ELSEVIER

Journal of Biotechnology 66 (1998) 101–107

JOURNAL OF Biotechnology

Review article

Mycotechnology: the role of fungi in biotechnology[1]

J.W. Bennett *

Department of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118, USA

Received 24 February 1998; accepted 8 April 1998

Abstract

Fungi have been important in both ancient and modern biotechnological processes. Processes and products that utilize fungi include baking, brewing, and the production of antibiotics, alcohols, enzymes, organic acids, and numerous pharmaceuticals. The advent of recombinant DNA technology and large scale genomics analysis has placed yeasts and filamentous fungi in the forefront of contemporary commercial applications. The term 'mycotechnology' is introduced here to describe the enormous impact of fungi on biotechnology. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Fungi; Biotechnological processes; Recombinant DNA technology; Genomics analysis; Mycotechnology

1. Introduction and definitions

Fungi are lower eukaryotes classified by modern biologists into their own kingdom, sometimes called 'The Fifth Kingdom' based on their absorptive mode of nutrition. Fungi secrete a wide range of powerful enzymes into the environment and then absorb these 'pre-digested' foodstuffs back into their cells. Diverse in morphology, physiology and ecology, many of the best known fungi have a negative impact on human welfare as agents of plant disease (e.g. smuts, blights, wilts, rusts), biodeterioration (rots and mildews) or as animal pathogens (e.g. as producers of toxins and mycoses). Fungi range from microscopic molds and yeasts to macroscopic mushrooms and truffles. Several species of macrofungi are considered delicacies and are gathered or cultivated for the human food supply. However, it is the microscopic species, which include genera such as *Aspergillus*, *Penicillium* and *Saccharomyces*, which are best known for their positive impact on hu-

* Corresponding author. Fax: +1 504 8656785; e-mail: jbennett@mailhost.tcs.tulane.edu

[1] Based on a lecture held at the symposium, 'Progress in US Biotechnology', at the 8th European Congress on Biotechnology (ECB8) in Budapest, Hungary, August 1997.

PII S0168-1656(98)00133-3

man affairs. They have been harnessed for their metabolic activities, either as producers of degradative enzymes or synthesizers of useful metabolites. These fungi form an important cornerstone of modern biotechnology.

Biotechnology has been defined in many ways. The Spinks Commission in the United Kingdom was one the first groups to adopt a formal definition: "Biotechnology is the application of biological organisms, systems, or processes to manufacturing and service industries". The European Federation of Biotechnology uses a similar but somewhat broader definition: "…the integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application of the capabilities of micro-organisms, cultured tissue cells, and parts thereof". The definition used by both the US National Institutes of Health and Food and Drug Administration is wordier still: "Biotechnology is the application of biological systems and organisms to technical and industrial processes. The technologies employed in this area include: classic genetic selection and/or breeding for the direct in vitro modification of genetic material, e.g. recombinant DNA, or gene splicing, and other novel techniques for modifying genetic material of living organisms, e.g. cell fusion, and hybridoma technology, etc." For a compilation of these and other formal definitions of biotechnology see Bennett et al. (1997).

To recapitulate, most definitions of biotechnology are comprehensive and encompass fermentation processes from wine to penicillin, as well as a broad spectrum of contemporary methodologies that grow out of recombinant DNA technology. I use the coined term 'mycotechnology' to include the many biotechnological processes, both old and new, that rely on fungal products and processes. This survey will emphasize filamentous fungi.

2. 'Pre-modern' mycotechnology

Modernism is a general term used to describe 20th century attempts to break with the traditions of the 19th century. In painting, abstraction replaced direct representation; in architecture functionalism replaced ornamentation; in literature, new stylistic forms replaced traditional narrative. In everyday life, the extensive application of the discoveries of basic science revolutionized the way people lived. The adjectives 'premodern', 'modern', and 'postmodern' are used here as a descriptive device to survey the enormous number of process and products encompassed under the rubric of fungal biotechnology.

