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Review Articles

Recent trends in Fungal Biotechnology - Review

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Abstract

Fungal biotechnology has become an integral part of human welfare. Nature represents a formidable pool of bioactive compounds and is more than ever a strategic source for new and successful commercial product. Among the microorganisms, fungi are well recognized to produce a wide variety of most valuable pharmaceutical chemicals, agrochemicals and industrial products. Recent advances made in genomics, proteomics and combinatorial chemistry show that nature maintains compounds that are the essence of bioactivity, within the host and environment. So the major challenging task is to explore the unexplored fungal wealth in our country and reveal their potential applications. The screening of marine fungi for novel bioactive compounds has yielded several novel metabolites, some of which are being commercially developed for medicinal or agricultural use. Sadly the data generated by pharmaceutical companies in screening for bioactive compounds if often 'lost' to science due to the need for industrial secrecy. Fungal enzymes are widely used in industry and, many vitamins and food supplements rely on fermentation processes using terrestrial fungi. Due to their slow growth rates it is unlikely that marine fungi will replace their faster – growing terrestrial counterparts in this respect. Many important industrial products are now produced from fungi using fermentation technology. A wide range of enzymes are excreted by fungi and play an important role in the breakdown of organic materials and many of these enzymes are now produced commercially. Most of these enzymes are used in food processing. Fungi are good candidate for employing them in degrading refractory substrates, cellulose, lignin, chitin, keratin and other substrates. Fungi like *Aspergillus niger* and *A. oryzae* are regarded as safe by the food and drug administration.

Introduction

Microbial cells produce a variety of enzymes and help in microbial growth and respiration including other cellular activities. At times, these enzymes may themselves become fermentation products, so that one of them is specifically interested in obtaining high level of the enzymes (Bell *et al*., 1972). Qualitative screening of degrading enzymes in marine fungi was reported by Rohrmann and Molitoris (1992).

The use of enzymes in food preservation and processing predates modern civilization. Fermentation of common substrates such as fruits, vegetables, meat and milk provide a diverse array of food in the human diet. Beer, wine, pickles, sausage, salami, yogurts, cheese and buttermilk are all fermented products. Irrespective of their origin, these fermented food products are, in fact, result of the enzymatic modification of constituents in the substrate. The use of enzymes in food industry also involves a range of effects including the production of food quality attributes such as flavors and fragrances and control of colour, texture, and appearance besides affecting their nutritive value.

Molecular techniques and Phylogeny

The study of systamatics and population biology of filamentous fungi entered a new era with the introduction of molecular biological techniques. This new approach to fungal systematics has been accelerated by the relative simplicity of these techniques and the use of particular regions of the relatively small fungal genome. Fungal molecular systematics has increased our understanding of taxonomic groupings and evolutionary histories within different groups of fungi. The evolution among the polyphyletic assemblage of organisms once considered to be fungi has been well documented with the advances of molecular techniques (Bruns *et al*., 1991,

1992; Barr, 1992; Berbee and Taylor, 1993, 1995). Molecular techniques used in fungal systematics can be divided into two main areas such as Protein and DNA analysis.

Advances in molecular techniques have made a large impact in many areas of mycology, one of which is fungal phylogeny and this includes the lower fungi, Chytridiomycetes to those of Ascomycetes

and Basidiomycetes. Other studies have increased our knowledge of the taxonomy of certain fungi, which have been previously assessed only by morphological characters (Nishida *et al*., 1995). DNA sequences have been used both as phylogentic characters and as a measure of dating the evolution of different groups of fungi, filling the gaps of information from the lack of fossil records (Berbee and Taylor, 1993,1995). More intensive work must be emphasized in molecular marine mycology to solve the phylogenetic relationships among several genera, which were assessed earlier by morphological and ultrastructural work.

Advances in molecular technology have enabled rapid strides to be taken in the area of amplication and detection technologies of important fungal pathogens, detection of fungi in the natural environment as well as geographical evolution of specific strains of fungi (Goodwin and Annis, 1991; Levesque *et al*., 1994; Jungeh Ulsing and Tudzynski, 1997; Sreenivasaprasad *et al*., 1996; White, 1996; Jeng *et al*., 1997; Maurer *et al*., 1997; Pei *et al*., 1997; Liew *et al*., 1998). With the large number of DNA sequence data within sequence database centers, the possibility of designing species or generic specific primers for fungi is relatively easy. This will greatly increase the detection of fungi from the natural environment but will depend on the time and amount of funding available to the molecular mycologist.

