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1 Research article

2

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

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27 **Keywords**: Biosensor, CebR, cellobiose, *Streptomyces*, synthetic biology, wood decay

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30

31 **Abstract**

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

46

## 47        1. Introduction

48        Thanks to its intrinsic mechanical properties, wood is used since the Neolithic era as a  
49        material for construction, furnishing, heating or wood-derived products such as paper.  
50        Nowadays, there is also a regain of interest for this material as it is renewable and a source  
51        of biomass for conversion into bio-ethanol [1]. As a material, wood is highly resistant to the  
52        environmental conditions such as rain, sun or other abiotic stresses and the main threat  
53        regarding its use comes mostly from the attack by lignolytic organisms. This wood bio-  
54        degradation represents a major economic problem. For instance, in the US only, it costs  
55        more than 5 billion USD each year to homeowners and almost 10% of the annual product of  
56        forest is used to replace the degraded products [2]. Being able to detect the early wood bio-  
57        degradation is thus crucial. During storage, it would avoid to use contaminated wood for  
58        construction. When the wood is already used as a material it would enable to apply early  
59        curative measures before any significant loss of mechanical properties. However, to our  
60        knowledge, there is no tool available to achieve this goal and wood degradation is so far only  
61        assessed by resistance testing and visual expertise only relevant and operating on wood  
62        decayed at an advanced level.

63        Wood bio-degradation in forest ecosystems is mainly the fact of diverse fungi [3] classified  
64        according to the type of decay they cause (e.g. white-rots, brown-rots, soft-rots, see for  
65        review [4]). If rot-fungi have distinct specificities in term of ecology or degradation strategies,  
66        they nevertheless all degrade and use as a carbon source the cellulose that represents 40%  
67        to 50% of the plant dry weight [5]. Two main degradation strategies exist during the wood  
68        degradation by fungi: enzymatic pathways involving cellulases or oxidative mechanisms  
69        using Fenton reaction and polysaccharide monooxygenases [6,7]. However, a canonical  
70        degradation pathway is common for all of them where the cellulose fiber is shortened into  
71        simpler forms: the cellobiose (two  $\beta$ -1,4 linked glucose units) or sometimes into  
72        cellodextrines (generally from three to six  $\beta$ -1,4-linked glucose units) [7,8]. These cellulose  
73        degradation by-products will be hydrolyzed later on into glucose by the action of  $\beta$ -  
74        glucosidases [8]. Thus, the initial release and presence of cellobiose and cellodextrines is a  
75        common denominator and can be considered as a signature of the attack of wood by rot-  
76        fungi.

77        The *Streptomyces* are filamentous spore forming soil dwelling bacteria that are generally not  
78        able to degrade native wood, but are considered as essential to recycle biomass polymers in  
79        environment thanks to their large-enzymatic arsenal able to degrade wood by-products  
80        [9,10]. The detection of these compounds and the activation of the enzymatic pool to  
81        degrade them has been linked to the regulator CebR of the Lacl family in *Streptomyces*

82 *griseus* [11], *Streptomyces reticuli* [12], *Streptomyces* sp. ActE [13] and *Streptomyces*  
83 *scabies* [14]. Since its characterization by Schlösser *et al.* [12], and Marushima *et al.* [11], it  
84 is known that the CebR transcriptional repressor prevents gene expression from binding a  
85 conserved 22 bp hairpin motif (*cebR*-box) found in the transcriptional region of its targets. In  
86 presence of inducer molecules such as cellobiose or in some cases cellodextrines or  
87 cellulose, the CebR repression is alleviated and enables the expression of the controlled  
88 genes. So far, the complete CebR regulome is not well-known. However, a transcriptomic  
89 analysis of the *Streptomyces* sp. ActE (Sirex) (a symbiotic strain that helps the pine-boring  
90 woodwasp *Sirex noctilio* to deconstruct wood biomass) has shown that, in presence of wood  
91 derived compounds, the most up-regulated genes were under the control of the CebR-  
92 system. These genes were mostly involved in the uptake (ABC transporter system) or in the  
93 production of cellulolytic and hemicellulolytic enzymes ( $\beta$ -glucosidases, cellulases,  
94 cellobiohydrolases, mannosidases) [13]. Moreover, CebR could induce other functions like  
95 the pathogenic factors of *S. scabies* in presence of cellobiose [15].

