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Research Article

Lignin-degrading Bacteria

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Abstract

Degradation of lignin by microorganisms was primarily highlighted in rot and brown-rot fungi, which are capable of producing certain extracellular lignolytic enzymes. Recently, studies on bacteria have also shown that these organisms likely possess a set of extracellular oxidative enzymes implicated in lignin degradation. Therefore, from a biotechnological point of view, lignolytic bacteria could have several novel applications regarding the valorisation and modification of the lignin biopolymers.

Introduction

Lignin, a polymer composed of phenylpropanoid units, associated with cellulose and hemicellulose in plant cell, is the most abundant aromatic substance present in the biosphere. Instead, it is composed of a number of chemically distinct subunits, or monolignols, the abundance of which can vary among species, among individuals, and even among cell types within an organism (Ralph et al., 2004). The decay of lignin in nature is a complex phenomenon not yet completely understood. The ability of lignin to resist degradation can be attributed to its distinctive polymeric structure (Weng et al., 2008) and its complicated aromatic polymer and high molecular weight make it resistant to microorganisms, resulting in the prevention of effective cellulose utilization (Wang et al., 2011). Indeed, complete degradation of lignin is mainly assured by microorganisms. However, only a few bacterial species are currently known to degrade lignin molecule. Furthermore, microbial degradation of lignin has been well studied in white-rot (*Phanerochaete chrysosporium* and *Trametes versicolor*) and brown-rot fungi (*Fomitopsis palustris*), but is much less well studied in bacteria (Bugg et al., 2011). Among these white rot fungi, basidiomycete, *P. chrysosporium* has become the most commonly used organism due to its ability to produce ligninolytic enzymes, fast growth and easy handling during culturing techniques (Barclay et al., 1993). However, there are some constraints that should be overcome by lignolytic organisms for attacking and decomposing the lignin. Fungi are

capable of overcoming these constraints by their extracellular lignolytic enzymes called ligninases, including heme-dependent lignin peroxidases, manganese peroxidases, versatile peroxidases (Camarero et al., 1999; Bhat, 2000; Coughlan, 1992), and multicopper-dependent laccases (Thurston, 1994).

White-rot fungi produce a range of extracellular lignolytic enzymes. The first molecular information on bacterial high molecular weight metabolite formation was reported with the isolation of a secreted bacterial heme peroxidase from a gram-positive bacterium, *Streptomyces viridosporus* T7A (Pieper, 2005; Adav et al., 2010). Thus, there are indications that bacteria use similar types of extracellular lignin-degrading enzymes to fungi (Bugg et al., 2011). But in general, the heme peroxidases from studied lignolytic bacteria have been found to be less oxidatively powerful compared to the fungal enzymes involved in lignin degradation (Brown et al., 2014). However, it is possible that the major lignolytic enzymes in bacterial systems is not the heme peroxidases. Beyond the enzymes used by fungi, bacteria also contain a suite of oxidative enzymes that could modify lignin for breakdown by hydroxylation or demethylation, such as cytochrome P450s, non-heme iron enzymes, or Mn- and Cu-containing oxidases (Brown et al., 2014). As indicated before, owing to the physical properties of lignin and its incorporation into the cell wall, current approaches used for its

removal from biomass are sufficiently expensive and energy intensive (Hamelinck et al., 2005). Although certain lignocellulolytic fungi are able to secrete industrial quantities of extracellular enzymes, bacterial enzyme production can be more cost-efficient (Woo et al., 2014). This is because they grow more rapidly, produce multi-enzyme complexes with increased functionality and higher specificity, and can tolerate larger and more diverse environmental stress (Gilkes et al., 1991; Maki et al., 2009). Lignocellulolytic bacteria could also potentially allow better separation of lignin from cellulose and thereby increase the value of both lignin, which is currently a waste product, and cellulose [14]. Moreover, It has been shown that fungi despite of their high capability of lignin-degrading, they are sometimes not stable in practical treatment under extreme environmental and substrate conditions (Hatakka, 1994). In contrast, bacteria, in particular, deserve to be studied for ligninolytic potential because of their immense environmental adaptability and biochemical versatility. This article will focus on the diversity of lignolytic bacteria.

