Chapter 10
Exploration of Microorganisms Producing Bioactive Molecules of Industrial Interest by Solid State Fermentation

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Abstract The prospect of biomolecules using microorganisms in fermentation processes is widely used, in this context to solid state fermentation (SSF) has advantages such as the possibility of using agricultural and industrial waste and reduction of water waste. Studies show that different microorganisms can be used in SSF; actinomyces and fungi are the most used due to growth in media with low water activity. Among the highlight biomolecules produced are antibiotics, anticarcinogenic agents, anticoccidians, antiviral, neuroactive, antioxidants, and enzymes. The enzymes are produced in greater scale among the different classes; hydrolases have gained importance because of cellulases, hemicellulases, proteases, chitinases, lipases, and phytases. Cellulases are a complex capable of acting on cellulosic materials, promoting its hydrolysis to release sugars, of which glucose is the one with largest industrial interest. Xylanolytic enzymes act on xylan, hemicellulose components, which may be attached to the cellulose and lignin in the plant cell wall. The study of chitinase has been stimulated by their possible involvement as agents of defense against pathogenic organisms that contain chitin,

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such as insects, nematodes, and fungi. Proteases catalyze the hydrolysis of peptide bonds of proteins and may have activity on ester and amide bonds. Lipases allow catalysis of the hydrolysis and synthesis, often in chemo, regal, or enantioselective reactions. Furthermore, phytase catalyzes the hydrolysis of phytate to phosphate and inorganic phosphorus, increasing the bioavailability of phosphorus for monogastric animals.

Keywords Enzymes • Solid state fermentation • Bioprospecting • Fungus • Actinomycetes • Biomolecules

10.1 Introduction

The exploitation of biodiversity rises as a new exploitation method of biological natural resources, generating bioprospecting, that is defined as the method to determinate, evaluate, and explore legally and systematic life diversity in particular location, whose main goal is seek for genetic and biochemical resources for commercial purposes. Therefore, microorganisms are versatile for molecules production with biological activities by fermentation processes, such as the solid state fermentation (SSF).

The SSF is widely used for obtaining biotechnological products, and it has become an interesting alternative to reduce the processes cost. This type of fermentation can use agricultural and agro industrial waste as substrates, which present low value, are nutrient rich, and have restricted water availability that helps to select contaminants, especially bacteria and yeasts. The obtainment of the final product by SSF is easier and the amount of waste is minimized (Lima et al. 2003). The use of surplus/waste as substrate for SSF allows the reduction of the final product cost and the implementation of a closed, sustainable, and environmentally friendly product chain. Among these substrates we can mention sawdust, bagasse from sugarcane of the sugar and alcohol industry and straw, bark and bran from cereal and fruit production. For biotechnological processes, the microorganisms are widely required because they are, in most cases, unicellular; when they are multicellular, they are poorly differentiated; they simplify cultivation in fermenter; they have rapid absorption of nutrients, fast metabolism, and high versatility, transforming different compounds and producing a wide variety of products. Microorganism is considered viable for a process when it is able to grow on cheap substrates; it is genetically stable, but liable for genetic manipulation; it provides high production yields on large scale, and, also, recovered at low cost; it does not produce incompatible substances with the target final product and it is not pathogenic. Actinomyces and fungi are the most used by SSF, since they grow under low water activity conditions.

Actinomyces were originally classified as fungi, as they present aerial hyphae, however, detailed studies of the cell wall composition, particularly the lipid membrane and the composition of its peptidoglycan, classified them as true aerobic bacteria. Molecular taxonomy studies created the class *Actinobacteria*, which includes all gram-positive bacteria with guanine and cytosine content greater than

