Journal of Biotechnology 239 (2016) 39–46

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Whole-cell biosensor of cellobiose and application to wood decay detection

Maxime Toussaint[a,b], Cyril Bontemps[a,b,**], Arnaud Besserer[c], Laurence Hotel[a,b], Philippe Gérardin[c], Pierre Leblond[a,b,*]

[a] Laboratoire Dynamique des Génomes et Adaptation Microbienne, UMR 1128, Université de Lorraine, Vandœuvre-lès-Nancy, F-54506, France
[b] Laboratoire Dynamique des Génomes et Adaptation Microbienne, UMR 1128, INRA, Vandœuvre-lès-Nancy, F-54506, France
[c] Laboratoire d'Etudes et de Recherches sur le Matériau Bois, EA4370, Université de Lorraine USC INRA, Faculté des Sciences et Technologies, 54506, Vandœuvre-lès-Nancy, F-54506, France

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 3 October 2016
Accepted 6 October 2016
Available online 8 October 2016

Keywords:
Biosensor
CebR
Cellobiose
Streptomyces
Synthetic biology
Wood decay

ABSTRACT

Fungal biodegradation of wood is one of the main threats regarding its use as a material. So far, the detection of this decaying process is empirically assessed by loss of mass, when the fungal attack is advanced and woody structure already damaged. Being able to detect fungal attack on wood in earlier steps is thus of special interest for the wood economy. In this aim, we designed here a new diagnostic tool for wood degradation detection based on the bacterial whole-cell biosensor technology. It was designed in diverting the soil bacteria *Streptomyces* CebR sensor system devoted to cellobiose detection, a cellulolytic degradation by-product emitted by lignolytic fungi since the onset of wood decaying process. The conserved regulation scheme of the CebR system among *Streptomyces* allowed constructing a molecular tool easily transferable in different strains or species and enabling the screen for optimal host strains for cellobiose detection. Assays are performed in microplates using one-day culture lysates. Diagnostic is performed within one hour by a spectrophotometric measuring of the cathecol deshydrogenase activity. The selected biosensor was able to detect specifically cellobiose at concentrations similar to those measured in decaying wood and in a spruce leachate attacked by a lignolytic fungus, indicating a high potential of applicability to detect ongoing wood decay process.

1. Introduction

Thanks to its intrinsic mechanical properties, wood is used since the Neolithic era as a material for construction, furnishing, heating or wood-derived products such as paper. Nowadays, there is also a regain of interest for this material as it is renewable and a source of biomass for conversion into bio-ethanol (Wang et al., 2013). As a material, wood is highly resistant to the environmental conditions such as rain, sun or other abiotic stresses and the main threat regarding its use comes mostly from the attack by lignolytic organisms. This wood bio-degradation represents a major economic problem. For instance, in the US only, it costs more than 5 billion USD each year to homeowners and almost 10% of the annual product of forest is used to replace the degraded products (Schultz and Nicholas, 2008). Being able to detect the early wood bio-degradation is thus crucial. During storage, it would avoid to use contaminated wood for construction. When the wood is already used as a material it would enable to apply early curative measures before any significant loss of mechanical properties. However, to our knowledge, there is no tool available to achieve this goal and wood degradation is so far only assessed by resistance testing and visual expertise only relevant and operating on wood decayed at an advanced level.

Wood bio-degradation in forest ecosystems is mainly the fact of diverse fungi (Rajala et al., 2012) classified according to the type of decay they cause (*e.g.* white-rots, brown-rots, soft-rots, see for review (Schwarze et al., 2000)). If rot-fungi have distinct specificities in term of ecology or degradation strategies, they nevertheless all degrade and use as a carbon source the cellulose that represents 40%–50% of the plant dry weight (Howard et al., 2004). Two main degradation strategies exist during the wood degradation by fungi:

