Fungi: Nature’s Gift for Organic Industrial Development

Odewale, J. O., Okolo, E. C., Odigie, E. E. and Enonuya, D. O. M.
Nigerian Institute For Oilpalm Research (NIFOR), P. M. B. 1030,
Benin City. 300001 Edo State, Nigeria.

Introduction

Fungi are living organisms. The word fungi embraces a large group of non-photosynthetic lower eukaryotes formerly considered as part of the plant kingdom, but was later assigned their own kingdom, the “fifth kingdom” on the basis of their characteristic absorptive mode of nutrition. There are two major divisions within this fungal kingdom (Whittaker, 1969; Kendrick, 1985).

They, like other eukaryotes, have nuclei, mitochondria, 80s ribosomes and chromosomes. Fungal cells may be haploid or diploid; the nuclei within a mycelium may be genetically identical (monokaryotic) or comprise of a mixture of different genetic types (heterokaryotic). Many fungi are microscopic, but the best known species form macroscopic fruiting structures such as mushrooms. Fungi are ubiquitous in terrestrial environments, and many fungi are capable of growing in environments hostile to most other forms of life (Jennings, 1993). Fungi are the only eukaryotes that include members with thermophilic properties of 60 to 62°C optimal growth temperatures (Tansey and Brook, 1972).

It was the study of fungi that paved way for the study of the principles of genetics when Acetabularia was used to prove that inheritance is located within the nucleus.

Fungi in relation to man have been considered both as a foe and a friend. As a foe because they cause spoilage of food and other valuable materials, it is a pathogen, an allergen and also produces toxins including some extremely deadly ones such as botulinus toxin. Fungi are considered as friends when they are involved in the areas of mineralization, mycorrhizae, food and fodder production, medicine, religious and mystical ceremonies, science and research, biotechnology, biological control, pollution control and as biological weapons.

The twentieth century is recognized as the golden age of industrial microbiology, and has yielded a myriad of products made by fermentation processes. Some of such products include solvents, antibiotics, enzymes, vitamins, amino acids, polymers and other essential compounds (Demain, 1999).

The use of fungi in biotechnology is becoming very important; biotechnology is the use of organisms or their fractions for the production of biomass or metabolites on a sustainable, industrial scale. As at 1995, 25% of food consumed by man world-wide was already produced by biotechnological processes and fungi play important roles in these. Cheese is produced through the use of the fungus Penicillium camemberti and
the brand of cheese produced is named after it “Camembert”. Other fungal products from milk include Kefir, Yoghurt, and so on and so forth.

In many respects, fungi are nature’s gift for organic industrial development because of their importance and positive roles in nature for the benefit of man. Recently, they are important tools in science and research, as they are increasingly used to fight the increasing myriad of problems of our modern society in biological control of pests, pathogens and pollution via bioremediation.

**Advantages**

Fungi were involved in early biotechnological processes for conservation and improvement of the taste of products of perishable food materials. For example, conversions of milk, a nourishing and easily perishable natural food into stable, delicious and nutritious foods like cheese and yoghurt; conversion of ground cereals with yeast under the right conditions to Bread. The conversion of fruit juices and other items to such alcoholic beverages as table wines, beer and alcohol involves the use of fungi, yeasts of pure cultures, while the cultivation of edible mushrooms is dependent on waste products like straw and other plant biomass residues, thus conserving the arable land whilst deriving food materials from waste.

Currently, 4 million tons of cultivated mushrooms like *Agaricus bisporus*, *Pleurotus ostreatus*, *Lentinus edodes*, *Auricularia auricular-judae*, *Volvariella volvacea* et cetera (Molitoris, 1995) are traded per annum. *Penicillium* and *Aspergillus* strains have the ability to catalyse very specifically one or few step transformations in steroids and are used in the production of birth control pills.

In antibiotics production, four hundred antibiotics are currently used in human medicine and fungi produce about a quarter of these. The pioneer antibiotic, penicillin, from which a wide variety of other antibiotics were derived, was obtained from *Penicillium notatum*, a fungus.

Fungal enzymes, mostly extracellular cellulases, lipases, proteases, esterases, are produced commercially and are indispensible tools in the laundry products and the food industry and research. These enzymes are used for a wide variety of applications from biochemical assays and analysis, medical diagnosis to food processing and laundry powders for cleaning textiles with difficult stains without damage to the fabric. Citric acid is a tricarboxylic organic acid of fungal origin, with an annual global production of 600,000 metric tons. It is mostly used in the food and beverages industry. However, amino acids are also commercially produced from fungi and have wide applications in food and dietary supplements.

