Chapter 13

Industrial Enzymes and Metabolites from Actinobacteria in Food and Medicine Industry

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Abstract

Microbial enzymes are known to play a crucial role as metabolic catalysts, leading to their use in various industries and several applications. These enzymes are very useful for industrial applications as they increase the reaction rates by several times than normal chemical reactions.

The biochemical heterogeneity, ecological diversity and capacity of the actinobacteria to produce secondary metabolites make them an ideal objective for the production of enzymes.

Members of the actinobacteria *Micromonospora* contains 32 species, are distributed in nature and have been isolated from different environments of different geographical zones, and also form associations with plants on its leaves, roots, rhizospheres and from nitrogen-fixing root nodules of actinorhizal and leguminous plants.

The present review mainly contemplates on enzymes and metabolites of actinobacterial genus *Micromonospora*.

Micromonospora L5, isolated from *C. equisetifolia* nitrogen-fixing nodules, produces hydrolytic enzymes, cellulose, xylanase, pectinase, and also secretes chitinase. The production of these enzymes allows *Micromonospora* L5 to play a potential role to succeed for second generation biofuel production and on the composting process to meet the need in the energy crisis and solve the problem of the increasing amount of organic domestic wastes.

Keywords: *Micromonospora*, hydrolytic enzymes, biofuel, composting, industry

1. Introduction

Microbial enzymes are known to play a crucial role as metabolic catalysts, leading to their use in several industries. The demand for industrial enzymes and for novel natural products is on a continuous rise due to the growing need for sustainable, ecological and economic solutions. Microbes have been serving as one of the largest and useful sources of many enzymes.

Microbial enzymes are very practical and friendly with the environment for industrial applications as they work under mild reaction conditions (e.g., temperature, pH, atmospheric conditions). Additionally microbial enzymes are highly specific and generally increase the reaction by several times than normal chemical reactions. On the other hand, many industrial processes require high temperature, low pH and high pressure, and have low catalytic efficiency. Furthermore, the use of organic solvents leads to organic wastes and pollutants.

Actinobacteria have been known for a longtime as powerful degraders of the dominant portion of plant biomass, lignin, cellulose, xylene, pectin and other complex polysaccharides. The availability of the whole genome sequence data has opened new insights in comparing genomes; current advances in genome sequencing indicate that the potential of bacteria (including the actinobacteria) to degrade certain components of lignocellulose is widespread. From 5,123 analyzed sequenced bacterial genomes for cellulose utilization or degradation 24% synthetized cellulases and β glucosidases [1]. Later results confirmed the potential importance of actinobacteria in lignocellulose degradation [2].

The biochemical heterogeneity, ecological diversity, and ability of the actinobacteria to produce secondary metabolites make them an ideal source for the production of enzymes [3], a source of antibiotic discovery [4], and a source of novel natural products [5]. As a source of novel natural products, 18 from 20 actinobacteria isolated from the soil of the Biosphere Los Petenes in the Mexican Caribbean, have shown activity against human pathogenic bacteria and fungi including *Escherichia coli, Salmonella entiriditis, Salmonella typhymurium, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Helminthosporium* sp. and *Candida albicans* [6]. This fact suggests a sustainable solution for the growing need to solve the medical problem of the pathogenic bacteria becoming resistant to the available industrial antibiotics.

Additionally actinobacteria represent the most suitable biotechnologically procaryotes for the production of a wide range of bioactive metabolites. These filamentous bacteria and their enzymes have an array of biological industrial and environmental applications e.g. soil decontamination [7], biological control of plant diseases [8], and decomposition of organic matter and domestic wastes [9,10].

Actinobacteria are key components of the soil environment and are important contributors to the sustainability of agricultural systems. The increasing energy demands have focused worldwide attention on the utilization of renewable resources, particularly agricultural and forest residues. Lignocellulose, xylan and pectin represent the dominant portion of plant biomass in terrestrial ecosystems and are considered to have great potential as a cheap and renewable feedstock for biofuel production. Alternative and renewable fuels derived from lignocellulosic biomass offer the potential to mitigate global climate change and reduce the

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dependence on fossil fuels. In addition, the decomposition of these compounds in soil environments is an essential process of the carbon cycle.