Lignolytic bacteria

Insolubility of lignin and its lack of stereoregularity contribute to making it a substrate that is difficult for the microflora to degrade (Vicuna, 1988). Furthermore, the need to gain access to the substrate by penetration of plant tissues constitute an additional challenge for microorganisms, bacteria in particular. Actinomycetes and fungi, accomplish this task by hyphal invasion of the various cell wall layers (Vicuna, 1988). Nonfilamentous bacteria, originally thought to be unable to fulfill this requirement, have been found to erode wood fibers through both tunneling (Nilsson et Daniel, 1983; Daniel et Nilsson, 1987) and cavitation (Nilsson et Singh, 1983). In contrast, information available indicates that lignin is fairly recalcitrant to bacterial attack, at least under laboratory conditions (Vicuna, 1988). For this reason, some authors believe that bacteria play a secondary role in lignin biodegradation in natural environments (Janshekar et Fiechter, 1983; Kirk et Farrell, 1987). However, bacteria seem to play a leading role in decomposing lignin in aquatic ecosystems (Vicuna, 1988). Both fungi and bacteria have been observed to metabolize lignin; however, their differential reactivity with this substrate indicates that they may utilize different chemical strategies for its breakdown (Brown et Chang, 2014). Although the metabolism of lignin is not as complete compared to fungal systems, it is clear that bacteria can react with lignin and possibly produce smaller aromatics that can be imported into the cell for aromatic catabolism, which is also widespread in soil bacteria (Burlage et al., 1989; Masai et al., 1999). Bacterial lignin degradation mainly occurs under aerobic conditions. Lignin-degrading activity under aerobic conditions has been reported from diverse groups of bacteria (Zimmermann, 1990). Indeed, bacterial systems have been found to be less oxidatively powerful compared to lignolytic fungal systems to date (Brown et Chang, 2014). The few bacterial species currently known to degrade cellulose and

lignin are within *Pseudomonas* (order Pseudomonadales), *Cellulomonas* (order Actinomycetales), *Streptomyces* (order Actinomycetales), and other genera within the order Actinomycetales (Lynd et al., 2002; Pérez et al., 2002) and are likely employing extracellular laccases and peroxidases (Woo et al., 2014). Many studies suggest that *Pseudomonas* sp. is the most efficient lignin degradation bacterium (Yang et al., 2007). It not only has the ability to degrade nature lignin but it also degrades aromatic rings (Das et al., 2012). Thus, bacteria of other genera, including *Alcaligenes*, *Arthrobacter*, and *Nocardia*, readily degrade the single-ring aromatic compounds that build up the lignin macromolecule (Dagley, 1971). Clostridiales was previously regarded as a degrader of cellulose and hemicellulose, but in recent years, it was also reported participating in lignin degradation (Chamkha et al., 2001). There are several reports of bacteria isolated from termite guts that have aromatic degradation capability (Geib et al., 2008) such as *Rhodococcus erythropolis* (Chung et al., 1994), *Microbacterium* sp., *Brucella melitensis*, *Ochrobactrum* sp. and *Sphingomonas* sp. (Wenzel et al., 2002), *Burkholderia cepacia* (Kato et al., 1998), *Burkholderia* sp. and *Burkholderia* sp. (Harazono et al., 2003). However, there are conflicting reports on the ability of termite gut micro-organisms to break down lignin (Bugg et al., 2011). Furthermore, bacteria isolated from wood-boring beetles possess in vitro lignolytic activity such as *Paracoccus* spp., *Escherichia* spp., *Yersinia* spp., *Rahnella* spp., *Pantoea* and *Erwinia* (Schloss et al., 2006; Vasanthakumar et al., 2006; Delalibera et al., 2007). Recently a bacteria consortium containing at least nine genera (Clostridiales, *Geovibrio thiophilus*, *Desulfomicrobium*, *Pseudomonas* sp., *Azoarcus* sp., *Thauera*, *Paenibacillus* sp., *Cohnella* sp., *Acinetobacter* sp., *Microbacterium*, and uncultured bacterium) was found mainly responsible for lignin degradation, based on various screening procedures (Wang et al., 2013). Some other bacteria are known to date, to have activity for lignin breakdown are listed in Table 1.

Conclusion

According to several previous studies, Lignin degradation which is a complex process with synergism among more enzymes, is mainly assumed by both bacteria and fungus. Many bacterial strains can solubilize and metabolize lignin compounds but their ability to mineralize polymeric lignin is limited compared to fungi. Furthermore, it was found that bacterial consortiums were more effective in lignocelluloses degradation than other isolates (Wang et al., 2011). Recent studies on lignin degradation in nature may provide novel resources for the delignification of dedicated bioenergy crops and other sources of lignocellulosic biomass (Weng et al., 2008). Indeed, Lignocellulolytic bacteria have promised to be a fruitful source of new enzymes for next-generation lignocellulosic biofuel production (Woo et al., 2014).

Table 1. Lignolytic bacteria and their substrate-degrading

Bacteria species	Substrate-degrading	References
<i>Streptomyces viridosporus</i> T7A	depolymerize lignin	(Ramachandra et al., 1988)
<i>Pseudomonas paucimobilis</i> SYK-6	break down various dimeric lignin compounds	(Katayama et al., 1988)
<i>Pseudomonas putida</i> mt-2 <i>Rhodococcus jostii</i> RHA1	Lignin degradation	(Ahmad et al., 2010)
<i>Azotobacter</i> sp. HM121	lignin mineralization and solubilization	(Morii et al., 1995)
<i>Aneurinibacillus aneurinilyticus</i>	depolymerize lignin	(Raj et al., 2007)
<i>Bacillus</i> sp.	degrade 37% kraft lignin by culture at 30 °C for 6 days	(EL-Hanafy et al., 2008)
<i>Paenibacillus</i> sp	degrade kraft lignin	(Chandra et al., 2007)

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