.

Alcohol

Alcohol is still a fungal product of major importance. It has changed, however, insofar as it is not only used in beverages, but is used increasingly as a component of medicines and for preparative and synthetic processes in industry. More recently, however, the amount of alcohol produced from prokaryotes, is increasing.

Steroids

Steroids became very important since the advent of the anti-baby pill and also for other medical purposes. Fungi such as several *Penicillium* as *Aspergillus* strains by virtue of their ability to catalyse very specific one- or few-step transformations in steroids became essential in the production of these drugs.

Antibiotics

Since Alexander Fleming in 1928 discovered the production of the first antibiotic, Penicillin, from the imperfect fungus *Penicillium notatum*, everyone knows about the importance of these metabolites in medicine. About 6000 antibiotics are known today, at least by their formula, but only about 400 of them, for various reasons, are used in human medicine. Fungi produce about a quarter of them. Constantly new antibiotics are looked for and found, a necessity by the fact that pathogenic microorganisms constantly acquire resistance against the known and administered antibiotics.

Enzymes

Quite a number of fungal enzymes are produced commercially and have become indispensable tools in industry and research (Table 5). As the table shows, they are used for a wide variety of applications from biochemical analysis, over medical diagnosis to food processing.

Organic acids, amino acids, nucleic acids

Among the organic acids of fungal origin, citric acid is leading with an annual production of over 600.000 tons. It is mainly used in the food and beverage industry. But also amino acids and nucleic acids are commercially produced from fungi and find wide application.

Ergot alkaloids

Fungal alkaloids, mainly originating from ergot, *Claviceps purpurea*, have been mentioned earlier mainly as poison, an abortivum and occasionally as medicinal drug. The latter use nowadays is their main importance. These alkaloids are

produced now in increasing amount by saprophytic culture or even by biosynthesis using certain – even genetically engineered – strains. These alkaloids find now wide medicinal application e.g. in gynaecology, against migraine and in the treatment of heart and circulatory diseases.

Tab. 5 Important industrial enzymes from fungi (Molitoris 1991)

Enzyme	Use
OXIDOREDUCTASES (EC 1.)	
Glucose oxidase	Taste intensifier
Catalase	Removal of hydrogenperoxide
HYDROLASES (EC 3.)	
Esterases (Ec 3.1)	
Lipase	Aroma development, aid for extraction
Ribonuclease	Taste intensifier
Glucosidas (EC 3.2)	
α -Amylase	Baking and brewing industry
β -Amylase	Starch hydrolysis
Dextranase	Cosmetic industry (tooth paste)
Glucoamylase	Starch hydrolysis, brewing industry
Hemicellulase	Food industry
Invertase	Production and processing of sweets
Lactase	Milk- and baking industry
Melibiose	Sugar production from sugar beets
Naringinase	Beverage industry (removal of bitter taste)
Pectinase	Beverage industry (fruit juices)
Xylanase	Food industry
Cellulase	Food-, cellulose-, paper-industry, SCP production
Peptide hydrolases (EC 3.4)	
Protease	Food industry, medicine (peptic preparation, digestion)
Rennin	Milk industry (cheese production)

Research

Genetics

Because fungi possess a relatively low level of organisation and are easy to handle in the laboratory, fungi represent increasingly important tools in modern research, especially in genetics. The different types of life cycles, the different ways of genetic inheritance and e.g. the possibility to identify by tetrade analysis in certain

ascomycetes the stepwise production of the meiosis products, are advantages of this group of microorganisms. Successes in this area of research are documented e.g. by the first Nobel Prize given for fungal research in 1958 for physiology and medicine to BEADLE and TATUM for their work with the red bread mould *Neurospora crassa*.

Metabolism

Fungal metabolites are indispensable substances in modern research. Fungal antibiotics e.g. already mentioned as highly efficient medicaments, are used also in research. The antibiotics Penicillin, whose action against many pathogenic bacteria is based on its effect on the bacterial cell-wall synthesis, is used to analyse and identify specific steps in this important morphogenetic process.

Other

Biological control of pathogens

To reduce the need for chemicals in control of pests and pathogens, recently much work has been done to find biological control measures. These methods would reduce the need for chemicals, which are expensive, relatively unspecific and also cause environmental problems because of their toxicity and recalcitrance. Again, in this quite modern aspect of biotechnology, fungi are involved, particularly because they show a high specificity of action (Burge 1988).

Nematodes, a deadly pest for mushroom culture, can efficiently be controlled by certain fungal preparations (Stirling 1988), such as from *Arthrobotrys robusta* ("Royal 300").

Some of the entomopathogenic fungi, fungi pathogenic against insects, can be used for control of insect pest, such as "Mycotal" and "Vertalec", preparations from *Verticillium lecanii*, which is used in the green houses industry (Quinlan 1988).

And finally, fungi may be used to fight fungal diseases as the Deuteromycete *Trichoderma* can be used against a number of pathogenic fungi, in particular if used as "integrated control" in connection with other control measures (Papavizas and Lewis 1988).

Pollution control

As already indicated above, biological control measures using fungi in turn help in reducing the need for chemicals in pest and pathogen control. They therefore are one new and important aspects in the involvement of fungi in the ever more necessary measures for the protection of our environment.

Recycling

Recycling in other word, making use of materials which otherwise would contribute to pollution or are at least useless, can consider as another aspect of environment protection. Two methods from mushroom cultivation might serve here as example.