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

100

## 101 **2. Material and Methods**

### 102 2.1) Plasmids, strains and media

103 Plasmids and strains used in this work are presented in Table 1. *Escherichia coli*  
104 strains were grown in LB medium [16] at 37°C and *Streptomyces* at 30°C either on solid SFM  
105 medium [20 g mannitol, 20 g soy flour and 20 g bacto-agar per liter] or in liquid modified HT  
106 medium (HT\*) [1 g yeast extract, 1 g beef extract, 5 g mannitol, 2 g bacto-tryptone and 0.02 g  
107 COCL<sub>2</sub> per liter, pH=7.3]. For the biosensor tests, cellobiose (Alfa Aesar, Karlsruhe,  
108 Germany) and cellodextrines (Elicityl, Crolles, France) were added after autoclaving in liquid  
109 modified HT at the appropriate concentration. Avicel Ph-101 (Sigma, St. Louis, USA), a  
110 crystalline form of cellulose, was added in liquid medium before autoclaving. Media were  
111 supplemented with apramycin (50  $\mu\text{g}\cdot\text{ml}^{-1}$ ) for the selection of strains transformed with the  
112 pIB139 derived plasmids or with ampicilline (100  $\mu\text{g}\cdot\text{ml}^{-1}$ ) for the selection of the *E. coli*  
113 transformed with pGemT-easy (Promega, Madison, USA).

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## 117 2.2) Wood infection, leachate preparation and cellobiose quantification

118 Wood degradation was performed according the European standard EN 113, 1996 [17] with  
119 some modifications. Spruce (*Picea abies* L.) wood specimens (40x15x5 mm) were sterilized  
120 at 103°C for 2 days. Then wood specimens were placed under sterile conditions in petri  
121 dishes containing one-week old mycelium of the lignolytic *Poria placenta* fungus. Wood  
122 specimens were incubated at 22°C and 70 % relative humidity for 16 weeks. For leachate  
123 preparation, *P. placenta* mycelium was carefully removed from the wood by hand scratching.  
124 Eight wood specimens were impregnated under vacuum with 100 ml of PBS buffer pH 7.4 for  
125 15 min. For cellobiose quantification, 20 µl of leachate were run on HPLC equipped with a  
126 refractometer. Sugars were separated on a Shodex sugar KS-803 column (Waters SAS,  
127 Guyancourt, France) at 80°C with a flow of 0.8 mL.min<sup>-1</sup> using HPLC grade water as solvent.  
128 Cellobiose and glucose were detected after 12.4 min and 13.2 min of elution, respectively.  
129 The cellobiose concentration present in the leachate was estimated by peak area integration  
130 and comparison with a cellobiose standard calibration curve. The cellobiose calibration curve  
131 ranged from 2.92 mM to 29.3 µM. Specificity and detection of free glucose resulting from  
132 wood cell wall degradation was detected by comparison with a calibration curve build from  
133 glucose concentrations ranging from 5.56 mM to 55.6 µM.

## 134 2.3) DNA manipulations

135 Polymerase chain reactions were performed with the Dream Taq polymerase  
136 (ThermoFisher Scientific, Waltham, USA) for fragments under 1.5 kb in size or Taq  
137 polymerase Takara (Takara Bio Inc., Kusatsu, Japan) for larger fragments. PCR primers are  
138 listed in Table S1. Ligation reactions were carried out with T4 DNA ligase (ThermoFisher  
139 Scientific, Waltham, USA). DNA was digested and purified from gel matrix respectively with  
140 restriction enzymes and the GeneJet Gel Extraction Kit purchased from ThermoFisher  
141 Scientific (Waltham, USA). The kits were used according to supplier's recommendations.  
142 Alkaline lysis plasmid extractions, plasmid electroporations and DNA extractions were  
143 performed as described in [18].

## 144 2.4) Functional characterization of biosensors

145 Functional screenings of the biosensors efficiency were performed by streaking of 2 µL of  
146 spore suspension (ca 10<sup>8</sup>-10<sup>9</sup> spores/mL) of each biosensor on HT\* plates supplemented  
147 with 5 g.L<sup>-1</sup> of cellobiose and incubation at 30°C for 2 days. Controls without cellobiose or

148 with crystalline cellulose (Avicel) were performed in parallel. After growth, plates were  
149 sprayed with a 0.5 M catechol solution and incubated for 10 to 20 min in the dark. The  
150 visualization of a yellow color in the mycelium and in its surrounding medium attested the  
151 detection of cellobiose by the biosensor after confrontation with the control plates *i.e.* without  
152 cellobiose and supplemented with crystalline cellulose. A positive result in both latter cases  
153 would constitute false positives.

