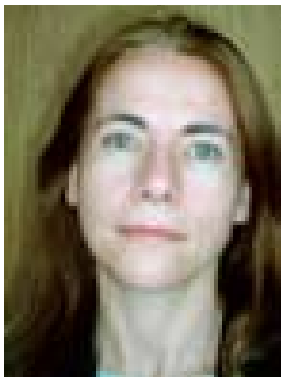
Microbial lignocellulolytic enzymes: industrial applications and future perspectives

Lara D Sette

Microbial Resources Division
and Brazilian Collection of
Environmental and Industrial
Microorganisms (CBMAI)
Center of Chemical, Biological &
Agricultural Research (CPQBA)
Campinas State University 6171
CEP: 13083-970, Campinas, Brazil
Tel: (55) 19 38847500
Fax: (55) 19 38847811
E-mail: lara@cpqba.unicamp.br

Valéria M de Oliveira

Microbial Resources Division
and Brazilian Collection of
Environmental and Industrial
Microorganisms (CBMAI)
Center of Chemical, Biological &
Agricultural Research (CPQBA)
Campinas State University 6171
CEP: 13083-970
Campinas, SP, Brazil



Maria Filomena A Rodrigues

Laboratory of Industrial
Biotechnology
Center for Processes and
Products Technology, Institute of
Technological Research – IPT/SP
Brazil

The demand for microbial industrial enzymes is ever increasing due to their use in a wide variety of processes. Lignocellulolytic enzymes have potential applications in a large number of fields, including the chemical, fuel, food, agricultural, paper, textile and cosmetic industrial sectors. Lignocellulosic biomass is an abundant renewable resource composed of cellulose (a polymer of glucose that represents the major fraction of lignocellulose), hemicellulose (also a sugar polymer) and lignin (a complex phenylpropane polymer). Lignocellulosic material can be broken down by microorganisms into its sugar components, thereby providing a readily fermentable substrate [1]. One of the most significant potential applications of lignocellulolytic enzymes is fuel production from agricultural and forest wastes as an alternative renewable energy resource. The need to reduce carbon dioxide emissions provides an additional incentive for the development of processes for production of fuels from lignocellulosic biomass and has attracted the interest of biotechnologists and microbiologists in recent decades.

Lignolytic enzymes

Lignin represents about 15-25% of lignocellulosic biomass and is a large cross-linked macromolecule composed of three monomers: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol [2]. The enzymes responsible for lignin degradation are mainly lignin-peroxidase (LiP), manganese peroxidase (MnP), laccases and hydrogen peroxide-producing enzymes. A wide variety of microorganisms, including fungi, actinomycetes and bacteria, have been implicated in lignin biodegradation. Among them, white rot fungi have received extensive attention due to their powerful extracellular lignin-degrading enzymatic systems [3] which are responsible for degradation of the lignin polymer into low molecular weight compounds that can be assimilated by other microorganisms [4]. Other groups of fungi have also been reported as producers of lignolytic enzymes [5].

Although lignolytic enzymes play a crucial role in the global carbon cycle, one of the most important current applications of these enzymes is related to environmental remediation, a process in which biological systems are used to degrade or neutralise pollutants [5] or to decolourise dyes [6]. There are many studies in the literature about dye decolourisation and degradation of recalcitrant molecules, such as herbicides, pesticides and polycyclic aromatic hydrocarbons (PAH), by microbial lignolytic enzymes. Among the lignolytic enzymes, laccases have significant potential for industrial use, mainly in the food, chemical and cosmetics sectors [7].

Cellulolytic enzymes

Cellulose, the most abundant constituent of the plant cell wall, is a linear polysaccharide composed of ß-1,4-linked glucose molecules. Efficient hydrolysis of cellulose requires the action of a complex of cellulolytic enzymes that work synergistically:

exo-1,4- β-D-glucanases, endo-1-4- β-D-glucanases and 1,4- β-D-glucanases [8].

The production of cellulases by fungi and bacteria is widely disseminated in nature. Among fungi, the genera Trichoderma, Aspergillus, Penicillium, Humicola and others are described as cellulase producers. The fungus *Trichoderma reesei* has been extensively studied and is used for the industrial production of cellulase [9, 10].

Cellulases can be used in textile, pulp and paper industries. At present, there is an increasing demand for these enzymes, especially for bioconversion of agricultural resources [11] and ethanol production [12]. Therefore, development of more efficient enzyme preparations at low cost is required. Production of highly active and stable cellulolytic complexes depends on the microorganism and the procedure used to obtain the enzyme, especially in relation to the composition of media for cultivation; this can affect the growth and product yield due to substances that can act as inducers, activators, inhibitors and repressors [10].

Future perspectives: microbial metagenomics

Cultured microorganisms have been the most common resource for biotechnological exploration of novel natural products and processes [13]. These microbial compounds span a wide range of industrially important enzymes including the lignocellulolytic enzymes. Traditionally, enzymatic activities have been accessed by isolation and cultivation of microorganisms from environmental samples. However, in the last 2 decades, scientists have realised that only 1-10% of the microbial diversity is retrievable from an environmental sample by current culturing techniques [14, 15]. Culture-independent molecular biology-based methods involving the direct isolation of the total microbial community DNA (the 'metagenome') provide an opportunity to explore the metabolic potential of organisms that cannot be isolated by cultivation. The strategy involves cloning large DNA fragments (20kb up to >500kb) from an environmental sample followed by analysis of the resulting metagenomic libraries to search for novel biological activities (Figure 1) [16, 17]. This technique had been used to detect a wide range of biocatalysts from unculturable biodiversity [18, 19].