Fungal biotechnology

Amylase from fungi

The decomposition of starch by marine fungi was demonstrated by Barghoorn (1944) for the representative of the genera *Ceriosporopsis, Corollospora, Lulworhtia, Phialophorophoma* and *Zalerion* and by Nilsson (1974b) for *Humicola alopallonella*. Denaturation of bacterial and fungal α- amylase by heat acid and urea was also investigated in the presence of added calcium ions (Hagihara *et al*., 1956).

Iqbal and Zafar (1994) reported a new matrix petiolat felt-sheath of palm (*Livistona chinesis*) immobilize the cells of *Aspergillus niger* for the production of aplha amylase. Growth of immobilized culture was 19% greater than free cells. Different types of immobilized cell systems have also been used for Glucoamylae (GA) production (Li *et al*., 1984; Fiedurek and Szczdrak, 1995; Shimada *et al*., 1998; Ariff and

Webb, 1996). Mycelia of *A. niger* were immobilized on various seeds such as wheat rye, barley, mustrad etc., for GA production. Enzyme productivity was 1.6 times higher in immobilized system than by free cells (Fiedurek and Szczdrak, 1995). Shimada *et al*. (1998) described a system using immobilized cells of *Saccharomyces cerevisiae* for the production of GA. The system was capable of accumulating high quantity of GA. Ariff and Webb (1996) compared the influence of different fermenter configuration and modes of operation on GA production by *Aspergillus awamori*.

Goto *et al.* (1998) studied the amylase from fungi easy to manufacture then the amylases from the bacteria and *Streptomyces.* Among the fungi *Aspergillus oryzae* and *Aspergillus niger* have been well studied. Arora *et al*. (2000) studied that the potato waste was fermented by *Rhizopus oryzae* under solid substrate fermentation and yielded a dry biomass of 25 g containing 17- 18% protein and 70% dry matter digestibility (*in - vivo*) and 3.2 g crude alpha amylase enzyme. Mangrove derived fungi (35 sp.) was screened for amylase

activity using starch agar and all the fungi showed zone of clearance on starch agar plates (Sivakumar and Ravikumar, 2006c).

Protease from fungi

Pisano *et al*. (1964) screened 14 marine fungi for their geletinase activity and found such activity in the culture filtrate of 13 isolates, *Halosphaeria mediosetigera* produced the highest level of gelatinase. Sguros *et al*. (1973) concluded that the *Culcitalana achtaspora, Halosphaeria mediosetigera* and *Humicola alopallonella* were probably insignificantly proteolytic, lipolytic, nucleolytic or ligninolytic.

The extracellular protease production was studied in many *Aspergillus* species. Klapper *et al*. (1973a,b) reported about this enzyme production and the factors affecting their synthesis and release from *A. oryzae* NRRL 2160. Fukushima *et al*. (1989) studied continuous protease production in a carbon limited chemostat salt tolerant *A. oryzae*. In the same way Battaglino *et al*. (1991) also reported the culture requirements for the production of protease by *Aspergillus oryzae* in solid-state fermentation.

Alkaline protease production from *Aspergillus niger* was also reported by Singh *et al*. (1973); Bathomeuf *et al*. (1992). Monod *et al*. (1991) also studied the enzyme production in *Aspergillus fumigatus, A. sojae* by Nasuno and Ohara (1971),

A. nidulans by Stevens (1985), Cohen (1973) and *A. melleus* (Luisetti *et al*., 1991). Malathi and Chakraborty (1991) reported about the production of alkaline protease from new *Aspegillus flavus* strain by solid substrate fermentation.

Dahot (1993) reported that *Penicillium expansum* was grown on 1% rice husk fine powder medium and along with 1% glucose, raffinose, maltose and molasses and corn steep liquor for the production of protease. The other *Penicillium* species like *P. lilacinces* and *P. griseofulvin* was also known to produce alkaline protease reported by Kitano *et al*. (1992). Dozie *et al*. (1994) studied the production of alkaline protease by *Chrysosporium keratinophilum*.