#### 154 2.5) Biosensor microplate tests

155 Spore suspensions of the *Streptomyces* S4N27 biosensor strain were realized on SFM  
156 complemented with apramycin [16] and stored in a 20% (v/v) glycerol solution at -20°C. To  
157 perform biosensor qualitative and quantitative tests in presence of cellobiose, cellodextrines  
158 or wood leachates,  $5 \cdot 10^5$  spores were inoculated into 3 ml of modified HT medium cultures  
159 complemented with the sugars at the selected concentrations or with 1 ml of wood leachate.  
160 Cultures were incubated at 30°C for 16-18 hours under agitation (200 rpm). Cells were  
161 harvested by centrifugation at 4,500 rpm for 5 min, washed with 1 ml of 20 mM phosphate  
162 buffer pH 7.2 and suspended in 1 ml of sample buffer (100 mM phosphate buffer pH 7.5, 20  
163 mM NaEDTA pH 8, 0.1% Triton X100, 10% acetone [v/v]). Cells were then lysed on ice by  
164 sonication (three cycles of 20 s in Bioruptor Standard device, Diagenode, New Orleans,  
165 USA). The cell lysate was harvested by centrifugation for 6 min at 7,000 rpm at 4°C and 20  $\mu$ l  
166 were mixed with 300  $\mu$ l of assay buffer (100 mM phosphate buffer pH 7.5, 0.2 mM catechol)  
167 previously warmed at 30°C. Assay buffer was prepared just before use by adding the  
168 catechol from a 20 mM stock solution (in water) to the pre-warmed phosphate buffer [19].

169 The yellow catechol degradation signal resulting from XylE activity was quantified by  
170 spectrometry at OD 595 nm (Bioteck, Winooski, USA). The catechol dioxygenase activity  
171 was determined and normalized at 375 nm per min per milligram of protein and converted to  
172 milliunits per milligram [16,19]. The protein concentration was estimated by the method of  
173 Bradford [20] by using bovine serum albumin as a standard. For each condition, experiments  
174 were realized with at least three repeats. The result of each experiment was expressed as  
175 the expression of XylE in the assay relative to that observed in the controls (*i.e.*, in the  
176 absence of cellobiose) systematically run in parallel. The significance of the relative signal of  
177 the assay *versus* the controls was statistically assessed with a *t*-student test.

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## 182 **3. Results**

### 183 **3.1 *In silico* analysis to assess the potential of *Streptomyces* as biosensor** 184 **hosts**

185 CebR prevents gene transcription in binding a specific sequence called the *cebR*-box and  
186 this repression is alleviated by the recognition of the inducer molecules, *i.e.* the cellobiose  
187 (Figure 1A). The CebR sensor system has been previously found and reported as a highly  
188 conserved regulatory system in some *Streptomyces* species [11–13], notably in term of  
189 sequence identity of the *cebR*-box. The concept of the biosensor was to build a generic  
190 biosensor cassette that could be regulated in different genetic backgrounds, *i.e.* in  
191 *Streptomyces* strains potentially exhibiting different specificities or sensitivities towards the  
192 target molecules. In order to assess the range of utilization of this construction within the  
193 *Streptomyces* genus we investigated the distribution of the CebR system within the 42  
194 available fully sequenced genomes. BlastP searches (NCBI) were done using as query the  
195 CebR sequence of the fully characterized *Streptomyces reticuli* system CebR (CUW28692)  
196 [12] with a cut-off of 50% of identity in amino acid. Conserved regulatory motif  
197 TGGGAGCGCTCCCA the so-called “*cebR*-box” was also searched. All genomes but five  
198 (*Streptomyces albulus* NK660, *Streptomyces albulus* ZPM, *Streptomyces albus* DSM41398  
199 and *Streptomyces* sp. 769, *Streptomyces lydicus* A02) possessed of at least one CebR  
200 homologue with amino acid identity ranging from 63% to 96% and the *cebR*-box motif as well  
201 as conserved regulatory *cebR*-boxes. This analysis showed that the CebR-system is  
202 widespread in this genus and conserved enough to recognize similar *cebR*-boxes.  
203 Consequently, most of the *Streptomyces* strains (including presumably environmental ones)  
204 are likely to be able to regulate a construction based on a conserved *cebR*-box through their  
205 endogenous CebR protein and could be turned into biosensors.