* Corresponding author at: Laboratoire Dynamique des Génomes et Adaptation Microbienne, UMR 1128, INRA-Université de Lorraine, Faculté des Sciences et Technologies-Campus Aiguillettes, 54506 Vandœuvre-lès-Nancy, France.
** Corresponding author at: Laboratoire Dynamique des Génomes et Adaptation Microbienne, UMR 1128, INRA-Université de Lorraine, Faculté des Sciences et Technologies-Campus Aiguillettes, 54506 Vandœuvre-lès-Nancy, France.
E-mail addresses: cyril.bontemps@univ-lorraine.fr (C. Bontemps), pierre.leblond@univ-lorraine.fr, leblond@inra.fr (P. Leblond).

http://dx.doi.org/10.1016/j.jbiotec.2016.10.003
0168-1656/

enzymatic pathways involving cellulases or oxidative mechanisms using Fenton reaction and polysaccharide monooxygenases (Lynd et al., 2002; Phillips et al., 2011). However, a canonical degradation pathway is common for all of them where the cellulose fiber is shortened into simpler forms: the cellobiose (two β-1,4 linked glucose units) or sometimes into cellodextrines (generally from three to six β-1,4-linked glucose units) (Langston et al., 2011; Lynd et al., 2002). These cellulose degradation by-products will be hydrolyzed later on into glucose by the action of β-glucosidases (Langston et al., 2011). Thus, the initial release and presence of cellobiose and cellodextrines is a common denominator and can be considered as a signature of the attack of wood by rot-fungi.

The *Streptomyces* are filamentous spore forming soil dwelling bacteria that are generally not able to degrade native wood, but are considered as essential to recycle biomass polymers in environment thanks to their large-enzymatic arsenal able to degrade wood by-products (Bontemps et al., 2013; Bruce et al., 2010). The detection of these compounds and the activation of the enzymatic pool to degrade them has been linked to the regulator CebR of the LacI family in *Streptomyces griseus* (Marushima et al., 2009), *Streptomyces reticuli* (Schlösser et al., 2000), *Streptomyces* sp. ActE (Takasuka et al., 2013). Since its characterization by Schlösser et al. (Schlösser et al., 2000), and Marushima et al. (Marushima et al., 2009), it is known that the CebR transcriptional repressor prevents gene expression from binding a conserved 22 bp hairpin motif (*cebR*-box) found in the transcriptional region of its targets. In presence of inducer molecules such as cellobiose or in some cases cellodextrines or cellulose, the CebR repression is alleviated and enables the expression of the controlled genes. So far, the complete CebR regulome is not well-known. However, a transcriptomic analysis of the *Streptomyces* sp. ActE (Sirex) (a symbiotic strain that helps the pine-boring woodwasp *Sirex noctilio* to deconstruct wood biomass) has shown that, in presence of wood derived compounds, the most up-regulated genes were under the control of the CebR-system. These genes were mostly involved in the uptake (ABC transporter system) or in the production of cellulolytic and hemicellulolytic enzymes (β-glucosidases, cellulases, cellobiohydrolases, mannosidases) (Takasuka et al., 2013). Moreover, CebR could induce other functions like the pathogenic factors of *S. scabies* in presence of cellobiose (Francis et al., 2015).

Since cellobiose is a key product of cellulose hydrolysis and indirectly indicates fungal wood degradation, we designed and developed a *cebR*-box based biosensor expressed by *Streptomyces* in order to detect the presence of cellobiose, demonstrated its sensibility and specificity and showed its applicability in a case of wood degradation detection.