Ergot alkaloids are produced by saprophytic cultures or by biosynthesis using some genetically engineered strains. *Claviceps* spp produces Ergot alkaloids used for the
treatment of severe headache, cerebral circulatory disorder, uterine contraction, bleeding after child birth and also for prevention of implantation in early pregnancy (Bentley, 1997; Vining and Taber, 1979). These alkaloids are now of wide application in medicine for gynaecology, against severe headache and in the treatment of heart and blood circulatory diseases.

Fungi are also a very important tool in research especially genetics. This is possible because of their different types of life cycles, and different mechanisms of genetic inheritance, for example the possibility of identification through tetrad analysis in some Ascomycetes during the stepwise production of meiosis products, which turns out to be the advantage of this group of microorganisms. Much success in this has been achieved in Neurospora crassa by Beadle and Tatum in 1958 for which they won the Nobel peace prize in science.

**Other advantages**

**Biological Control of Pathogens**
To reduce the need for use of chemicals which are expensive, relatively unspecific and moreover cause environmental destabilization problem as a result of their toxicity and persistence, this modern aspect of biotechnology employs fungi because they have high specificity and consistency or precision of action (Burge, 1988).

**Nematodes**
In 1988, Stirling Corporation used a fungal preparation tagged ‘Royal 300’ from the fungus Arthrobotrys robusta to effectively control nematodes which are deadly pests for mushroom culture.

**Insects**
‘Mycotal’ and “Vertalec” preparations from Verticilium lecanii are enthomopathogenic fungi used for control of insect pests in green house agriculture (Quinlan, 1988).

**Fungi**
Fungi like Trichoderma spp. can be used against a number of other pathogenic fungi, especially when used in “integrated control” that is in combination with other measures (Papavizas and Lewis, 1988).

**Pollution Control**
From the above examples, biological control using fungi has helped to reduce the use of chemicals in pest and pathogen control. This has become a new area of employing fungi in environmental protection and control measures.

**Recycling**
In order to make use of waste vegetable or other organic materials which could contribute to environmental pollution, mushroom cultivation has also been profitable. For example, when Pleurotus ostreatus is grown on straw as a sole substrate, the spent Pleurotus compost was very nutritious, has a good smell and can be added to fodder for cattle; through this method straw is recycled. This recycled product gives rise to fruit bodies (ca
10% of substrate weight) and can partially replace peat in gardening (for orchids and acclimatization of biotechnology plantlets under acclimatization), and partially used as fodder.

Health

Through the use of yeasts and moulds, monologous and heterologous (especially mammalian) proteins and other metabolites like antibiotics, pigments and fatty acids are being produced. Fungi used in the food industry are selected based on production yields, regulatory issues and from those granted GRAS (Generally Recognized As Safe) status by the U.S. Food and Drug Administration (FDA). Several species of fungi have that status and are currently being used for large scale production of recombinant proteins, enzymes, co-enzymes, hormones and metabolites (Punt et al., 2002).

The yeast *Saccharomyces cerevisiae* is considered safe for the production of pharmaceutical proteins. This yeast has the characteristics of rapidly growing to a high cell density, secrete heterologous proteins into extracellular broth and more advanced knowledge of its genetics than that of any other eukaryotes.

Mammalian genes have been cloned and expressed in *S. cerevisiae*, examples of which include human epidermal growth factor (Brake et al., 1984) and human haemoglobin (Strohl, 1997). The first safe hepatitis B vaccine of commercial importance was obtained through the production of genes encoding surface antigen of the hepatitis B virus using yeast recombinant process (Miyanoahara et al, 1983; Valenzuela et al., 1982).

Taxol is a natural anticancer agent discovered in plants (Wall and Wani, 1995) and can also be produced by the fungus *Taxomyces andreanae* (Shuster and Connelley, 1999). It is approved for the treatment of breast and ovarian cancer and is the only anti-tumour drug that acts by blocking depolymerisation of microtubules.

Nigerian experience

In Nigeria, in the early years of independence and before, Palm sap (Palmwine) from *Raphia hookeri* or *Elaeis guineensis* were used in bread production as they were the locally available sources of yeasts. The yeast was also used in the production of beverage drinks from sorghum, millet, maize, etc. *Aspergillus tamarii* was used in the Nigerian Institute For Oil palm Research (NIFOR), Benin City for bio-remediation in the conversion of palm oil mill effluent solids (POMES) an environmental pollutant to a protein rich feed-stuff and partial replacement of maize in the broiler’s feed (Okpefa, 1993 and 1996).