The relevant aspects of actinobacteria and their ecological, economic, and industrial importance are described in the review [11].

In this chapter, we will highlight the importance of different enzymes with a special focus on the soil actinobacteria *Micromonospora.*

2. Micromonospora

The actinobacteria *Micromonospora* is a genus that contains 32 species [12,13], are gram positive filamentous bacteria, chemo-organotrophic and aerobic characterized by their high guanine-cytosine content in its genome, do not form aerial mycelium in agar plates and produce mycelial carotenoid pigments, white, orange, brown, and when colonies sporulate they appear black in color in certain strains. This bacterium forms branched and septate hyphae of about 0.25 to 0.6 μm in diameter.

These filamentous bacteria are distributed in nature, and have been isolated from different environments of different geographical zones e.g. coastal sediments in Wales [14], marine sediments in Mexico [15] and peat swamp forests in Thailand [16]. The genus have been found forming intimate associations with plants on their leaves [17], roots [18, 19] and various plant rhizospheres [20, 21] including rhizospheres of biofuel crops growing on marginal lands [22], from nitrogen-fixing root nodules of the actinorhizal plant *Casuarina equisetifolia* [23, 24] and *Coriaria myrtifolia* [25], and also from root nodules of the leguminous plants *Lupinus angustifolius* [26] and *Pisum sativum* [27]. Furthermore, *Micomonospora* inhabits nitrogen-fixing nodules in a systematic way [28]. This fact has opened up the question as to what is the ecological role of this bacterium in the plant. The genome of *M. lupini* Lupac 08 and *Micromonospora* L5 contains different genes for hydrolytic enzymes including chitinases [29] which are directly involved in the defense against fungal pathogens by hydrolyzing the cell walls indicating that these bacteria may confer protection to the plant.

Micromonospora also acts as a plant growth promoting rhizobacteria (PGPR) [30] through its ability to promote the growth of nitrogen-fixing symbioses such as *Discaria trinervis-Frankia* [31], *Lupinus albus-Bradyrhizobium canariense* [32] and *Medicago sativa-Sinorhizobium meliloti* [33]. It is supposed, that the actinobacteria produce bioactive metabolites, which are released into the culture medium confirming its role as a PGPR. *Micromonospora* in dual inoculation with other actinobateria in the *Lotus tenuis-Mesorhizobium loti* symbiosis showed to promote root nodulation in plants fertilized with high N levels [34], indicating a high potential of agronomic application since the N fertilization has a powerful inhibition of nodulation of the nitrogen-fixing plants.

The genus shows high biochemical versatility capable of utilizing many different carbon sources given its ability to produce a very rich array of secondary metabolites: antitumor

anthraquinones (lupinadicins A and B), antibacterial polyketides, and inhibitors of tumor cell invasion (lupinacidin C) [35, 36, 37].

2.1. Micromonospora L5

Micromonospora L5 (Figure 1) was isolated from *C. equisetifolia* nitrogen-fixing nodules. In the course of isolating the diazotroph Actinobacteria *Frankia* from surface-sterilized root nodules, we obtained the filamentous bacterium *Micromonospora* strain L5. *Frankia* is hard to isolate due to its very slow growth (generation time is 24–48 h) and a very frequent contaminant is *Micromonospora*.

Indirect evidence of nitrogen fixing genes was obtained by acetylene reduction activity and partial amplification of nifH-like gene fragments in the strain *Micromonospora* sp. L5. However, its genome was screened for the presence of nitrogen-fixing genes and the result was negative.