First, the oyster mushroom, *Pleurotus ostreatus*, can be grown on straw as sole substrate. It has been found in this country that spent *Pleurotus* compost, being very nutritious and having a good smell, can be used as an addition to the fodder for cattle. In this way straw, an almost costless agricultural surplus product is totally recycled, partially giving rise to fruitbodies (ca 10% of substrate weight), partially replacing peat in gardening, and partially being used as fodder.

Secondly, experiments in Germany have shown that edible mushrooms can be successfully grown on composted household garbage. The reason, however, why this method has not been introduced into practise is, that unfortunately the average content of heavy metals in ordinary household is too high and results in too high contents of these substances in the fruitbodies produced on this substrate.

FUNGI IN BIOTECHNOLOGY IN THE FUTURE

In the future many of the biotechnological uses of fungi are expected to continue, even at an increased scale, and new application will be added. Some of these products and areas are listed in table 6.

Tab. 6 Fungi in biotechnology in the future

Food
Metabolic products
Genetic engineering
Microbial leaching
Biological control of pests and pathogens
Environments protection
Research
Basic research
Space biology

Food

Besides using other fungi for the production of new types of food such as QUORN, constantly new strains and species of edible fungi are investigated for mushroom cultivation. An interesting example is the cultivation of the edible morel, *Morchella conica*, based on an US patent, which is reported to enter now an economically feasible stage (Coombs 1994).

Metabolic products

Constantly new, better and more metabolites will be produced in the future by fungal species, some of them modified by genetic engineering. This is true for work on the production of "Hirudin" (HOECHST AG, Germany), for human "Insulin" (NOVO, Denmark) and for new types of brewing yeasts. In all cases yeasts are used

because of the relative ease and efficiency of their genetic manipulation, cultivation and metabolic production.

Genetic engineering

Genetic engineering usually includes in principle the following steps. First: Isolation of the genetic information for the production of an important metabolite from a naturally producing organism (often a higher plant or an animal, usually slow growing and producing). Second: Transfer of that genetic information into another organism (usually a fast growing and fast producing microorganism). Third: Integration of this information into its genetic material. Finally these microorganisms, in our case, fungi, often yeasts, produce with modern fermentation technology the desired metabolites faster, in higher quantities and at much lower costs because of their simple growth requirements and modern fermentation technology.

Microbial leaching

Bacterial leaching of ores by now is a well established method to obtain economically valuable metals from ores too low in metal content for conventional mining. The principle is that the metal-containing ores are percolated with microorganism-containing liquids, the metals are solubilized by the bacterial metabolism and may be later extracted or precipitated. Recently, methods are being developed to employ also fungi (*Saccharomyces*, *Rhizopus*), for specific extraction of such valuable metals as manganese, uranium, gold and platinum (Gröger et al. 1987).

Biological control

A number of promising projects involving fungi are being investigated, e.g. control of the deadly chestnut blight or the Dutch elm disease, using hypovirulent strains of the respective fungal pathogens (Adams 1988).

Environment protection

A number of projects are concerned with the use of fungi for degradation of waste or toxic materials. Of particular interest are here the often highly toxic xenobiotics, such as polycyclic aromatic hydrocarbons, where research at the Czechoslovak Academy of Sciences and in our own laboratory has shown that fungi, particularly ligninolytic fungi, are able to degrade a high proportion of these substances in a relatively short time (Vyas et al. 1994a, Vyas et al. 1994b).

Other problems are caused by our indispensable plastic materials, because they are usually produced from the rapidly diminishing fossil resources and because of their lacking biological degradability (originally considered to be an advantage). As a possible solution new microbial biosynthetic thermoplasts from renewable resources (e.g. BIOPOL, ICI, England) have been developed. However, in order to use them generally and in large quantities, their complete bio-degradability (without toxic residues) has to be established first. Research concerning the ability

of fungi to degrade these materials is being conducted in our laboratory and the results look quite promising.

Research

Also in the future fungi will be involved in research. Among these projects are the elucidation of basic principles like the effect of gravity on living systems. Because of their low level of organisation and good handability, fungi again are investigated in the laboratory to solve these questions and because of new and interesting approaches a new aspects of the use of fungi in space biology research are presented.

The recent developments in space flight and the possibility to conduct research in orbit, recently provided the chance to investigate living systems under space conditions such as (near) weightlessness (microgravity), cosmic irradiation and low pressure. A few examples for the involvement of fungi in this type of research shall be presented, which in some cases may lead to new biotechnological processes (Molitoris 1990b).

Genetic engineering often involves optimisation of hybrid formation. Electro-fusion is such a method, where cells are brought into contact in an electric field, form chains and are hybridised by electric pulses. Experiments using yeast cells under simulated and actual space conditions (US/German space mission D-1, 1985) have shown a much higher yield of hybrid cells than on the ground since thermoconvection and sedimentation do not interfere.

Finally let us come back to a product where fungi were essential in biotechnology already in the past and still are important in the present and certainly will be also in the future, let us speak about beer: In the last US/German spacemission (D-2, 1994), space experiments with brewers yeast were conducted. Whether they were successful, the forthcoming publications will show.

Summarising the role of fungi in biotechnology in the past, in the present and in the future, we can state:

Fungi in biotechnology are important and their future certainly looks bright,
but it depends on us, on the scientists and the people who use our results,
whether our positive expectations may be fulfilled,
so that modern biotechnology with fungi
does not destroy but protects and improves
the blue planet earth on which we live.

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