In Date palm (*Phoenix dactylifera*), fungi infested date fruits hitherto treated as waste fruits and source of environmental pollution have been discovered to produce caramel, a product essential in soft drink factories for production purposes. The fungus is being purified for better caramel production in NIFOR, Benin City (Gold, 2008).

Recombination Fungi are the main sources of enzyme for industrial applications.
Future prospects
Great progress has been achieved using fungi as cell factories. According to Punt et al. (2002) there are four major fronts in which work on fungi as cell factory is currently under focus for Nigeria to be relevant in the global competition:

1. Development of alternative hosts among fungi that have been given GRAS (Generally Recognized as Safe) status by the U. S. Food and Drug Administration (FDA) and can be used in the food industry. Research is being focused on Aspergillus sojae, Aspergillus japonicus, Mortierella alpine and Fusarium veneratum among others.

2. Development of better molecular techniques to improve expression and secretion of non-fungal/exotic proteins in filamentous fungi.

3. The use of these molecular techniques to carry out metabolic engineering in order to modify and improve particular biosynthetic pathways and their output.

4. The utilization of techniques dealing with the overall analysis of gene expression.

Genetic Engineering

The genetic engineering protocol involves:

1. Isolation of genetic information (DNA) for the production of an important metabolite from a naturally producing organism (usually higher plant or animal, that are slow growing and low producing).

2. Transfer of the genetic information (DNA) into another organism (usually a fast growing and fast producing micro-organism).

3. Integration of this information into host genetic material.

Finally, these micro-organisms in this case fungi or yeast produce with modern fermentation technology, the desired metabolites faster, in higher quantities, higher relative purity and at much lower costs because of their simple requirements and modern technology.

Microbial Leaching

The standard process currently is that bacteria are used to obtain valuable metals from ores too low in metal content for conventional mining and enrichment processes. The procedure is that metal bearing ores are percolated with micro-organism-containing liquids, the metals are solubilised by bacterial metabolism and may be later extracted or precipitated. Recently, methods are being developed to employ fungi (Saccharomyces, Rhizopus) for specific extraction of such valuable metals as manganese, uranium, gold and platinum (Groger et al., 1987).
Biological Control
Promising projects involving fungi are being investigated; for example, control of the deadly chestnut blight a disease which reduces production, using hypovirulent strains of the respective fungal pathogens (Adams, 1988).

Environmental Protection
Vyas et al. (1994a and 1994b) showed that ligninolytic fungi, are able to degrade a high proportion of polycyclic aromatic hydrocarbons which are found in toxic wastes. This will encourage further research in future. The non-biologically degradable plastic materials produced from the rapidly diminishing fossil resources will be replaced by the newly emerging biosynthetic thermoplastics which are produced from renewable resources which have recently been developed. To produce them generally and in large quantities, their complete bio-degradability (without toxic residues) has to be established first.

Experiments
There is a current development of investigating the behaviour of living system under conditions in outer-space such as microgravity, cosmic irradiation and low pressure leading to new biotechnological processes which involve fungi as a result of the possibility of conducting research in orbit. This was a fall out of the space experiment conducted with brewer’s yeast in the US/German space mission D-2, 1994.

Genetics
Application of recombinant DNA technologies is of great utility in the production of new fungal metabolites. Progress in the area of metabolic engineering has led to the overproduction of limiting enzymes of important biosynthetic pathways which in turn has increased the production of the final products.

Thaumatin has been successfully expressed in *Penicillium roqueforti* and *Aspergillus niger* var. *awamori* at titres of 2-7mg/l. Thaumatin is a protein from the plant *Thaumatococcus danielli* with intense sweetness (about 3,000 times sweeter than sucrose), and has been approved as a food grade ingredient.

Potential Bio weapons
The United States of America Department of Health and Human Services (H. H. S.) and the United State Department of Agriculture (USDA) have each promulgated lists of pathogens they think pose risks as potential bio weapons. This is because fungal diseases are not easily or immediately detectable. This long incubation period is an advantage of biological weapons and will become a vital ingredient in the weapon industry in the future.

Fungi have unique features that make them manageable and threatening in the hands of determined antagonists. The features include their inexpensive mode of acquisition, easy mode of culture in large quantities without the use of technical equipment (e.g. large quantities of spores of *Histoplasma* species and *Coccidoides immitis* can be easily cultivated).

Fungal spores are easier to disseminate over large territories without the need of biological
warfare engineering and moreover, fungal spores resist desiccation, irradiation and heat and have the potential to be disseminated by aerosol. This means in a sense, they are naturally weaponized and ready to be deployed (Merry, 2008).