Aspergillus ustus (NIOCC 20) producing the highest amounts of the enzyme was selected for further studies. The growth yield was substantial at 30°C and 50°C at 1 bar and elevated hydrostatic pressures. The fungus produced alkaline, cold-tolerant protease when grown at 30°C and 1 bar pressure. The enzyme was active at combinations of 30°C and 50°C and 300 bar pressure. The enzyme was totally inhibited in the presence of 2 mM PMSF suggesting it to be a serine protease (Damare, *et al*., 2006).

Cellulase from fungi

Barghoorn (1944) was first to use marine Ascomycetes and Deuteromycetes to demonstrate their ability to grow on wood flour and regenerated cotton cellulose by measuring the rate of radial growth on agar medium. The clearing of cellulose – contianing agar by 14 marine fungi was also used by Henningsson (1976) as a measure of cellulase and xylanase production. Nilsson (1974a) employed several methods to assay the enzymatic activities 0f 36 wood inhabitating fungi, among them one marine species, namely, *Humicola alopallonella*. 12 of these fungi unable to degrade pure cellulose substrates in culture but produced characteristic soft-rot patterns, namely cavities in the secondary cell walls of wood.

A number of fungal species are known to produce cellulase enzymes. Among these are the Ascomycetes such as *Neurospora* and *Trichoderma.* Shoemaker *et al*. (1983) reported a variety of cellulase produced from *Trichoderma.* These enzymes are also produced *by Sporotrichum, Humicola, Thermoascus, Trichoderma ressei* and *T. koningii. Penicillium funiculosum* is a potent cellulase producer, has been studied earlier for various applications including cellulolysis of various cellulose substrates (Dighe *et al*., 1987; Betrabet and Paralikar, 1977).

Cellulolytic enzyme system can be produced by a number of different fungi, such as white rot fungi (Uzcategui *et al*., 1991; Thompson *et al*., 1998) soft rot - fungi (Kubicek *et al*., 1990) and anaerobic fungi (Barichievic and Calza, 1990).

Pectinase from fungi

Raghukumar *et al*. (1994) have investigated degradative enzyme pectin lyase production by fungi isolated from detritus of the leaves of the mangrove *Rhizophora apiculata*. Twenty-one higher filamentous fungi isolated from *Spartina alterniflora* and other salt march substrata were shown to capable of degrading cellulose, lipids, starch including pectin compounds (Gessner, 1980).

Lipase from fungi

Lawrence (1967) and Barockerhoff and Jensen (1974) have presented its comprehensive reviews. These lipases are being exploited due to their low cost of extractions, thermal and pH stability, substrate specificity, and activity in organic solvents. The chief producers of commercial lipases are *Candida cylindracea, Humicola lanuginose, Rhizopus delemar, R. japonicus, R. niveus and R. oryzae* (Godfredson, 1990).

In 1981, one group highlighted the lipolytic activity of thermophilic fungi of paddy straw compost (Satyanarayana and Johri, 1981). Systematic screening strategies were employed by (Bhaduria, 1989). This study reported *Aspergillus niger, Aspergillsus flavus, A. fumigatus* and *Penicillium glaucum* as the potential lipase producers isolated form the kernels of Chironji and Walnut.

Yadav *et al.* (1997) purified and characterized of a regiospecific lipase from *Aspergillus terreus.* The purified enzyme showed excelled temperature tolerance and was highly thermo stable. The enzyme showed good pH tolerance. Ionic detergents inhibited enzyme activity where as non-ionic detergents stimulated enzyme activity.

Lazer and Schroder (1992) investigated fungal lipases, which degrade lipids from palm oil. Among Mucorales, the lipolytic enzymes of the moulds *Mucor hiemalis, Mucor miehei, Mucor lipolyticus, Mucor pusillus, Rhizopus japonicus , R. arrhizus, R. delear, R. nigricans, R. microsporus* and *R. chinesis* have been studied.