2. Material and methods

2.1. Plasmids, strains and media

Plasmids and strains used in this work are presented in Table 1. *Escherichia coli* strains were grown in LB medium (Kieser et al., 2000) at 37 °C and *Streptomyces* at 30 °C either on solid SFM medium [20 g mannitol, 20 g soy flour and 20 g bacto-agar per liter] or in liquid modified HT medium (HT*) [1 g yeast extract, 1 g beef extract, 5 g mannitol, 2 g bacto-tryptone and 0.02 g $COCL_2$ per liter, pH = 7.3]. For the biosensor tests, cellobiose (Alfa Aesar, Karlsruhe, Germany) and cellodextrines (Elicityl, Crolles, France) were added after autoclaving in liquid modified HT at the appropriate concentration. Avicel Ph-101 (Sigma, St. Louis, USA), a crystalline form of cellulose, was added in liquid medium before autoclaving. Media were supplemented with apramycin (50 μg ml^{-1}) for the selection of strains transformed with the pIB139 derived plasmids or with ampicilline (100 μg ml^{-1}) for the selection of the *E. coli* transformed with pGemT-easy (Promega, Madison, USA).

2.2. Wood infection, leachate preparation and cellobiose quantification

Wood degradation was performed according the European standard EN 113, 1996 (EN 113, 1996) with some modifications. Spruce (*Picea abies* L.) wood specimens (40 × 15 × 5 mm) were sterilized at 103 °C for 2 days. Then wood specimens were placed under sterile conditions in petri dishes containing one-week old mycelium of the lignolytic *Poria placenta* fungus. Wood specimens were incubated at 22 °C and 70% relative humidity for 16 weeks. For leachate preparation, *P. placenta* mycelium was carefully removed from the wood by hand scratching. Eight wood specimens were impregnated under vacuum with 100 ml of PBS buffer pH 7.4 for 15 min. For cellobiose quantification, 20 μl of leachate were run on HPLC equipped with a refractometer. Sugars were separated on a Shodex sugar KS-803 column (Waters SAS, Guyancourt, France) at 80 °C with a flow of 0.8 ml min^{-1} using HPLC grade water as solvent. Cellobiose and glucose were detected after 12.4 min and 13.2 min of elution, respectively. The cellobiose concentration present in the leachate was estimated by peak area integration and comparison with a cellobiose standard calibration curve. The cellobiose calibration curve ranged from 2.92 mM to 29.3 μM. Specificity and detection of free glucose resulting from wood cell wall degradation was detected by comparison with a calibration curve build from glucose concentrations ranging from 5.56 mM to 55.6 μM.

2.3. DNA manipulations

Polymerase chain reactions were performed with the Dream Taq polymerase (ThermoFisher Scientific, Waltham, USA) for fragments under 1.5 kb in size or Taq polymerase Takara (Takara Bio Inc., Kusatsu, Japan) for larger fragments. PCR primers are listed in Table 2. Ligation reactions were carried out with T4 DNA ligase (ThermoFisher Scientific, Waltham, USA). DNA was digested and purified from gel matrix respectively with restriction enzymes and the GeneJet Gel Extraction Kit purchased from ThermoFisher Scientific (Waltham, USA). The kits were used according to supplier's recommendations. Alkaline lysis plasmid extractions, plasmid electroporations and DNA extractions were performed as described in (Sambrook et al., 2001).

2.4. Functional characterization of biosensors

Functional screenings of the biosensors efficiency were performed by streaking of 2 μl of spore suspension (ca 10^8–10^9 spores/mL) of each biosensor on HT* plates supplemented with 5 gL^{-1} of cellobiose and incubation at 30 °C for 2 days. Controls without cellobiose or with crystalline cellulose (Avicel) were performed in parallel. After growth, plates were sprayed with a 0.5 M catechol solution and incubated for 10 to 20 min in the dark. The visualization of a yellow color in the mycelium and in its surrounding medium attested the detection of cellobiose by the biosensor after confrontation with the control plates *i.e.* without cellobiose and supplemented with crystalline cellulose. A positive result in both latter cases would constitute false positives.

2.5. Biosensor microplate tests

Spore suspensions of the *Streptomyces* S4N27 biosensor strain were realized on SFM complemented with apramycin (Kieser et al., 2000) and stored in a 20% (v/v) glycerol solution at −20 °C. To perform biosensor qualitative and quantitative tests in presence of cellobiose, cellodextrines or wood leachates, 5.10^5 spores were