Potential Agents of Collaboration between Industries

A range of filamentous fungal materials that help with the healing of wounds have been jointly designed and produced by the British Textile Technology Group (BTTG) and Welsh School of Pharmacy. One of the products is the fabrication of mycelia network into plaster with a difference with chitin which maintains the rigidity and structure of fungal cell walls, speeds up the healing of wounds and encourages the growth of fibroblasts into the wound. The fibroblasts help build new tissue, just apply the plaster on the wound!

This process has the potential to heal chronic ulcers and bed sores in hospital patients and save hospital and nursing resources as it does not require frequent changes.

Immunosuppressive agents

*Tolypocladium nivenum* a mould which produces Cyclosporin A used in heart, liver and kidney transplant for its overwhelming success in immune-suppression of tissue-graft rejection in organ transplants. Meanwhile, several species of Penicillium produce 2-morpholinoethylester which was approved as a new immunosuppressant for kidney transplant in 1995 and for heart transplant in 1998 (Jose et al 2003).

Environmental Remediation

The growth of *Cladosporium resinae* on fuel, first reported during World War II when it caused plane crashes has become advantageous in the area of biodegradation of petroleum waste for environmental remediation (Kahlon, 1991) in oil spillage. Bioremediation is generally classified into three categories: 1 Target compound is used as a carbon source 2. Target compound is enzymatically attacked but is not used as a carbon source (cometabolism) and 3. Target compound is not metabolized, but rather taken up and concentrated within the organism (bioaccumulation).

Filamentous fungi are good bio-degraders because the rapidly-ramifying mycelial growth habit gives a competitive advantage over cells.

Food

New strains and species of edible fungi are investigated for mushroom cultivation as new type of food. For example, *Morchella conica*, based on US patent has entered an economically feasible stage (Coombs, 1994).

Metabolic products

More metabolites will be produced in future from fungal species, and some of them are modified by genetic engineering. An example is the production of “Hirudin” (HOECHST AG, Germany), for human “Insulin” (Novo, Denmark) and for new types of brewers’ yeast. Yeasts are used in these cases because of the ease and efficiency of genetically manipulating them,
Table 1: Table of Application Areas: 1998

<table>
<thead>
<tr>
<th>Areas of Application</th>
<th>Percentage</th>
<th>$ Billion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>Detergent</td>
<td>34.0</td>
<td></td>
</tr>
<tr>
<td>Textile</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>Leather</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Pulp and paper</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Non-Pharmaceutical Proteins</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Diagnostic and Therapeutic</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics (Biosynthetic)</td>
<td>44.0</td>
<td></td>
</tr>
<tr>
<td>Semi-synthetic</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>Fungal Statin</td>
<td>15.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Economically beneficial examples of fungal degradation

<table>
<thead>
<tr>
<th>Process</th>
<th>Substrate</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-cell protein production</td>
<td>Alkanes</td>
<td>Yeasts, e.g., <em>Candida tropicalis</em></td>
</tr>
<tr>
<td></td>
<td>Brewery wastes, molasses</td>
<td><em>Saccharomyces cerevisiae, S. carlsbergensis</em></td>
</tr>
<tr>
<td></td>
<td>Sulfite waste liquid</td>
<td><em>Candida utilis, Paecilomyces variotii</em></td>
</tr>
<tr>
<td>Solid-waste treatment</td>
<td>Sludge/sewage</td>
<td>Consortia of bacteria and fungi, usually</td>
</tr>
<tr>
<td></td>
<td></td>
<td>uncharacterized</td>
</tr>
<tr>
<td></td>
<td>Pulp and paper mill effluent</td>
<td><em>Coriolus versicolor, Phanerochaete chrysosporium</em></td>
</tr>
<tr>
<td>Wastewater treatment</td>
<td>Distillery waste</td>
<td>Yeasts, especially <em>Candida utilis</em></td>
</tr>
<tr>
<td></td>
<td>Kraft bleaching effluent</td>
<td><em>Phanerochaete chrysosporium</em></td>
</tr>
<tr>
<td></td>
<td>Tannery effluent</td>
<td><em>Aspergillus, Penicillium</em></td>
</tr>
</tbody>
</table>
in large scale cultivation and metabolite extraction.

At the current level of fungal biotechnology, less than 5% of known fungal species occurring in nature have been utilized. Meanwhile, soils and marine environments contain thousands of unknown microbial species of which many are fungi. Based on this, we can say that the future of fungal biotechnology will be revolutionary in outlook and impact on human existence.

In conclusion, naturally occurring fungi, as is or genetically modified have been beneficial to man in so many different ways for organic industrial development. It is now that it will become an even more useful gift in future as they are more aggressively exploited in due course.

REFERENCES