55 %. Within this new class, actinomyces with capacity to produce mycelium are classified as *Actinomycetales* and include 10 subclasses and 34 families. Each year, new proposals are presented in literature of new species, genera, or families, and so, the classification of these organisms is constantly renewed (Stackebrandt et al. 1997; Stackebrandt 2000). Actinomyces of genus *Streptomyces*, the most commonly isolated and studied, are considered important microorganisms for industrial production and they have been described as the main antibiotics producers. Species of this genus are noted for producing more than half of the 10,000 bioactive compounds documented until 2001 (Anderson and Wellington 2001). Due to its high metabolic diversity, actinomyces have also been explored as major producers of many bioactive substances (Korn-Wendisch and Scheider 1992). The Kingdom Fungi consists of about 1.5 million species of which 77,000 species are known. These microorganisms have important ecological functions in nature, such as decomposition of organic material and reduction of mineral discharge to environment, immobilization and nutrient release, association with plants that can vary from beneficial to pathogenic, release of organic acids for the soil, among others. They are capable of degrading various substances with aid of exoenzymes to achieve required solubility and shape to be transported and incorporated by the cells. These enzymes are amylases, pectinases, xylanases, lipases, cellulases, and proteases, which are important for many applications in promising industrial processes (Silva et al. 2008). Furthermore, they produce other metabolites as antibiotics, chelating agents, and others (Hawksworth et al. 1996; Fransson et al. 2004; Klein and Paschke 2004).

10.2 Production of Bioactive Substances by Microorganisms

The production of bioactive compounds by SSF may be conducted as shown in Fig. 10.1.

Actinomyces are producers of antibiotics (Bull 2004; Berdy 2005; Strohl 2004), antitumor agents (Olano et al. 2009), and immunosuppressive agents (Mann 2001). The *Streptomyces* have the ability to produce many bioactive compounds. Around 23,000 antibiotics have been discovered from microorganisms. It is estimated that about 10,000 of them have been isolated from actinomyces (Okami and Hotta 1988).

Regardless of its chemical structure, these bioactive substances can be classified as peptides, quinones, macrolides, terpenes, polyketides, among others (Li and Piel 2002; Salmon et al. 2003). Most peptides derived from *Streptomyces* species are cyclical and contain elements such as chromophores or amino acids in its structure. Peptides include ciclomarine A, which can be obtained from *Streptomyces* with great anti-inflammatory and antiviral activity (Renner et al. 1999), and piperazimicines A-C, which are cytotoxins isolated from *Streptomyces*

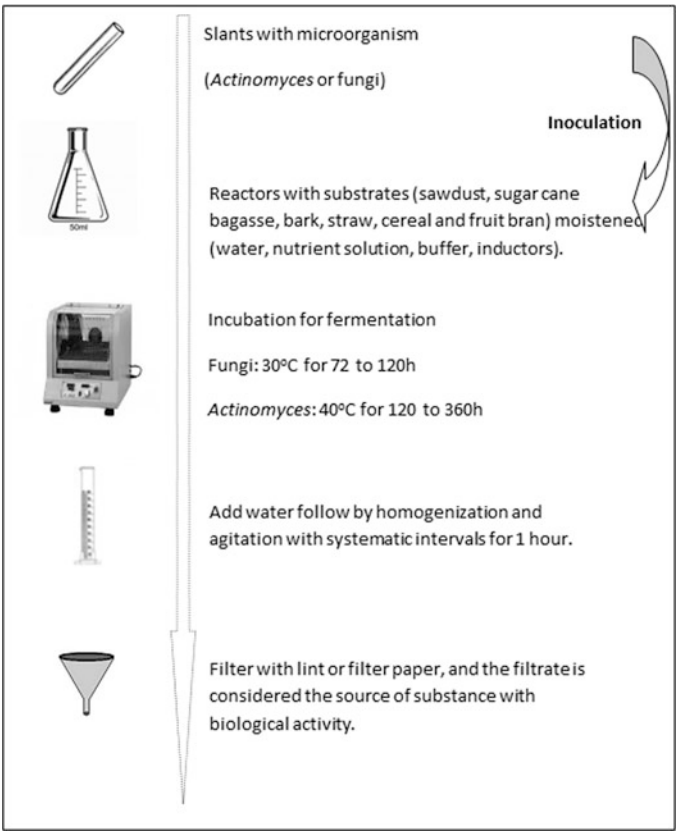

Fig. 10.1 Solid state fermentation process. Microorganisms kept in slants are sterile inoculated in reactors containing substrate previously sterilized. Afterwards they are incubated for fermentation, followed by water addition, homogenization and filtration to obtain the substances with biological activity