206

### 207 **3.2 Construction of the biosensor**

208 The biosensor design is depicted in Figure 1B. Briefly, the concept consists to put a reporter  
209 gene under the transcriptional control of CebR. The *xyIE* gene from *Pseudomonas putida*  
210 which was successfully used in *Streptomyces* was chosen [16,19]. It codes for a catechol  
211 dioxygenase that converts the colorless substrate catechol to an intensely yellow  
212 hydroxymuconic semialdehyde. The genomic DNA of *Streptomyces coelicolor* KC900 which  
213 includes a *xyIE* gene was used as a PCR template with the primer couple consisting of the  
214 forward Biosens\_F and the reverse Biosens\_R primers (Table S1, Figure 1B.I). The



215 Biosens\_F primer sequence includes a *cebR*-box and a ribosome binding site (RBS). The  
216 amplification thus places the *cebR*-box and a RBS site upstream of the coding region of *xyIE*  
217 (Figure 1B.I). The 955 bp PCR product was subcloned in the pGEM-T Easy vector,  
218 introduced in *E. coli* DH5 $\alpha$  and its sequence checked by sequencing. After releasing from  
219 pGEM-T Easy by a double restriction digestion *Bam*HI-*Eco*RI present in the Biosens\_F and  
220 Biosens\_R primer sequences respectively. This DNA fragment was further cloned  
221 directionally into the multicloning site of the pIB139 vector also digested with the same  
222 couple of endonucleases (Figure 1B.II). This cloning step resulted in the positioning of the  
223 *xyIE* gene downstream the strong constitutive promoter *PermE*\* [21,22]. This construct could  
224 then be mobilized into *Streptomyces* cells by intergeneric conjugation using the intermediary  
225 of *E. coli* S.17 host strain. The vector pIB139 was chosen for its site-specific integration  
226 system targeting the bacteriophage  $\Phi$ C31 attachment site [16]. The integration into the  
227 chromosome secures the stability of the construct. A single copy is expected to be inserted.

228 Nine *Streptomyces* species were chosen as receptors and the insertion of the construct was  
229 selected by the presence of apramycin in the culture medium. The absence of endogenous  
230 catechol dioxygenase activity was checked on plate in presence of catechol which is the  
231 XylE substrate (data not shown). Three of them were reference strains (*Streptomyces*  
232 *ambofaciens* ATCC 23877, *Streptomyces coelicolor* M145 and *Streptomyces lividans* TK23).  
233 The seven others were environmental strains (*Streptomyces* sp. S9N29, S2N2, S6N6,  
234 S9N14, S4N22, S1N3 and S4N27). These strains were chosen for their cellulolytic properties  
235 and their representativeness of the taxonomic diversity of a forest soil [9]. The presence of  
236 CebR was expected and highly likely considering the wide distribution of the CebR system in  
237 sequenced genomes (see above). After conjugation with *E. coli* S17.1 harboring the  
238 biosensor construct, *Streptomyces* transconjugants were selected on SFM plates with  
239 apramycin and nalidixic acid which counter selects *E. coli*. For each *Streptomyces* strain,  
240 eight transconjugants were selected. The integration into the  $\Phi$ C31 attachment site was  
241 checked by PCR for *S. ambofaciens* ATCC 23877. For environmental strains, the insertion of  
242 the construct was checked by PCR amplification of the *xyIE* gene (data not shown).