Kamini *et al.* (1997) studied the fungal strain isolated from curd. The fungal strain was identified as *Aspergillus niger.* Tributyrin was the substrate for examining lipase production on agar plates. A holozone of 9mm diameter around a colony in the tributyrin agar plate clearly indicated the production of lipase. The initial lipase activity was 8U ml^{-1} at 72 hrs in the culture supernatant of the basal medium, which indicated the extra cellular nature of the lipase.

Prabhakar *et al.* (2002) reported the effect of cultural conditions on the production of lipase by Fungi. They found that selected organism *Aspergillus niger, A. flavus, A. japonicus* and a fungi isolated from the contaminated ghee belonging to the genus, *Aspergillus* spp. were tested for the

production of lipase on four different media by submerged fermentation technique.

Xylanase from fungi

Nilsson (1974a) demonstrated a xylanase in *Humicola alopallonella*, whereas mannose was absent. Barghoorn (1944) found that *d* –xylose, produced by the hydrolysis of xylan, was used by the 8 species of marine fungi tested. Pectin was used as a carbon by the same species Barghoorn (1944). Leightley and Eaton (1977) determined the ability to degrade wood cell wall components of several marine fungi belonging to the genera *Cirrenalia, Culcitalana, Halosphaeria, Humicola, Nia* and *Zalerion*. They compared fresh water and terrestrial fungi and found production of cellulase, xylanase and mannanase in all species tested.

Neurospora crassa has also the ability to ferment D – glucose, D- xylose and treated cellulosic substrates directly to ethanol (Deshpande *et al*., 1984). Most microbial hemicellulolytic system contain beta xylosidase, which has been purified and characterized from many fungi *Aspergillus niger* (Rodonova *et al*., 1983), *A. fumigatus* (Kitpreechavanich *et al*., 1986), *Trichoderma viride* (Matsuo and Yasui, 1984b), *Emericella nidulans* (Matsuo and Yasui, 1984a) and *Chaetomium trilaterale* (Uziie *et al*., 1985). Screening and production of xylanase enzyme required in the hydrolysis of different xylan was investigated using strains of 35 species of fungi isolated from mangrove samples (Sivakumar and Ravikumar, 2006a).

Phosphatase from fungi

Phosphatases fall into the category of "extracellular enzymes" which are secreted and actively pass through the cytoplasmic membrane, and are associated with the producers. So, their function is involved in chemical communication of microorganisms with the surrounding microenvironment.

Both alkaline and acid phosphatases have been found as external and internal enzymes in microorganisms (Siuda, 1984). There exists a relationship between pH and synthesis and release of phosphatase congregation of organisms producing the enzymes and phosphatase stability and conformation (Herbien and Neal, 1990).

Lignin degrading enzymes from fungi

Lignin is an amorphous high molecular – mass composed of phenylpropane subunits interconnected by variety of non – hydrolysable bonds. The relatively few groups of microorganisms that can degrade the macromolecule. The most efficient degraders are the white rot fungi (Orth and Tien, 1995; Paul and Clark, 1989). Safari Sinegani *et al*. (1999) assessed the production of lignin – degrading enzymes by the imperfect fungi *Aspergillus terreus* and *Trichoderma reesei* and yeast in the N-ethyl alanine, benzyl alcohol and benzaldehyde. In contrast to low biomass of the yeast, the Mnp- and LiP activiites of this fungus were much higher of the Deuteromycetes *A. terreus* and *T. ressei*. Among the fungi *A. terreus* reduced pH of its culture media significantly. Laccase activity of *A. terreus* was higher than 2 and 1.35 time of *T. ressei* and the yeast respectively. All the fungi had the highest MnP and LiP activities and fungal biomasses were significantly low in the benzaldehyde treated media.

DeSouza-Ticlo *et al*. (2006) studied that the carbon and nitrogen sources in the growth medium play an important role in the production of lignin-degrading enzymes in the white-rot Basidiomyceteous fungi. The role of nutrient nitrogen sources in growth media on production of lignin-degrading enzymes namely laccase, lignin peroxidase and manganese peroxidase as well as on the decolorization of industrial effluents like black liquor, molasses spent wash and textile mill effluents was studied using the Basidiomycetous fungus NIOCC No.2a isolated from mangrove wood. The amount of extracellular peroxidases increased by several fold in the presence of effluents whereas in their absence they were of negligible quantity. Some of the effluents had an inhibitory effect on laccase production.