sp. Though, piperazimicine A showed high cytotoxicity against tumor cells in vitro (Miller et al. 2007) and salinamides A and B, produced by *Streptomyces* sp. CNB-091, showed anti-inflammatory activity (Moore et al. 1999). Quinones are compounds with conjugated dione cyclic in its structure and they are common constituents and biologically relevant molecules. As an example: C-glycoside himalomicines A and B complex and tetracenomycin D. The first is the anthraquinone with fridamicine E chromophore, precursor of anthracycline antibiotic obtained from *Streptomyces* sp. 6921, that show great antibacterial activity (Maskey et al. 2004), while the second, the anthraquinone antibiotic produced by *Streptomyces corchorusii* AUBN (Adinarayana et al. 2006) showed cytotoxic activity against hepatic carcinoma cells. Terpenes (large and diverse class of hydrocarbons) are biosynthetically derived from units of isoprene, with molecular formula C_5H_8. *Streptomyces* sp. NPS008187, isolated in Alaska, synthesized three

new pyroles sesquiterpenes which showed antibacterial activity (Macherla et al. 2005). Carotenoids are tetraterpenos mostly know, and they can be obtained from strains such as *Streptomyces griseus* (Lee et al. 2001).

The polysaccharides produced by basidiomycetes fungi are extensively studied in China and Japan, due to its medicinal and tonics attributes. Examples include *Agaricus blazei* which produces substances with anticarcinogenic activity (Mizuno et al. 1990); *Flammulina velutipes* that produces elements which helps to reduce cholesterol (Miles and Chang 1997); extracts of *Ganoderma lucidum* which are immune system boosters and promoters of tonic effects for cardiac system (Hikino et al. 1985); *Lentinula edodes* biomolecules with anti-HIV effect (Chihara 1992); and others. Filamentous fungi (ascomycetes) of *Penicillium* genus are fairly flexible for antibiotics production by fermentation processes alone or associated with chemical modification, such as penicillin G produced by *P. chrysogenum*; griseofulvin of *P. griseofulvin* used for infections treatment of skin, hair, and nails; cyclosporin, used as an immunosuppressant in transplant surgery; and fusidic acid, used to help control the infection by *Staphylococcus aureus* resistant to methicillin. Duarte et al. (2012) described the achievement of marine fungi molecules from different genus such as *Penicillium*, *Fusarium*, *Trichoderma*, *Hupocrea*, *Phoma*, and *Scopulariopsis*, among others with cytotoxic, antifungal anticoccidial, antiviral, and neuroactive activity. Whereas Xiong et al. (2009) studied the production of antibacterial compounds by *Cladosporium* sp., Meenupriya and Thangaraj (2011) describe the bioactive molecules obtained from marine organisms present anticancer, antimicrobial, and anti-inflammatory activity. Thus, these researchers obtained molecules from *Aspergillus ochraceus* with activity against microorganisms that cause humans diseases.

10.3 Production of Enzymes by Microorganismis

Enzymes include a abundant class of substances produced by actinomyces and fungi. The advantages of using microorganisms for enzyme production in replacement of the traditional animal and vegetable sources are relatively high performance, low cost, and susceptibility to genetic manipulation. Currently, microorganism enzymes are used in food processing, manufacture of detergents, textile and pharmaceutical industries, medical therapy, molecular biology, biofuels industry, wastewater treatment, environmental preservation, bioremediation, and biological control. These microorganisms have a wide ecological and biochemical diversity, and furthermore, they have a high capacity for production of secondary metabolites. Therefore, they can be considered an excellent source for finding new enzymes with new specificities and different biochemical characteristics. They are capable of producing several enzymes that can be considered promising for biotechnological applications, including oxidoreductases, transferases, hydrolases, lyases, isomerases, and synthases. Hydrolases are noteworthy, because these are cellulases, hemicellulases, proteases, chitinases, phytases, and lipases, whose features and applications are described below.