### 243 **3.3 Screening for optimal host strain for the biosensor**

244 In order to confirm the efficiency of the constructed strains as biosensors, they were  
245 functionally tested for their ability to emit a yellow color on plates complemented with  
246 cellobiose (5 g.L<sup>-1</sup>) as an inducer molecule. In comparison with plates without cellobiose, all  
247 transformed clones for 5 strains (*S. ambofaciens* ATCC 23877, *S. coelicolor* M145, *S. lividans*  
248 TK24, *Streptomyces* S4N27 and *Streptomyces* S6N6) exhibited a bright yellow coloration of  
249 mycelium and in the surrounding solid medium, indicating that the cellobiose was able to

250 unlock the CebR repression and to activate the expression of *xyIE*. In order to choose the  
251 optimal host strain among them, several screenings were performed (Table S2) and the  
252 strain S4N27 was selected for the following reasons. First, it visually exhibited the highest  
253 yellow color on plate (Figure 2) suggesting an optimal production of the XylE protein.  
254 Secondly, no detection signal was obtained in presence of crystalline cellulose (Avicel),  
255 indicating that the presence of none-degraded cellulose could not lead to false positive.  
256 Further specificity and sensitivity experiments were carried out on mycelium grown in liquid  
257 medium in order to ensure quantitative and reproducible assays. For that purpose, mycelium  
258 samples were sonicated to release the intracellular XylE content. Consequently, another  
259 selection criterion was the ability to lyse the mycelium. One of our candidate environmental  
260 *Streptomyces* strain, S4N27, was typified by a planktonic growth mode while other species  
261 grew as typical dense mycelium pellets difficult to lyse. For that reason, we finally selected  
262 strain S4N27 as the best host biosensor for further tests (Table S2).

### 263 **3.4 Sensitivity and specificity of cellobiose detection**

264 Tests were performed in liquid cultures to enable the quantification of the XylE activity of the  
265 biosensor S4N27 in presence of cellobiose. Its sensitivity was tested with cellobiose  
266 concentrations ranging from 1.5  $\mu\text{M}$  to 15 mM and after 10 min of reaction. Controls without  
267 cellobiose were performed for each experiment and exhibited a background signal  
268 presumably corresponding to the basal *xyIE* expression from the construction as no  
269 endogenous dioxygenase activity was observed in wild-type strains devoided to the  
270 construction. As the latter signal was equivalent in all controls, the *xyIE* activity of the assays  
271 was expressed as a relative expression of this baseline and did not raise an issue regarding  
272 the interpretations of the results. A significant detection was observed starting from 15  $\mu\text{M}$  of  
273 cellobiose (equivalent to 5  $\text{mg}\cdot\text{L}^{-1}$  concentration). This is in the same range than a leachate  
274 produced from spruce wood in degradation (see below) as measured by HPLC. However, no  
275 significant signal could be detected at 1.5  $\mu\text{M}$  (Figure 3). An increasing signal was observed  
276 in response to cellobiose concentration to reach a plateau from 80  $\mu\text{M}$ . The stability of the  
277 colorimetric reaction was assessed every two minutes for 1 h at 80  $\mu\text{M}$  of cellobiose. Since 4  
278 minutes and until the end of the experiment, all the measured intensity ratios were statically  
279 identical with a value close to 2.4 that showed there was no signal loss over time (data not  
280 shown). The chemical reaction was thus stable during this lap of time and biochemical  
281 instability of the reaction could not technically impair the liability of the measures.

282 At the same 80  $\mu\text{M}$  concentration of cellodextrines (triose, tetraose, pentaose and hexose),  
283 no signal could be observed (data not shown) showing that cellobiose was the specific signal  
284 sensed by the CebR system in the S4N27 strain.

285

286

287 3.5 Application of the biosensor for wood degradation detection.

288 In order to test whether our biosensor was able to detect the presence of cellobiose in a  
289 context of wood degradation, spruce sticks were infected by the lignolytic fungi *Poria*  
290 *placenta* during a period of 7 weeks to allow an effective fungal attack. Wood leachates were  
291 extracted and tested on the biosensor along with control leachates collected from uninfected  
292 spruce sticks. The *xyIE* expression for infected spruce was 1.8 relative to the control in the  
293 same range than the ratio observed using pure cellobiose as inducer (Figure 4). The  
294 cellobiose presence was checked and its concentration estimated in leachates by HPLC.  
295 While no cellobiose was detected in the uninfected control, a concentration of 15  $\mu$ M was  
296 measured in the infected wood. This result confirmed that the biosensor was able to detect a  
297 low cellobiose concentration in a complex wood leachate and consequently enabled the  
298 detection of the fungal decaying process.