Fungal metabolites

Amino acids, Lipids, and Fatty acids from fungi

Schafer and Lane (1957) demonstrated 12 amino acids in *Lulworthia* sp. and Perters *et al*. (1975) found the following common to 10 species of marine fungi; alanine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine,serine, threonine, tyrosine and valine.

Lipids are important fungal components both in terms of structure and membrane constitution. Many studies have demonstrated the importance of lipids for development, sporulation and germination and their involvement in various physiological process (Rattray, 1975;van Etten and Gottlies, 1965, Weete, 1980, 1981; Weete *et al*., 1973).

Most fungi contain 5 to 32% lipids depending on culture conditions, developmental stage and species. The lipid content of spores of many fungi ranges from 5 to 17% dry weight, but spores some species, such as rusts contain up to 35% lipid (Shen, 1966). The major factors influencing the extent of lipid production are the nature and proportion of carbon (C) and nitrogen (N) as nutrients sources in the medium. In fungi in general, lipids have been reported to be important for germination, in addition to having other functions (Cochrane *et al*., 1963; Owens, 1955; Turain and Bianchi, 1972). Smith and Silverman (1973) reported a 30 to 40% decrease in lipids during the early phase of germination. In a study on *Rhizopus stolonifer* (Weete *et al*., 1973) was observed that spore having

a low concentration of lipid required a new synthesis of lipid during the early stages of spore germination compared to spores with a high concentration of lipid.

Linoleic acid has also been detected in large amounts in *Penicillium atrovenetum*, where it represents 66% of all fatty acids (van Etten and Gottlies, 1965). The study of fatty acid composition has been used for the identification of species of entomopathogenic fungi (Latge and Bievre, 1980; Tyrrell and Weatherston, 1976; Tyrrell, 1967,1968, 1969). However, studies on lipid and fatty acids at the physiological and genetic level may permit the selection of strains for environmental persistence and expression at the epizootic level and may provide information for their large-scale production and utilization.

A separation of the two conjugated isomers may be obtained using the ability of lipases produced by fungus *Geotrichum* to selectively hydrolyse the cis – 9, trans – 11 –18; 2 methy ester (Hass, 1999).

Glyceride fatty acids, in particular, oleic, palmitic and linolic acids were isolated from *Corollospora maritima* and *Zalerion maritimum* by Block *et al*. (1973) and Kirk *et al*. (1974). A number of these and other fatty acids were also determined in *Buergenerula spatinae* and *Dendtyphiella salina* (Schultz and Quinn, 1973). Szaniazlo and Mitchell (1971) compared the hyphal wall compositions of marine and terrestrial species of the genus *Leptosphaeria* and found qualitatively identical compositions in both groups. The walls consisted of glucose, mannose, galactose, glucosamine, amino acids, and traces of galactosamine.

Molecular studies and phylogeny

Protein profile of fungi

Gel electrophoresis of soluble proteins, particularly isozymes, has proves a powerful tool in the investigation of a wide range of questions concerning the taxonomy, evolution and population genetic structure of a large number of animals and plant species. Isozyme variations were analyzed between different species and formae speciales of the genus *Puccinia* (Burdon and Marshall, 1981). Similar isozyme studies have been carried out for *Ceratocyatis coerulescens* (Harrington *et al*., 1996), the Harpellales (Grigg and Lichtwardt, 1996) and *Ganoderma* species (Gottlieb *et al.*, 1998). To date, no isozyme studies have been reported for marine fungi but Pointing *et al*. (1999) has studied the production of extracellular enzymes of five lignicolous mangrove fungi.

In fungi, the synthesis of heat shock proteins is a rapid process. In *Fusraium oxysporum* began to synthesis after 10 minutes of heat treatment (Freeman *et al*., 1989), while for *Saccharomyces cerevisiae*, a peroid of 20 to 30 minutes was required. The synthesis of HSPs peaked at 60 minutes after heat treatment of *Achyla* (Silver *et al*., 1983) and *Neurospora crassa* (Plesofsky – Vig and Brambl, 1985b). However, under high temperature treatments, fungi differ from plants and animals in needing a long recovery time before synthesizing normal proteins (Plesofsky – Vig and Brambl, 1985a).