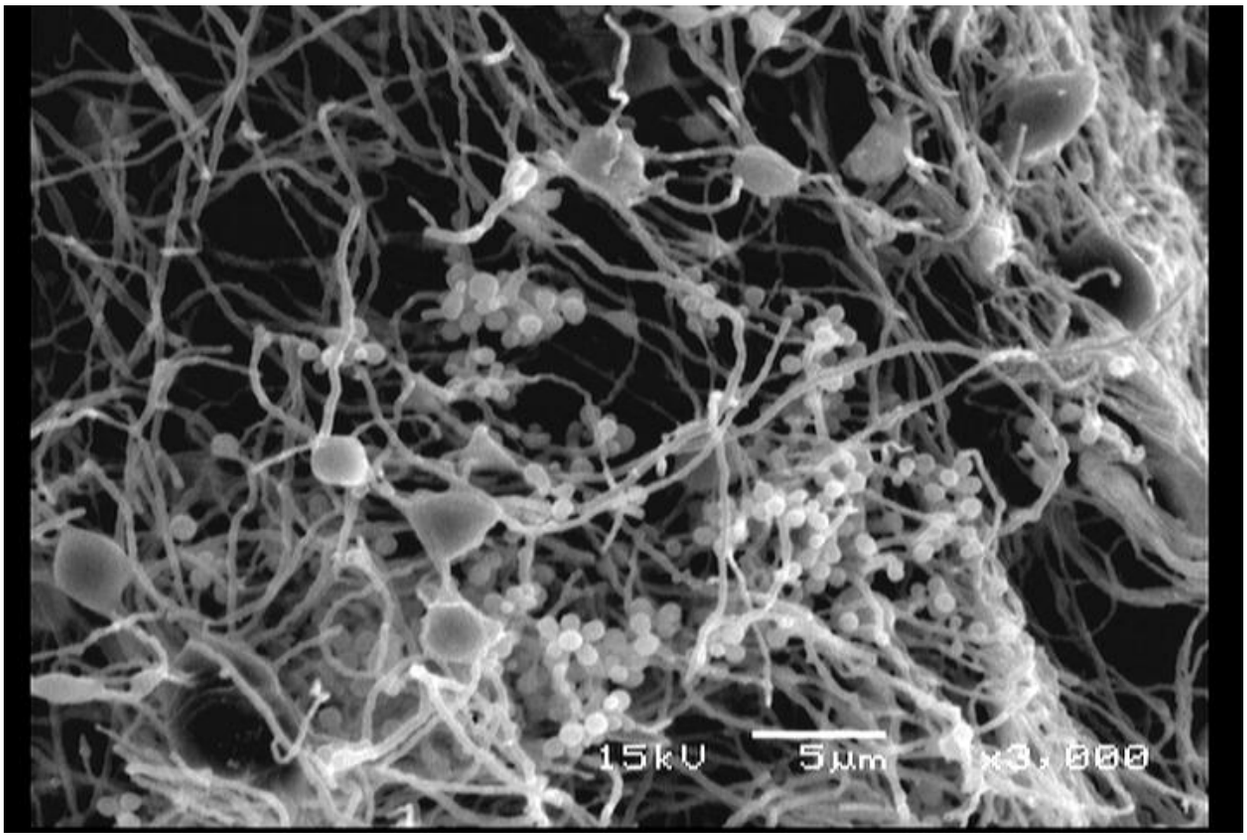

Figure 1. Scanning Electron Microscope view of *Micromonospora* L5. Branched hyphae are observed as well as microspores and large single spores.

The complete genome of *Micromonospora* L5 [38] (NCBI Reference Sequence NC_014815.1) allowed us to find the sequences of different hydrolytic enzymes, cellulases, xylanases, pectinases, and through the BIOCYC Database Collection Enzymes we found the different pathways of the biodegradation of the enzymes (Figures 2, 3, 4).