10.3.1 Cellulases

Cellulases are enzymes consisting of complex capable of acting on cellulosic materials, promoting its hydrolysis. These biocatalysts enzymes are highly specific, acting synergistically to release sugars, where glucose is the one with greater industrial interest due to the possibility of its conversion to ethanol, sweeteners, phytohormones, organic acids, etc. The steps involved in cellulose degradation by cellulase are not fully understood, but it is formed as a multienzyme system including three enzymes that act together for hydrolysis of cellulose: endoglucanases (EC 3.2.1.4), which cleave randomly cellulose polymer by changing the degree of polymerization; cellobiohydrolases (EC 3.2.1.91), which hydrolyze the polymer at its nonreducing end, releasing cellobiose; and cellobiases (β-glucosidase, EC 3.2.1.21), which are responsible for cleavage of small chain, both celloligosaccharides and cellobiose, until glucose (Fleuri and Lima 2013). The prospect of cellulose degradation (most abundant polymer in nature present in vegetable cells) is linked to program implemented in Brazil in 1970 that meant to replace gasoline with ethanol from sugarcane. Consequently, research for agriculture and new technologies have been greatly intensified, leading Brazil in a favorable position in terms of secure energy sources. However, only a part of the biomass produced is used for bioenergy production, as one-third of the sugarcane is used for sugar production, one-third is residue, which is burned to produce electricity, and the other third of the remaining residue is left in the field and decomposed by microorganisms (Zanin et al. 2000; Soccol et al. 2010). However, a significant increase in ethanol production may be possible if new technologies converting the polysaccharides of the two-thirds of the remaining biomass of the entire process in bioethanol. For the last four decades, much effort is being made to development of second-generation bioethanol, through abundant and renewable lignocellulose biomass by physical, chemical, and enzymatic treatments, isolation and/or combined (Hahn-Hägerdal et al. 2006; D'Souza-Ticlo et al. 2010; Soni et al. 2010). The raw lignocellulose materials include agribusiness, municipal waste, and wood from angiosperms and gymnosperms. The agro industrial materials are important for its residue character, after processing raw materials with high value, and the natural capacity that Brazil has for generation of these products, that is: sugarcane bagasse and straw, soybean straw, rice straw, and corncobs. Among the mentioned biomass, bagasse from sugarcane is predominant in Brazil, producing, in 2007, 147 million tons of wet mass (Chandra et al. 2010). Furthermore, these materials may also be used for solid state fermentation (SSF), since they are inexpensive materials and they have shown effective results for biocatalysts and bioactive compound production (Lever et al. 2010; Sukumaran et al. 2009; Bhattacharya and Banerjee 2008; Lin and Tanaka 2006; Mishima et al. 2006). The polysaccharides present in lignocellulose biomass must be hydrolyzed with acid (in the presence of high temperature and pressure) and/or cellulases and other enzymes to release fermentable sugar in a high yield. Pre-treatment help to hydrolyse the lignin and to solubilize the cellulose partly, so the enzyme can act on the molecule and available

all the remaining hexoses and pentoses. The process of enzymatic conversion of lignocellulose into ethanol is affected by the use and purchase of cellulases preparation, since they are marketed by a small number of suppliers and have high cost. For this process to become economically viable, large-scale production of cellulases at low cost, using agro-industrial residues as substrate, is necessary (Maclean and Spatari 2009; Chandra et al. 2009).

Actinomyces produce cellulase with high activity and stability in extreme temperature and pH conditions (Lima et al. 2005; Jang and Chen 2003). Such cellulases exhibit great activity in a wide range of pH, between 4.0 and 8.0, which it is also promising (Lima et al. 2005; Jang and Chen 2003; George et al. 2001; Bhat 2000). The proportion of current total production of cellulases as additives for detergents for laundry industry market exceeds 30 %. Due the increase of environmental pressure on paper and textile industries, it is assumed that cellulases should play an important role in the development of clean technology, both for denim processing and for discoloration of paper for recycling purposes (George et al. 2001). Currently, one of the main applications for cellulases are textile industry, where the need of high temperatures (50–65 °C) and alkaline pH requires the use of thermostable enzymes for efficient jeans treatment (Bhat 2000).