299

300

#### 4. Discussion

301 The aim of this study was to create a biosensor dedicated to cellobiose detection. We based  
302 its construction on a microbial cellobiose sensor: the *Streptomyces* CebR system. Since, its  
303 characterization [10,11], it is known that the CebR transcriptional repressor prevents gene  
304 expression from binding a conserved *cebR*-box. In presence of cellobiose it releases its  
305 action and enables the expression of the repressed genes such as genes coding cellulolytic  
306 enzymes [11,12] or pathogenic factors in *S. scabies* [15]. The presence of this system has  
307 been assessed in few *Streptomyces* so far [11–13,15], but thanks to an *in silico* prospection  
308 of the CebR-system, we showed that it was actually highly prevalent in this genus. We also  
309 showed that the CebR harboring *Streptomyces* all had highly conserved *cebR*-boxes,  
310 suggesting that their CebR regulators were prone to recognize similar ones.

311 We took advantage of this conserved genetic scheme to design a whole-cell biosensor. Most  
312 microbial sensors are generally based on a specific promoter cloned upstream a reporting  
313 gene [23,24]. Once integrated in the cell, the promoter initiates the transcription of the  
314 reporting gene when the cell comes in contact with the inducer signal. As CebR is a  
315 transcriptional repressor, we adapted this strategy in consequence and cloned a canonical  
316 *cebR*-box in the promoter region of a constitutively expressed reporter gene in order to  
317 regulate its expression by CebR. The *cebR*-box was cloned between the promoter and the  
318 transcription start of the gene as it is found in some *Streptomyces* [11]. Considering the  
319 widespread distribution of CebR among *Streptomyces*, we decided to integrate the construct  
320 in various *Streptomyces* and relied on the endogenous CebR for its regulation. As these  
321 CebRs were naturally present in the strains they had the advantage to be perfectly adapted  
322 to the recipient strain and thus to offer an optimal regulation of the construct. The construct  
323 was successfully transferred and integrated with the pIB139 vector in different *Streptomyces*  
324 both model strains and environmental isolates, and its good regulation in presence of  
325 cellobiose was observed in one third of them. For the other strains, the absence of a visual  
326 signal during this screening could be due to various reasons such as a low *xyIE* expression,  
327 a diffusion problem of the dioxygenase or non-recognition of cellobiose. All together, these  
328 results showed that our biosensor design was efficient and enabled to create a promiscuous  
329 construct that could be easily transferred and regulated in most *Streptomyces* to turn them  
330 into potential biosensors. More generally, this strategy that consists in diverting without a  
331 *priori* a conserved sensor system in different strains to control a same conserved molecular  
332 construction could be probably applied to other systems and in other bacteria. A main  
333 advantage is that it allows for a same construction the screening of different strains with  
334 different sensitivity or sensibility towards the molecule of interest and might ease the  
335 construction of whole-cell biosensors. A further development to our approach could be to

336 introduce the construction in a strain that does not harbor the CebR system (e.g. *E. coli* or a  
337 *cebR*-free *Streptomyces*) along with a heterologously expressed *cebR* gene to control it.  
338 Previous biosensors, principally amperometric enzyme-based, were able to detect cellobiose  
339 [23–25]. They generally used cellobiose hydrolases that are catalytic enzymes able to  
340 degrade cellobiose. However, these enzymes have a large spectrum of molecule recognition  
341 and the biosensors built with them were used to detect other compounds such as glucose,  
342 lactose or catechol, but never specifically and directly the cellobiose [25]. Regarding the  
343 CebR system, some cross specificity for cellodextrines in addition to cellobiose has been  
344 identified in *S. griseus* [11]. Thus we did test the specificity of the *Streptomyces* S4N27  
345 biosensor strain towards these compounds and got no positive detection, indicating a  
346 specificity of cellobiose recognition with our biosensor like in *S. reticuli* [12].