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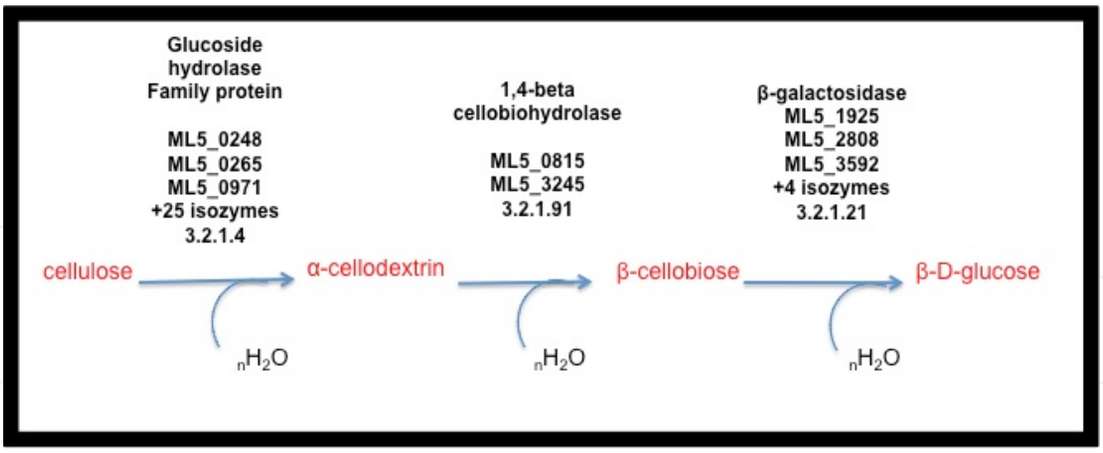

Figure 2. The different pathways of the hidrolysis of cellulose of *Micromonospora* L5 according to BIOCYC Database Collection. Letters in black color indicate the enzymes and its access number in the genome.

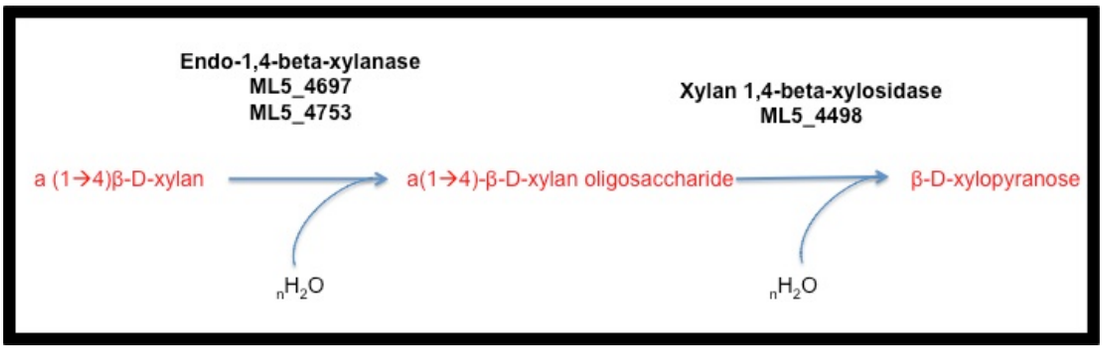

Figure 3. The different pathways of the hidrolysis of xylene of *Micromonospora* L5 according to BIOCYC Database Collection. Letters in black color indicate the enzymes and its access number in the genome.