The main commercial cellulase preparations are obtained from filamentous fungi, such as *Aspergillus niger* (Cellulocast of Novozyme) and *Trichoderma reesei* (Megazyme). Among cellulases producing fungi, we can name genus *Aspergillus*, *Trichoderma*, *Penicillium pinophilum*, *Sporotrichum*, *Fusarium*, *Talaromyces*, *Thermoascus*, *Chaetomium*, *Humicola*, *Neocallimastix*, *Piromonas*, and *Sphaeromonas* (Fleuri and Lima 2013).

10.3.2 Xylanases

Xylanases enzymes act on xylan, hemicellulose components, which may be associated to cellulose and lignin in the plant cell wall. Xylan is formed by xylose units linked with β-1,4 glycosidic bonds; they, also, may have arabinose, glucuronic acid or 4-methyl ether, and acetic, *p*-cumaric, and ferulic acids (Brienzo et al. 2008). Among the xylanases enzymes, there are β-1,4 endoxylanases (β-1,4-D-xilanil-xylan hydrolase, EC 3.2.1.8), which depolymerize xylan by random hydrolysis of main skeleton, and β-xylosidases (β-1,4-D-xilosidic-xylo hydrolase, EC 3.2.1.37), which hydrolyze small oligosaccharides (Collins et al. 2005). Xylanase's most important application is in the pulp and paper industry where high temperatures (55–70 °C) and alkaline pH of the pulp substrate requires utilization of thermostable enzymes for efficient bleaching (Beg et al. 2001; Collins et al. 2005; Saha 2003). However, other applications, such as food industry can be mentioned like: dough preparation (Collins et al. 2005), for clarification of beer and juices, and partial hydrolysis of xylan in animal feeds. Nascimento et al. (2003) found that the xylanase extract obtained from *Streptomyces malaysiensis* showed biochemical characteristics (temperature 50–65 °C and pH 6.0–8.0) with great potential for

pulp and paper industry. Beg et al. (2000) showed optimal values of temperature, range between 50 and 75 °C and pH from 6.0 to 9.0 for strain *Streptomyces* QG-11-3. Most known thermostable xylanases are produced by strains of *Thermatoga*, with half-life of 90 min at 95 °C (Sunna and Antranikian 1997). However, very significant thermostability of xylanases has been studied in many *Streptomyces* strains, including *Streptomyces* sp. T7 with stability at 50 °C, at pH 6.0 for 6 days (Deng et al. 2005). Costa et al. (2000) described the production of xylanase complex using *Penicillium janthinellum* with sugarcane bagasse hydrolyzed as substrate.

10.3.3 Proteases

Proteases (EC 3.4.21.12) catalyze hydrolysis of peptide bonds of proteins, and they may have activity on ester and amide bonds. The proteolytic enzymes synthesized by microorganisms have become significant for research because of its wide application at different industries and medicine, as well as its involvement in microbial metabolism. They are used in leather industry, pharmaceutical and food industries, in hydrolysis of substrates used for microbiological growth and parenteral nutrition preparation, detergents, and cosmetics. Proteases enzyme preparations are particularly important in medicine for burns cleaning and removal of necrotic tissue and blood clots lysis (Landau and Egorov 1996). Proteases can also be applied for monogastric animals feed aimed at reduction of anti-nutritional agents of vegetable ingredients, increased digestibility, increasing endogenous enzymes activity, and reduction of environmental pollution (García et al. 2000). Actinomyces and fungi produce a variety of extracellular peptidases, including endopeptidases (serine and metallo-peptidases, specially) and exopeptidases (amino- and carboxypeptidases) with specificity for many substrates. Peptidases obtained from actinomyces, such as serine-peptidases from *Streptomyces exfoliates*; serine and metallo-peptidases from *Streptomyces lactamdurans*; and serine-peptidase from *Streptomyces pactum* are involved in the nitrogen protein sources assimilation, in degradation of aerial mycelium, in sporulation processes, and in production of antibiotics (Kim and Lee 1996). Peptidases and other enzymes used in detergent formulations may have high activity and stability in a wide range of pH and temperature. Serine and metallo-peptidases have been described for the genus *Streptomyces*, as observed with strains *Streptomyces* sp. 594, *Streptomyces malaysiensis* AMT-3, and *Streptomyces alboniger* (Born 1952). However, the nature and characteristics of each component of the peptidase complex from *Streptomyces* has not been extensively studied. Likewise, thermostable actinomyces produce peptidase with major role in degradation of keratin components, such as chicken feathers present in the poultry industry waste (De Azeredo et al. 2004). Specifically, keratinase produced by actinomyces can have great biochemical characteristics with pH ranging between 6.0 and 9.0 and optimal temperature between 50 and 70 °C, as observed for some species (Gushterova et al. 2005). Fungi are, also, capable for protease production. Zanphorlin et al. (2011) used