Beer, wine, bread, koji, and other fermented foods and beverages have been part of the human diet for millenia. Their origins are lost in antiquity (Table 1). It is clear from the historical record, however, that people indirectly knew of microorganisms such as molds and yeasts by their activities. Scientific study of these activities, aided by the microscope, led to the disciplines now called microbiology and biochemistry. For example, after Pasteur observed that living organisms could always be seen under the microscope during sugar fermentations, the organisms producing them—mostly yeasts—were called 'organized ferments'. The changes which occurred in solutions without any detectable microorganisms were called 'unorganized ferments'. Later, when it became apparent that the 'unorganized ferments' were generally the metabolic products of 'organized ferments', Kuhne suggested the new word: 'enzyme' (en = in,

Table 1
'Premodern' examples of mycotechnology

Process	Organism
Asian food fermentations	
Ang-kak	*Monascus purpurea*
Miso	*Aspergillus oryzae*
Ontjam	*Neurospora crassa*
Soy sauce	*Aspergillus oryzae*, *A. sojae*
Tempeh	*Rhizopus niveus*
Brewing and baking	*Saccharomyces cereviseae*, *S. carlbergensis*
Mold-ripened cheeses	*Penicillium roqueforti*, *P. camembetii*
Mushroom cultivation	*Agaricus bisporus*, *Auricularia* sp., *Flammulina velutipes*, *Lentinus edodes*, *Pleurotus* sp., *Volvariella volvacea*

After Gray (1970), Chang and Hayes (1978), and Hesseltine (1983).

Table 2
'Modern' examples of mycotechnology

Process or product	Organisms
Antibiotics and other pharmaceuticals	
Penicillins	*Penicillium chrysogenum*
Cephalosporin	*Cephalosporium acremonium*
Cyclosporin (immunosuppressive)	*Tolypocladium inflatum*
Ergot alkaloids	*Claviceps purpurea*
Griseofulvin	*Penicillium griseofulvin*
Mevalonin	*Aspergillus terreus*
Enzymes	
α-Amylases	*A. niger*, *A. oryzae*
Cellulase	*Humicola insolens*, *Penicillium funiculosum*, *Trichoderma viride*
Glucoamylases	*Aspergillus phoenicis*, *Rhizopus delemar*, *R. niveus*
Glucose oxidase	*A. niger*
Invertase	*A. niger*, *A. oryzae*
Laccase	*Coriolus versicolor*
Pectinase	*A. niger*, *A. oryzae*, *Humicola insolens*
Proteinases	*A. oryzae*, *Aspergillus melleus*, *R. delemar*
Rennin (microbial)	*Mucor miehei*, *M. pusillus*
Organic acids	
Citric acid	*A. niger*
Itaconic acid	*A. terreus*

After Turner (1975), Bigelis (1985), and Godfrey and West (1996).

zyme = yeast). Ultimately, 'enzyme' came to mean all 'unorganized ferments', not just those produced by yeasts (Lutman, 1929).

3. 'Modern' mycotechnology

The enzymes, alcohols, organic acids, and pharmaceuticals from filamentous fungi are central to the development of modern biotechnology. Some examples of major industrial fermentation products and their producing species are given in Table 2.

One of the best studied of the early enzymes was diastase (amylase), traditionally isolated from germinating barley and used for the malting step in beer manufacture. Jokichi Takamine, in 1894, was probably the first to realize the technical possibilities of enzymes from molds and to introduce these fungal enzymes to industry. Takamine used the Japanese koji mold, *Aspergillus oryzae*, to produce diastase. Takamine inoculated mold spores on steamed rice or wheat bran and grew his cultures in thin layers. With a series of patents starting in 1894, he protected his processes and suggested new uses for partially purified fungal diastase as a substitute for malting enzyme, and as a digestive aid for the treatment of dyspepsia (Takamine, 1894; Underkofler, 1954). During the early years of the Twentieth Century, similar processes were developed for numerous other enzymes. By 1983, there were approximately 30 different classes of enzyme in common commercial use, of which approximately half were of fungal origin. The number of commercial enzymes is growing at a rapid rate; an excellent summary of industrial enzymology has been compiled by Godfrey and West (1996).

Another filamentous fungal product central to the development of modern biotechnology is citric acid. Originally isolated from citrus fruits, since the end of the nineteenth century it was known that citric acid was also made by filamentous fungi. The development of a commercial process by Pfizer, in Brooklyn, New York, USA, soon led to widespread use of the compound in the food and beverage industry. Additional uses included tablets, cosmetics, detergents, antifoaming, textile treatment, and as a preservative for stored blood. Many aspects of modern fermentation technology were developed in the context of improving citric acid yields by manipulating culture conditions, by developing submerged processes, and improving product recovery (Crueger and Crueger, 1982).