347 We designed the biosensor to detect wood degradation. Fungal attack is one of the main  
348 threats regarding the use of wood as a material. Generally, such attack is empirically  
349 discovered when the decaying process had already altered the wood mechanical properties.  
350 Even in the case of standardized tests aiming to assess the efficiency of protective  
351 treatments against fungal attack, professionals rely on 4 month experiments after infection  
352 where they measure the wood mass loss (EN 113) [17]. In order to assess whether our  
353 biosensor could bring a quicker answer in comparison to such tests, we performed a similar  
354 experiment as in the EN113 norm in infecting a spruce stick with *Poria placenta*, but for half  
355 of the normal time of the test. The application of the biosensor in this real context was  
356 successful and enabled to detect cellobiose in the wood attacked by the xylophagous fungus,  
357 but not in the uninfected control and by extension revealed the ongoing wood decaying  
358 process at this stage, dividing by two the time of the EN113 test. Such experiments are  
359 preliminary and despite further experiments are still needed to insure the full applicability of  
360 the biosensor in that context, this study led to a patent (PCT/EP2016/052417) regarding the  
361 exploitation of this biosensor. In future experiments, other types of wood-rots and kinds of  
362 wood should be tested but according to the fact that cellobiose remains a common  
363 denominator of fungal wood degradation process; it should be detected in most cases.  
364 Another aspect to be tested will be the kinetic of degradation to assess at which stage the  
365 biosensor would be applicable. Yet, as cellobiose is produced since the beginning of the  
366 decaying process, degradation of wood should be detected in the earlier steps.

367 In conclusion, we created here a whole-cell biosensor in diverting a widespread sensor of  
368 cellobiose in *Streptomyces*. According to the conservation of this system, we designed a  
369 construct optimized for being transferable and regulate by most *Streptomyces*. After  
370 selection of a biosensor strain, we have been able to detect cellobiose even in complex

371 solutions such as a wood leachate. The application of this biosensor to detect wood  
372 degradation appears thus very promising for the wood industry.

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## **5. Reference:**

- 376 [1] Wang, L., Littlewood, J., Murphy, R.J., Environmental sustainability of bioethanol  
377 production from wheat straw in the UK, *Renew. Sustain. Energy Rev.* 2013, 28, 715–725.  
378
- 379 [2] Schultz, T.P., Nicholas, D.D., Introduction to Developing Wood Preservative Systems and  
380 Molds in Homes, *Dev. Commer. Wood Preserv*, American Chemical Society, 2008, pp. 2–8.  
381
- 382 [3] Rajala T., Peltoniemi M., Pennanen T., Mäkipää R., Fungal community dynamics in  
383 relation to substrate quality of decaying Norway spruce (*Picea abies* [L.] Karst.) logs in boreal  
384 forests, *FEMS Microbiol. Ecol.* 2012, 81, 494–505.  
385
- 386 [4] Schwarze, F.W.M.R., Engels, J., Mattheck, C., Fungal Strategies of Wood Decay in  
387 Trees, Springer Science & Business Media, 2000.  
388
- 389 [5] Howard, R.L., Abotsi, E., Van Rensburg, E.J., Howard, S., Lignocellulose biotechnology:  
390 issues of bioconversion and enzyme production, *Afr. J. Biotechnol.* 2004, 2,602–619.  
391
- 392 [6] Phillips, C.M., Beeson, W.T., Cate, J.H., Marletta, M.A., Cellobiose dehydrogenase and a  
393 copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by  
394 *Neurospora crassa*, *ACS Chem. Biol.* 2011, 6, 1399–1406.  
395
- 396 [7] Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S., Microbial Cellulose Utilization:  
397 Fundamentals and Biotechnology, *Microbiol. Mol. Biol. Rev.* 2002, 66, 506–577.  
398
- 399 [8] Langston, J.A., Shaghasi, T., Abbate, E., Xu, F., et al., Oxidoreductive cellulose  
400 depolymerization by the enzymes cellobiose dehydrogenase and glycoside hydrolase 61,  
401 *Appl. Environ. Microbiol.* 2011, 77, 7007–7015.  
402
- 403 [9] Bontemps, C., Toussaint, M., Revol, P.-V., Hotel, L., et al., Taxonomic and functional  
404 diversity of *Streptomyces* in a forest soil, *FEMS Microbiol. Lett.* 2013, 342, 157–167.  
405
- 406 [10] Bruce, T., Martinez, I.B., Maia Neto, O., Vicente, A.C.P., et al., Bacterial community  
407 diversity in the Brazilian Atlantic forest soils, *Microb. Ecol.* 2010, 60, 840–849.  
408
- 409 [11] Marushima, K., Ohnishi, Y., Horinouchi, S., CebR as a Master Regulator for  
410 Cellulose/Celooligosaccharide Catabolism Affects Morphological Development in  
411 *Streptomyces griseus*, *J. Bacteriol.* 2009, 191, 5930–5940.  
412
- 413 [12] Schlösser, A., Aldekamp, Schrempf, T., H., Binding characteristics of CebR, the  
414 regulator of the ceb operon required for cellobiose/celotriose uptake in *Streptomyces reticuli*,  
415 *FEMS Microbiol. Lett.* 2000, 190, 127–132.  
416
- 417 [13] Takasuka, T.E., Book, A.J., Lewin, G.R., Currie, C.R., et al., Aerobic deconstruction of  
418 cellulosic biomass by an insect-associated *Streptomyces*, *Sci. Rep.* 2013, 3, 1030.  
419
- 420 [14] Padilla-Reynaud, R., Simao-Beaunoir, A.-M., Lerat, S., Bernards, M.A., et al., Suberin  
421 Regulates the Production of Cellulolytic Enzymes in *Streptomyces scabiei*, the Causal Agent  
422 of Potato Common Scab, *Microbes Environ. JSME.* 2015, 30, 245–253.  
423
- 424 [15] Francis, I.M., Jourdan, S., Fanara S., R. Loria, et al., The Cellobiose Sensor CebR Is the  
425 Gatekeeper of *Streptomyces scabies* Pathogenicity, *mBio.* 2015, 6, e02018–14.  
426  
427