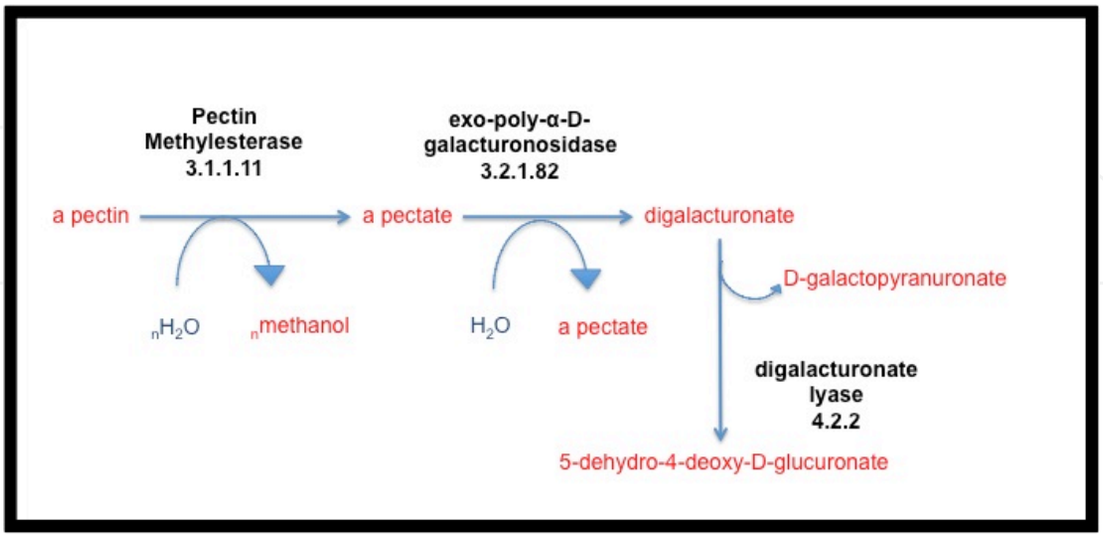

Figure 4. The different pathways of the hidrolysis of pectin of *Micromonospora* L5 according to BIOCYC Database Collection. Letters in black color indicate the enzymes and its access number in the genome.

In addition, the genome of *Micromonopora* L5 contains genes for chitinases. The pathway of the degradation of pectin is shown in Figure 5. The production of chitinases indicates that this strain may confer protection to the plant by hydrolyzing the cell walls of fungal pathogens.

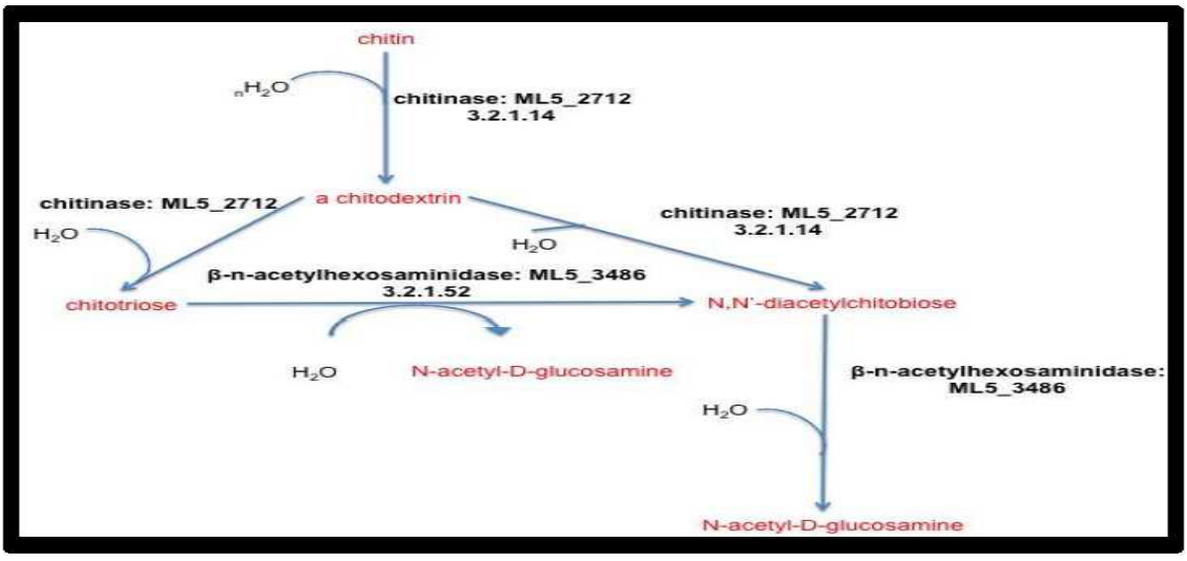

Figure 5. The different pathways of the hidrolysis of chitin of *Micromonospora* L5 according to BIOCYC Database Collection. Letters in black color indicate the enzymes and the access number of enzymes in the genome.

Production of all these enzymes was observed under laboratory conditions and activity was visualized after 8 days of incubation at 28oC and 37oC as shown in Figure 6.

The enzymes endo-β-1,4-glucanase, Exo-β-1,4-glucanase and β-glucosidase of *Micromonospora* showed to be very active at 28oC as well at 37oC (Table 1). The production of 1,4 celobiohydrolase by *Micromonospora* L5 supports its ability as a powerful degrader of cellulose since this enzyme is the most important in the hydrolysis of cellulose.