Without a doubt, however, it was the discovery of penicillin, and the subsequent development of penicillin into a 'wonder drug' that was the turning point in the development of modern industrial microbiology (Wainwright, 1990). Penicillin triggered searches for other secondary metabolites with antibacterial activity, as well as stimulating research on fungal physiology, fermentation technology, and industrial strain development. So many new antibiotics were discovered during the 1940s and 1950s that it has been called The

Golden Age of Antibiotics. The research group associated with Selman Waksman at Rutgers University and Merck Corporation, in New Jersey, was particularly successful in screening for antibacterial metabolites. Many of the new antibiotics discovered by the Waksman group came from soil bacteria, especially actinomycetes. The filamentous nature of both molds and actinomycetes challenged chemical engineers to develop industrial-scale sterile fermentation procedures. Both batch and continuous methods were perfected (Smith and Berry, 1975; Demain, 1981). After the recombinant DNA revolution, these processes required only minor modification for the production of genetically engineered microbial products.

A development growing out of the Gold Age of Antibiotics was the systematic search for drugs with activities other than anti-infective. Laboratories in Japan under the leadership of Umezawa were particularly innovative in establishing new screens to look for anti-tumor, anti-hypertensive, immunostimulant, anti-diarrheal, anti-mutagenic and other such biological activities (Umezawa, 1982). Of these, the immunosuppressant cyclosporins and the anti-hypertensive mevalonins are two of the most important pharmaceuticals to be discovered from filamentous fungi (Von Wartburg and Trabor, 1986; Monghan and Tkacz, 1990).

4. 'Postmodern' mycotechnology

Recombinant DNA and its cognate technologies have revolutionized biology. As used here, the term 'postmodern' mycotechnology refers to the new biotechnological developments spawned by marrying the techniques of gene splicing and other post-recombinant DNA methodologies to those of conventional industrial mycology. Some examples of the developments coming out of this hybrid mix are listed in Table 3.

The expression of heterologous proteins in filamentous fungi has received considerable attention. Currently, the major production hosts are *Aspergillus nidulans*, *A. niger*, *A. oryzae*, and *Trichoderma reseii* (Davies, 1991). *Neurospora crassa* is also under development (Rasmussen-Wilson et al., 1997). Originally, researchers hoped to produce high levels of mammalian proteins in filamentous fungi. For bovine prochymosin (rennin) this was achieved for several species (Davies, 1991). However, the levels of expression and secretion for most mammalian gene products have been lower than hoped. The molecular steps of the fungal secretion pathway, post-translational modification of metabolites, and the release of proteins from hyphae into their environment are understood only in a descriptive sense (Wosten et al., 1991). There is a need for continued research on the molecular biology of filamentous fungal gene expression and secretion.

Secondary metabolic pathways are also amenable to molecular analyses. Predictably, the penicillin family of antibiotics was the first group of compounds to benefit from the new methodologies. After cloning the gene for isopenicillin *N*-synthase, it was found that many of the genes for the pathway were clustered; these were isolated in rapid order (Skatrud, 1991). It was discovered that certain high-yielding strains contained multiple copies of the genes for key enzymes in the penicillin pathway. In some cases it has been possible to engineer portions of the pathway using unusual precursors and production hosts, thereby embellishing nature's chemical diversity (Skatrud, 1992).

Table 3
'Postmodern' examples of mycotechnology.

Development	Examples
Expression of heterologous genes	Fungal, plant, mammalian
Amplification of homologous genes	Antibiotic pathways, enzymes
Manipulation of secondary pathways	New semi-synthetic antibiotics; hybrid antibiotics
Large scale genomics	*Aspergillus nidulans*; *Neurospora crassa*; *Magnaporthe grisea*; *Phytophthora infestans*
DNA chips	High-density DNA arrays for screening gene expression
'Mining' fungal biodiversity for new pharmaceuticals	Sampling environmental DNA genomics-based screening

Polyketides are another group of secondary metabolites that have benefited from molecular analyses. To date, most of the research on mixing-and-matching polyketide synthases has been conducted in actinomycetes (Kao et al., 1994); however, these strategies will also be useful in fungi. The permutations generated by the use of different starter units; adjustment of oxidation and stereochemistry during elongation; and the introduction of various post-polyketide modifications lead to an almost astronomical number of theoretically possible different molecules. By using engineered modular polyketide synthases, one can create 'unnatural natural products'.

The development of fungal genetic transformation systems has been used to bring the power of genetic analysis to species lacking sexual and parasexual cycles (Esser, 1997). Traditional fungal fermentations have been improved and genetic engineering makes it possible to tailor fungal enzymes for specific purposes. Both homologous and heterologous genes have been introduced into fungal production hosts. These genes can be manipulated in various ways in order to improve the properties of the enzymes or to increase yields. Enzymes with wider substrate range, temperature optima, and the like can be produced (Kinghorn and Lucena, 1994).