wheat bran moistened with casein and nutrient for protease production using fungus *Myceliophthora* sp. The enzyme showed optimum pH and temperature of 9.0 and 40–45 °C, respectively. Rojas et al. (2009) studied fungal proteases obtained from *Eladia sacculum* in biodeterioration processes. Cabaleiro et al. (2002) studied protease production by fungi *Phanerochaete chrysosporium* and *Phlebia radiata* in SSF using nylon sponge and corncob. Proteases obtained from this process were distinguished by microbial growth time and activity, and they are of different classes.

10.3.4 Chitinases

Chitin is linear polymer of β-1,4-*N*-acetylglucosamine, which is the most abundant natural amino polysaccharide. Moreover, it is present in cell wall of most fungi and it is the main constituent of insects and crustaceans exoskeleton (Fleuri et al. 2009a). The hydrolysis of chitin occurs by action of enzyme complex involving two enzymes: chitinase or poly (1,4-*N*-acetyl-β-D-glicosaminida) glucan hydrolase (EC 3.2.14), which breaks randomly internal bonds in the chitin chain, generating oligomers and disaccharides, and β-*N*-acetyl-glucosaminidase or β-*N*-acetyl-β-D-hexosaminide-*N*-acetyl-hexosamino hydrolase (EC 3.2.1.52), which cleaves nonreducing terminal unit, releasing *N*-acetylglucosamine. The first ones have higher affinity for larger molecules, while the others prefer small oligomers, including quitobiose (Merzendorfer and Zimoch 2003). Study of chitinase has been increasing because its contribution as defense agents against pathogenic organisms that have chitin, such as insects, nematodes, and fungi (Sahai and Manocha 1993). Besides, chitinases can be used as a protective agent against pathogenic fungi, in protoplast preparation, and production of biologically active substances as aminoglucanooligossacarides (Fleuri et al. 2009a, b). Han et al. (2008) observed application of chitinase in medicine (hypocholesterolemic action and antihypertensive), in agriculture (anti-phytopathogenic), in bioremediation, and in maintenance of food quality. It is estimated that there are between 10 and 25 different chitinases. Tikhonov et al. (1998) produced and purified chitinases from *Streptomyces kurssanovii*. Brzezinska et al. (2012) studied the degradation of chitin substances with chitinase from *Streptomyces rimosus*, which was isolated from soil. Many fungi genus (*Beauveria* sp., *Aspergillus* sp., *Thermoascus* sp., *Chaetomium* sp. *Trichoderma* sp.) are able to produce chitinases by SSF.

10.3.5 Lipases

Lipases are enzymes that are increasing at the biotechnological enzymes scenario. They are very versatile, allow catalysis of hydrolysis and synthesis of chemical reactions; often in chemo, regal, or enantioselective, lipases are applied in many industries such as food industry, pharmaceuticals, fine chemicals, oil chemistry,