Enzyme	Enzymatic activity in IU/ml			
	pH 7.0		pH 8.0	
	Temperature		Temperature	
	28°C	37°C	28°C	37°C
Endo-beta-1,4-glucanase	0.800	1.946	0.675	1.688
Exo-beta-1,4-glucanase o celobiohydrolase	0.425	1.114	0.345	0.834
Beta-glucosidase	0.655	1.611	0.415	1.245

Table 1. Quantitative cellulolytic, xylanolytic, and pectinolytic activity of *Micromonospora* L5 after 7 days of culture.

The production of these enzymes also allows *Micromonospora* L5 to play an active role in the degradation of organic matter on its natural habitat, in the carbon cycle and during the composting process of organic domestic wastes. High amounts of solid organic waste are produced all over the world and require safe treatment. The increase of organic waste that

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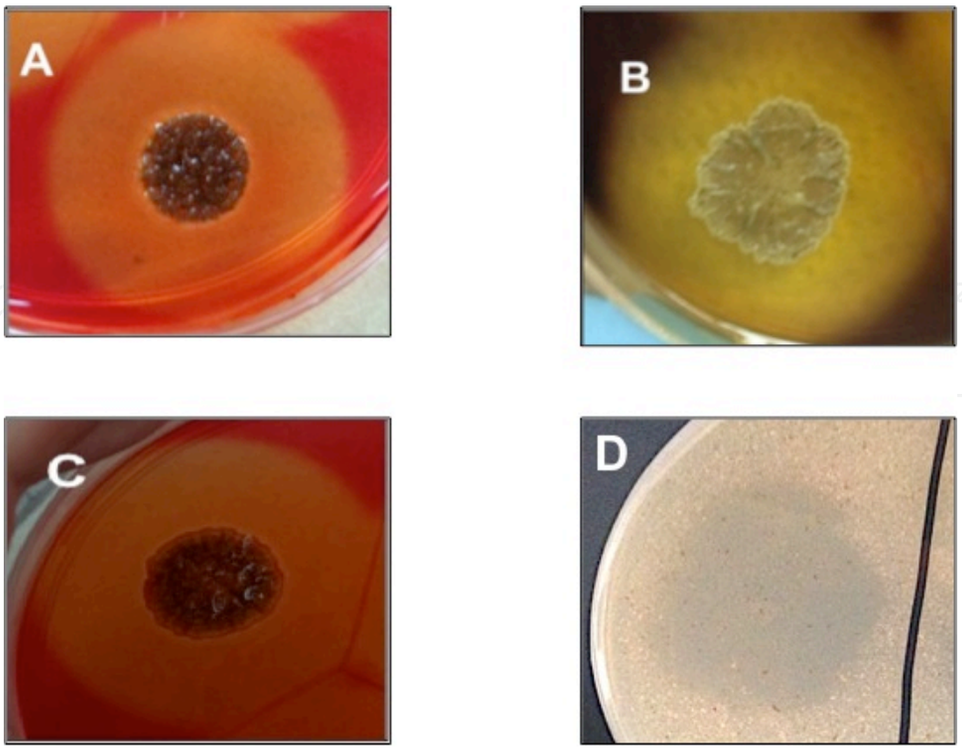

Figure 6. Expression of (A) cellulolytic, (B) pectinolytic, (C) xylanolytic and (D) Chitinase genes of *Micromonospora* L5 at 7 days after inoculation and at a temperature of 37oC.

contains polymerized hydrocarbons requires an efficient composting process. An alternative for improving this process is the search for microorganisms to accelerate the degradation of the organic residues.

3. Enzymatic hydrolysis and applications

3.1. Cellulose

Bioconversion of cellulose, nature's most abundant polysaccharide is accomplished by the enzyme cellulase. Sources of bioconversion of cellulose are wastes of the wood industry, agroindustry, and domestic and garden wastes [39].