Even as traditional a field as mushroom cultivation has been stimulated by the new mycotechnology. Many delectable species cannot be cultured and must therefore be collected during their sporadic fruitings in nature. *Coprinus cinereus* (Pukkila, 1993) and *Schizophyllum commune* (Raper and Horton, 1993) have been used as models to study the genetic basis of fruit body formation in these species. It is predicted that improved strains of commercially cultivated mushroom can be obtained using similar breeding strategies based on molecular tools. Moreover, it is hoped that the isolation of genes associated with mushroom development in these laboratory models will facilitate understanding of fruiting in exotic wild species as well.

The acceleration and automation of DNA sequencing has made large-scale genomics possible, and genomics is changing all of biology. The first complete DNA sequence of an organism was for the bacterium *Haemophilus influenzae* Rd (Fleischmann et al., 1995); since then seven additional genomes have been completed and it is estimated that about 100 species are under investigation. The first complete eukaryotic genome was the yeast *Saccharomyces cerevisiae* (Dujon, 1996). The yeast genome consists of 16 chromosomes encompassing 12067266 base pairs (excluding repeats). Many yeast genes have mammalian homologues and this functional compatibility has been exploited in studying cancers and other human diseases (Botstein et al., 1997). On the other hand, most of the open reading frames identified by the sequencing project had gone unnoticed, i.e. they had no known phenotypes.

Genomics projects have been initiated for the yeasts *Schizosaccharomyces pombe* and *Candida albicans* as well as for several filamentous fungal species, including the genetic models *Aspergillus nidulans* and *Neurospora crassa* (Bennett, 1997). The large amount of classical genetic and biochemical information available for these species will make it easier to correlate sequence data with phenotypes.

Microbial genomics promises to revolutionize not only basic biology but also to accelerate the drug discovery processes by guiding clinical trials. Correlations between sets of genes and drug activities open new avenues for screening. Using photolithography and technology derived from the silica chip industry, immobilized oligonucleotides encompassing the entire yeast genome can be arrayed on a small surface creating a DNA microchip. Using probes to measure hybridization, the chip can be used as a reagent for measuring the expression of any number of genes. Genome-based diagnostic products are also likely. In conjunction with combinatorial chemistry and assay miniaturization, DNA chips can be used to identify important molecular targets for drug discovery. They also are being used to search for bioactive products using environmental DNA samples from organisms that have been overlooked in the past, including difficult-to-culture marine and macrofungi (Blanchard and Hood, 1996).

Like all of biotechnology, mycotechnology operates at the scientific frontier. New scientific ad-

vances drive new approaches in agriculture, industry, and medicine. The molecular biotechnology revolution has spawned social and political controversy. Sometimes regulatory issues overshadow the scientific issues. Although economic factors are not new to industrial microbiology, these factors have taken on certain urgency in the era of biotechnology start-up firms. They have also generated an enormous literature on financing, intellectual property, safety, and governmental regulation (for a review, see Moses and Cape, 1991).

5. Conclusion

Fungi, the industrial workhorses of traditional fermentations, are in the forefront of molecular biotechnology. These lower eukaryotes remain important models for basic biology and commercial manufacture. The yeast genome is the platform for discoveries in functional genomics and DNA microchip technology. In the next millenium, biotechnologists will continue to rely on yeasts and filamentous fungi to develop new paradigms for research and development.

References

Bennett, J.W., 1997. White paper: genomics for filamentous fungi. Fungal Genet. Biol. 21, 3–7.

Bennett, J.W., Heller, F., Case, C.L., 1997. Bio-tech-nol-o-gy, n.: The many definitions of biotechnology. Soc. Indust. Microbiol. News 47, 240–243.

Bigelis, R., 1985. Primary metabolism and industrial fermentations. In: Bennett, J.W., Lasure, L.S. (Eds.), Gene Manipulations in Fungi. Academic Press, New York, pp. 358–401.

Blanchard, A.P., Hood, L., 1996. Sequence to array: probing the genome's secrets. Nat. Biotech. 14, 1649.

Botstein, D., Cherfitz, S.A., Cherry, J.M., 1997. Yeast as a model organisms. Science 277, 1259–1260.

Chang, S.T., Hayes, W.A., 1978. The Biology and Cultivation of Edible Mushrooms. Academic Press, New York.

Crueger, W., Crueger, A., 1982. Biotechnology: A Textbook of Industrial Microbiology. Sinauer, Sunderland, MA.