Species within the genera *Penicillium* and *Aspergillus* have been used in several phylogenetic studies to establish whether the taxa within these individual genera belong to their respective genera and whether they form separate monophyletic groups (LoBuglio *et al*., 1993; Berbee *et al*., 1995; Verweij *et al*., 1995). Studies on fungal molecular systematics have proved the way for studies of fungi in fresh water and marine habitats (Chen *et al*., 1995; Spatafora *et al*., 1995; Fallah *et al*., 1997; Spatafora *et al*., 1998; Chen *et al*., 1999; Ranghoo *et al*., 1999). Differences in strains within species and geographical variations of single species have also been investigated for *Phytophthora striiformis* (Chen *et al*., 1993), *Fusarium oxysporum* (Appel and Gordon, 1995,1996).

Electrophoretic Karyotyping of Fungal DNA

A recent advance in gel electrophoresis technology has allowed megabase – sized DNA molecules to be efficiently separated. The successful separation of the chromosomes within *Saccharomyces cerevisiae* (Schwartz and Cantor, 1984; Carle and Olson, 1985) had led the way for similar electrophoretic karyotyping studies of other fungi. By the beginning of this decade, more than 25 species of fungi have been successfully karyotyped (Mills and McCluskey, 1990).DNA relatedness of the species of *Aspergillus* section Flavi was studied by Kurtzman *et al*. (1986) with the DNA reassociation method. The cot values calculation results showed 100% relatedness between *A. flavus* and *A. oryzae*. Similarly *A. parasiticus* and *A. sojae* were 91% related. The homology between these two groups was 70%.

Electrophorectic karyotyping of certain species of fungi such *as Tilletia*, *Ustilago* and *Phytophthora* show distinctive patterns when viewed by Pulse Field Gel Electrophoresis (PFGE) as their chromosome have variable base numbers and hence allows species to be easily distinguished. It has been considered to be wiser to use a combination of Electrophorectic karyotyping and other molecular techniques like RFLP to make taxonomic interferences (Mills and McCluskey, 1990). Taga *et al*. (1998) compared different ways to study the karyotype of *Nectria haematococca* and found that PFGE was the most effective tool. Species within the genera *Penicillium* and *Aspergillus* have been used in several phylogenetic studies to establish whether the taxa within these individual genera belong to their respective genera and whether they form separate monophyletic groups (LoBuglio *et al*., 1993; Berbee *et al*.,

1995; Verweij *et al*., 1995). Phylogenetic analyses within specific genera include those of *Gliocladium* (Rehner and Samules, 1994), *Colletotrichum* (Sheriff *et al*., 1994), *Fusarium* (O' Donnell, 1993; Appel and Gordon, 1995,1996; Waalwijk *et al*., 1996), *Puccinia* (Zambino and Szabo, 1993), *Phytophthora* (Lee and Taylor, 1992; Cooke *et al*., 1996; Crawford *et al*., 1996), *Pythium* (Chen, 1992), *Ganoderma* (Moncalvo *et al*., 1995) and *Laccaria* (Gardes *et al*., 1991).

The protein profile of fungi has been already carried out by Burdon and Marshall (1981), Harrington *et al*. (1996), Grigg and Lichtwardt, (1996) and Pointing *et al*. (1999). Fungal phylogenics have been traced on the basis of sequence of protein genes (Loomis and Smith, 1995, Paquin *et al*., 1995). Protein is used to infer phylogeny between organisms (Baladauf and Palmer, 1993; Hasegawa *et al*., 1993; Martin *et al*., 1993; Edlind *et al*., 1996; Zhou and Kleinhof's, 1996). The DNA analysis report has been used in phylogentic studies and genotypic approaches have been applied recently to clarify the taxonomic relationships within *Aspergillus* genus (Peterson, 1997; Varga *et al*., 1999, 2000a,b).