Complete enzymatic hydrolysis of cellulose requires synergistic action of three cellulase enzymes: endoglucanase, exoglucanase and beta-glucosidases. Cellulase enzyme systems have a higher activity than the sum of the individual activities of the enzymes, a phenomenon known as synergy collective activity. Cellulase systems are not only an accumulation of enzymes representing all three types, but act in coordination to efficiently hydrolyze cellulose [40].

The cellulose enzymes Endoglucanases III and Cellobiohydrolases I are used in detergents for cleaning textiles. A recent innovation in this industry is the use of cellulases along with protease

and lipase in the detergents [41], although certain enzymes (protease, amylase, lipase, cellulase, mannanase, and pectinase) have been used as catalysts in detergents since the 1960s.

On the other hand the importance of cellulases in the industry of the production of biofuels is the bioconversion of cellulose to molecules of glucose for the fermentation process. A critical step in the development of cellulosic fuels is determining the most favorable conditions for enzymatic saccharification to hydrolyze the cellulose in biomass to fermentable sugars. For a review of cellulases for biofuels see [42].

3.2. Xylan

Xylan is the second most abundant polysaccharide in nature. Xylanases have been reported from actinomycetes [43, 44].

The xylanolytic enzyme system is composed of an array of hydrolytic enzymes, endo-1,4-β-xylanase, xylan-1,4-β-xylosidase, α-glucosiduronase,α-larabinofuranosidase, and acetylxylan esterase.

The most successful application of xylanase is in the paper industry for prebleaching of kraft pulp (process of conversion of wood into wood pulp) to minimize the use of corrosive chemicals in the subsequent treatment stages of pulp [45]. Apart from its use in the paper industry, xylanases are also used as food additives to poultry [46] for the hydrolysis of arabinoxylanes contained in the forage crops conducting to a good nutrimental efficiency of the prime materials [47]. The use of xylanase in combination with pectinase and cellulase are utilized for clarification of fruit juices and degumming of plant fiber sources such as flax [48]. For a review of xylanases and their applications see review [49].

3.3. Pectin

Pectic substances are present in the primary cell wall and are the major component of the middle lamellae, they are responsible for the structural integrity and cohesion of plant tissues. Microbial pectinases are important virulence mechanisms in the phytopathologic process and in plant-microbe symbiosis. The endophytes from soil enter the host plant by colonizing the cracks formed by the emergence of lateral roots from where they spread to the intercellular spaces in the root.

Soil microbial pectinases also participate in the decomposition of dead plant material, contributing to the natural carbon cycle.

Considering the industrial pectinase production alone occupies about 25% of the overall manufacturing of enzyme preparations for food, the use of pectinolytic enzymes in the industry for juice improves the fruit juice yield. The crushing of pectin-rich fruits results in high viscosity juice, and pectinase addition in the extraction process decreases the juice viscosity and degrades the gel structure. In several processes, pectinolytic enzymes are applied together with other cell wall degrading enzymes such as cellulases and xylanases. The mixture

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of pectinases and cellulases has been reported to improve more than 100% the juice extraction yields [50]. For a review of the industrial application of microbial pectinolytic enzymes see [51].

Apart from its use in the food industry for juice production, pectinolytic enzymes are widely used in wine production. The use of pectolytic enzymes, as both clarifying and color extractors, to improve the chromaticity and stability of red wines, gives wines better chromatic characteristics that are more stable over time than the control wines. They show lower loss of red, lower increase in tonality, reach greater levels of brightness much earlier and remain less turbid. Also their chromatic intensity is maintained throughout the two years of storage at fairly acceptable levels [52].

3.4. Chitin

Chitin is the second most abundant natural polymer and distributed as a structural component of crustaceans, insects, other arthropods, and as a component of the cell walls of most fungi.

Chitinase has received attention due to its use as a biocontrol agent. Plant pathogenic fungi is the major problem for agricultural food production. Control of plant pests by the application of biological agents holds great promise as an alternative to the use of chemicals. Chitinases are directly involved in defense against fungal pathogens by hydrolyzing the cell walls. The chitinase genes can also be useful

in developing transgenic plants leading to the plant to develop resistance to various fungal and insect pests [53]. This enzyme may also be useful in the management of sea food waste industries.

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