Although cellulolytic enzymes offer great potential in many industrial applications, from the generation of bioethanol, a realistic long-term energy source, to the finishing of textiles [11, 12], to date there are only a few reports of metagenome-derived cellulases [20-22]. Recently, a soil metagenome-derived cellulase, Cel5A, was shown to be an endoglucanase (endo 1,4-β-glucan hydrolase) which was highly active towards soluble forms of cellulose [23]. This novel cellulase was remarkably stable over a wide pH and temperature range and in the presence of high salt concentrations and is therefore an ideal candidate for industrial applications.

Conclusions

The application of lignocellulolytic enzymes in biorefineries and ethanol production requires improved enzymes, the use of low cost resources and the manufacture of enzymes on-site to overcome the need for purification and stabilisation of enzyme preparations to reduce production costs. To address these problems, several studies have focused on the discovery of new lignocellulolytic enzymes, including screening for new enzyme-producing microorganisms, random mutagenesis of fungal strains and genetic engineering of microorganisms [8, 9].

The use of metagenomics to explore the vast biocatalytic potential locked within the uncultured portion of the biodiversity is a promising tool for the discovery of new and versatile lignocellulolytic enzymes, especially from extreme environments which usually harbour many enzymes with industrially relevant characteristics. In that sense, a wide range of different approaches are being exploited for the development of commercially

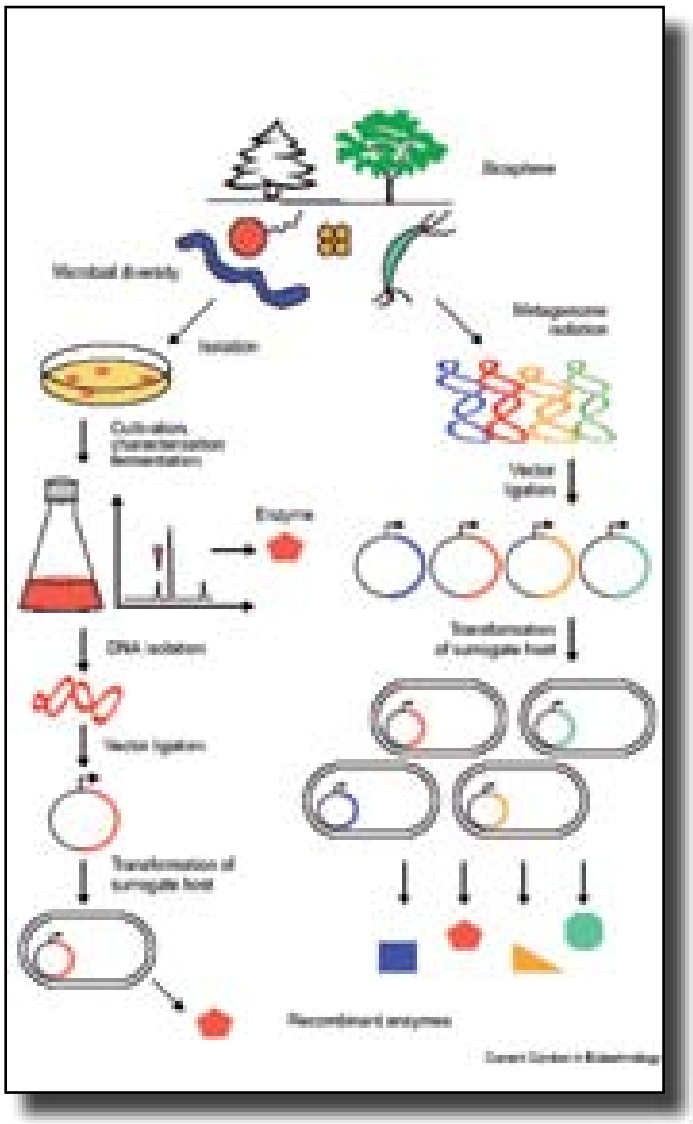


Figure 1. Culture and uncultured-based methods for new enzyme discovery (reprinted with permission, Elsevier) [17].

significant and low cost technologies to facilitate the production of enzymes with industrial utility.

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Lara D Sette is the CBMAI Curator and a Researcher Scientist at CPQBA/UNICAMP. Her research interests are in isolation, preservation and characterisation of filamentous fungi and their potential application for production of bioactive compounds.

Valéria M Oliveira is the head of the Microbial Resources Division at CPQBA/UNICAMP. Her main research areas are molecular microbial ecology and metagenomics, with major focus on the structure and function of bacterial communities associated to petroleum environments.

Maria Filomena A Rodrigues is the head of the Industrial Biotechnology Laboratory at IPT. Her research focuses on the development of bioprocesses, especially in isolation, screening and genetic manipulation of microorganisms for industrial application.

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