More recently, genotypic characterization have proven useful and several methods for detecting DNA polymorphism in fungi have been used to detect interspecific and intrasepcific variation in *Penicillium* and other genera (Castle *et al*., 1998; Chen *et al*., 1999; Dupont *et al*., 1999; Laroche *et al*., 1995). DNA relatedeness and reassociation of *A. flavus, A. sojae, A. oryae* and *A. parasiticus* has been tested and homology of species analysed (Kurtzman *et al*., 1986).

The results of fatty acid study was correlated with the studies of Hama *et al*. (2000). In their study HPLC analysis of very long chain fatty acids of yeast strain (*Saccharomyces cerevisiae*) BW 7 and *fah* 1 mutans were compared. Fatty acids were liberated from cellular lipids by alkaline treatment and UV – absorbing phenacyl derivaties were analysed by reverse phase HPLC. The peaks eluting between 30 seconds to 4 minutes includes the shorter chain fatty acid derivaties and excess derivatization reagents. Calvo *et al*. (2001) reported that the genetic connection between fatty acid metabolism and sporulation in *Aspergillus nidulans* with reference to linoeic acid. Pillai (2002) reported the incorporation of radiolabelled lipids precursors into Triacyl glycerol (TG) molecular species in *Morticella ramanniana* var *angulispora* an oleaginous fungus.

Protein from fungi has been studied by several authors (Plesofsky-vig and Brambl, 1985; Silver *et al*., 1983; Freemann *et al*., 1989). Lipids content of spore of fungi ranges from 5 to 17% dry weight but spores of some species such as rusts contain up to 35% lipid (Shen, 1966). In fungi lipids are important for germination, in addition to other functions (Cochrane *et al*., 1963; Owens, 1955; Turain and Bianchi, 1972). Low concentration of lipids observed in *Rhizopus stolonifer* (Weete *et al*., 1973).

The enzyme screening and their acitivity was well correlated with previous reports by Goto *et al*. (1998) who studied that the amylase enzyme production from the fungi *A. oryae* and *A. niger*. Alpha amylase enzyme production was carried out under the influence of nitrogen sources using *A. oryzae* by Pederson and Neilson (2000). Under solid substrate fermentation potato waste was fermented by *R. oryae* and yielded 3.2 g crude alpha enzyme (Arora *et al*., 2000). Alkaline protease enzyme production from *A. niger* was also reported by Singh *et al*., (1973). Bathomeuf *et al*. (1992) and Monod *et al*. (1991) also studied the enzyme production in *A. fumigatus, A. sojae* by Nausho and Ohara (1971), *A.nidulans* by Stevens (1985), Cohen (1973) and *A. melleus* (Luisetti *et al*., 1991). Alkaline protease enzyme production by solid substrate fermentation using *A. flavus* (Malathi and Chakraborthy, 1991). Fungi like *A. niger, A. flavus, A. fumigatus* and *P. glacum* are reported as potential lipase producers (Bhaduria, 1989). Kamini *et al*. (1997) screened for the production of lipase enzyme on tributyrin agar plates using *A. niger* and maximum halozone observed on agar plates which indicates the production of lipase.

Species of *Neurospora* and *Trichoderma* are known to produce cellulase enzyme (Gallo, 1978). Ojumu *et al*. (2003) reported that lignocellulose substrates used for the production of celluase enyzme using *A. niger* and cellobiose substrates for the production of cellulase enzyme using *Aspergillus* and *Trichoderma* (Romana *et al*., 1990).

Production of xylanses has been investigated using different fungi such as *Chrysosporium* (Eriksson and Rzedowski, 1969), *Sclerotium* (Sadana *et al*., 1980), *Aspergillus* (Stewart *et al*., 1983), *Aureobasidium* (Leathers *et al*., 1986), *A. niger* (Rodonova *et al*., 1983) and *A. fumigatus* (Kitpreechavanich *et al*., 1986). Safari Sinegani *et al*. (1999) reported that the lignolytic enzyme like laccase production using *A. terreus, T. ressei*, yeast and additional chemical compounds. In this, Manganse peroxidase and Lignin peroxidase activity were higher *A. terreus* and *T. ressei* than yeast and chemical compounds tested.

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