Davies, R.W., 1991. Expression of heterologous genes in filamentous fungi. In: Peberdy, J.R., Caten, C.E., Ogden, J.E., Bennett, J.W. (Eds.), Applied Molecular Genetics of Fungi. Cambridge University Press, Cambridge, pp. 103–117.

Demain, A.L., 1981. Industrial microbiology. Science 214, 987–991.

Dujon, B., 1996. The yeast genome project; what did we learn? Trends Genet. 12, 263–270.

Esser, K., 1997. Fungal genetics: from fundamental research to biotechnology. Prog. Bot. 58, 3–38.

Fleischmann, R.D., Adams, M.D., White, O., Clayton, R.A., Kirkness, E.F., et al., 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science 269, 496–512.

Godfrey, T., West, S. (Eds.), 1996. Industrial Enzymology, 2nd ed. Stockton Press, New York.

Gray, W.D., 1970. The use of fungi as food and in food processing. CRC Crit. Rev. Food Technol. 1, 1–225.

Hesseltine, C.W., 1983. Microbiology of oriental fermented foods. Annu. Rev. Microbiol. 37, 575–592.

Kao, C.M., Katz, L., Khosla, C., 1994. Engineered biosynthesis of a complete macrolactone in a heterologous host. Science 265, 509–512.

Kinghorn, J.R., Lucena, N. 1994. Biotechnology of filamentous fungi. In: Iberghin, L., Frontali, L., Sensi, P. (Eds.), ECB6: Proceedings of the 6th European Congress on Biotechnology, Elsevier, pp. 277–286.

Lutman, Benjamin F. 1929. Microbiology. McGraw Hill, New York.

Monghan, R.L., Tkacz, J.S., 1990. Bioactive microbial products: focus upon mechanisms of action. Annu. Rev. Microbiol. 44, 271–301.

Moses, V., Cape, R.E. (Eds.), 1991. Biotechnology: the science and the business. Harwood Academic Publishers, London.

Pukkila, P.J. 1993. Methods of genetic manipulation in *Coprinus cinereus*. In: Chang, S.T., Buswell, J.A., Miles, P.G. (Eds.), Genetics and Breeding of Edible Mushrooms. Gordon and Breach, Amsterdam, pp. 249–264.

Raper, C.A., Horton, J.S. 1993. A strategy for isolating mushroom-inducing genes in edible basidiomycetes. In: Chang, S.T., Buswell, J.A., Miles, P.G. (Eds.), Genetics and Breeding of Edible Mushrooms, Gordon and Breach, Amsterdam, pp. 285–295.

Rasmussen-Wilson, S.J., Palas, J.S., Wolf, V.J., Taft, C.S., Selitrennikoff, C.P., 1997. Expression of a plant protein by *Neurospora crassa*. Appl. Environ. Microbiol. 63, 3488–3493.

Skatrud, P.I. 1991. Molecular biology of the beta-lactam producing fungi. In: Bennett, J.W., Lasure, L.S. (Eds.), More Gene Manipulations in Fungi. Academic Press, San Diego, CA, pp. 364–395.

Skatrud, P.I., 1992. Genetic engineering of beta-lactam antibiotic biosynthetic genes in filamentous fungi. Tibtech 10, 17–20.

Smith, J.E., Berry, D.R. 1975. The Filamentous Fungi, vol. 1. Industrial Mycology. Wiley, New York.

Takamine, J. 1894. United States Patents 525,820 and 525,823.

Turner, W.B. 1975. Commercially important secondary metabolites. In: Smith, J.E., Berry, D.K. (Eds.), The Filamentous Fungi, vol. 1. Industrial Mycology. Wiley, pp. 122–142.

Umezawa, S.I., 1982. Low-molecular weight enzyme inhibitors of molecular origin. Annu. Rev. Microbiol. 36, 75–99.

Underkofler, L.A. 1954. Fungal amylolytic enzymes. In: Underkofler, L.A., Hickey, R.J. (Eds.), Industrial Fermentations. Chemical Publishing Company, NY, pp. 94–118.

Von Wartburg, A., Trabor, R., 1986. Chemistry of the natural cyclosporin metabolites. Prog. Alleg. 38, 28–45.

Wainwright, M. 1990. Miracle Cure—The Story of Antibiotics. Blackwell, Oxford, England.

Wosten, H., Moukha, S., Sietsma, J., Wessels, J., 1991. Localization of growth and secretion of proteins in *Aspergillus niger*. J. Gen. Microbiol. 137, 2017–2023.