detergents, and biodiesel (Barros et al. 2010). The participation of lipases in the world market of industrial enzymes has grown significantly; it is estimated that in the future they will have world significance comparable to peptidases today which count for 25–40 % of industrial enzymes sales (Hasan et al. 2006). Lipases act in the organic–aqueous interface; they catalyze hydrolysis of carboxylic-ester bonds and liberate fatty acids and organic alcohols. However, the reverse reaction (esterification) and also various transesterification reactions can occur in environments with restricted water (Freire and Castilho 2008). The transesterification term refers to radical change between an ester and an acid (acidolysis), or ester and alcohol (alcoholysis), or between two esters (interesterification). Their ability to catalyze these reactions with high efficiency, stability, and versatility make these enzymes very commercially important. Lipases are enzymes of the group of serine hydrolases (EC 3.1.1.3). Their natural substrates are triglycerides; however, its activity is increased when located at the interface polar/nonpolar, and they have higher affinity for long-chain fatty acids (Hasan et al. 2006). Among lipases obtained from actinomyces, there is *Streptomyces rimosus*, *S. coelicolor* (Côte and Shareck 2008), *S. fradiae*, *S. coelicolor* (Sharma et al. 2001), *S. exfoliatus*, *S. albus*, and *S. cinnamomeus* (Abramic et al. 1999). Bielen et al. (2009) and Abramic et al. (1999) reported that lipases have been traced from different microorganisms for different kinds of applications, and that streptomycetes have a large number of genes encoding different enzymes with many lipolysis activities. Among these actinomyces are cited *S. exfoliates*, *S. albus*, *S. coelicolor*, *S. rimosus*, and *S. exfoliatus*. Mander et al. (2012) studied the transesterification with the lipase obtained from *Streptomyces* sp. CS133 for production of biodiesel. Even with a wide variety of microbial lipases, use of these enzymes on industrial scale is still limited due to high production costs, low activity, and limited biochemical characteristics, which facilitates searching of other microbial lipases sources. Extracellular lipases from fungi *Rhizopus homothallicus* (thermostable) were obtained by SSF with sugarcane bagasse as substrate. Moreover, these authors mention that the yield of enzyme production by SSF is higher than liquid fermentation due to increased rate of biomass growth. There are lower protease production that can degrade other enzymes, as well as higher stability for pH and temperature of the enzyme obtained by this type of fermentation (Mateos Diaz et al. 2006). The main commercial lipase preparation is from *Aspergillus oryzae*, created from lipase clones derived from *Thermomyces lanuginosa* (Lipolase from Novo Nordisk) and lipase clones from *Rhizomucor miehei* (Lipozyme, Novo Nordisk S/A). They are especially applied as detergents and production of analogues of cocoa butter from cheap oil sources (Romdhane et al. 2010).

10.3.6 Phytases

Phosphorus is an important ingredient for various biochemical pathways, biological processes, and skeletal integrity. Vegetable ingredients are important sources of

phosphorus, and phytate (inositol hexaphosphate or IP6) is the mineral storage for plants. The amount can differ between plant species. However, phytic acid is not a suitable source of phosphorus for nonruminants animals, since 85 % of the phosphorus is bound to inositol making phytic acid or inositol hexametaphosphate, kept it chelated and unavailable. Diets fed to animals are supplemented with inorganic sources of phosphorus such as calcium phosphate or animal sources like meat and bone flour, due to the lack of availability of phosphorus and a possible deficiency of this mineral for animals in diets with vegetable ingredients. As result, diets for nonruminant animals have amount of phosphorus addition to nutritional requirements, with elimination of excess not absorbed by the animal. Furthermore, phytate acts as anti-nutrient associated to proteins, amino acids, lipids, and minerals, while it interacts with their digestive enzymes reducing activity, influencing digestion, and impairing nutrients utilization. In this sense, phytase catalyzes the hydrolysis of phosphate and phytic acid to phosphorus inorganic, increasing the bioavailability of phosphorus for monogastric animals. Phytase classification is based on first position of the phosphate to be hydrolyzed; named 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26). Supplementation of phytase in diet benefits animal nutrition and improves digestibility of protein, gross energy, and increases the availability of calcium, phosphorus, zinc, manganese, and magnesium. Furthermore, these enzymes improve phosphorus availability in 50 %, and it is important toll to reduce environmental excretion, because of better utilization of phytic phosphorus from vegetable sources, reducing utilization of inorganic sources. Main phytases are classified as their activity on determined pH. Acid phytases show better dephosphorylating between pH 5.0 and alkaline phytase in pH 8.0. All phytases show great pH between 4.0 and 6.0 (Kies et al. 2001; Lei and Stahl 2000). Phytase is produced in large commercial scale by recombination DNA techniques, from fungi of genus *Aspergillus niger*. Enzymes that blend with phytase from *Peniophora lycii*, *Schizosaccharomyces pombe*, and *Escherichia coli* are also found on the market.

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