- 428 [16] Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F., et al., Practical *Streptomyces*  
429 Genetics, *John Innes Center* Norwich, The John Innes Foundation, 2000.  
430
- 431 [17] CEN (1996) EN 113 Wood preservatives. Test method for determining the protective  
432 effectiveness against wood destroying basidiomycetes. Determination of the toxic values.  
433 European Committee for Standardisation, Brussels, Belgium  
434
- 435 [18] Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, *Cold Spring*  
436 *Harbor Laboratory Press*, Cold Spring Harbor, New York, 2001.  
437
- 438 [19] Ingram, C., Brawner, M., Youngman, P., Westpheling, J., xylE functions as an efficient  
439 reporter gene in *Streptomyces* spp.: use for the study of galP1, a catabolite-controlled  
440 promoter, *J. Bacteriol.* 1989, *171*, 6617–6624.  
441
- 442 [20] Bradford, M.M., A rapid and sensitive method for the quantitation of microgram  
443 quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 1976, *72*,  
444 248–254.  
445
- 446 [21] Bibb, M.J., J. White, J.M. Ward, G.R. Janssen, The mRNA for the 23S rRNA methylase  
447 encoded by the *ermE* gene of *Saccharopolyspora erythraea* is translated in the absence of a  
448 conventional ribosome-binding site, *Mol. Microbiol.* 1994, *14*, 533–545.  
449
- 450 [22] Schmitt-John, T., Engels, J.W., Promoter constructions for efficient secretion expression  
451 in *Streptomyces lividans*, *Appl. Microbiol. Biotechnol.* 1992, *36*, 493–498.  
452
- 453 [23] Bereza-Malcolm, L.T., Mann, G., Franks, A.E., Environmental sensing of heavy metals  
454 through whole cell microbial biosensors: a synthetic biology approach, *ACS Synth. Biol.*  
455 2015, *4*.  
456
- 457 [24] Park, M., Tsai, S.-L., Chen, W., Microbial Biosensors: Engineered Microorganisms as  
458 the Sensing Machinery, *Sensors.* 2013, *13*, 5777–5795.  
459
- 460 [25] Ludwig, R., Harreither, W., Tasca F., Gorton, L., Cellobiose Dehydrogenase: A Versatile  
461 Catalyst for Electrochemical Applications, *ChemPhysChem.* 2010, *11*, 2674–2697.  
462
- 463 [26] Hanahan, D., Studies on transformation of *Escherichia coli* with plasmids, *J. Mol. Biol.*  
464 1983, *166*, 557–580.  
465
- 466 [27] Simon, R., Priefer, U., Pühler, A., A Broad Host Range Mobilization System for In Vivo  
467 Genetic Engineering: Transposon Mutagenesis in Gram Negative Bacteria, *Nat. Biotechnol.*  
468 1983, *1*, 784–791.  
469
- 470 [28] Bruton C.J., Guthrie E.P., Chater K.F., Phage Vectors that Allow Monitoring of  
471 Transcription of Secondary Metabolism Genes in *Streptomyces*, *Nat. Biotechnol.* 1991, *9*,  
472 652–656.  
473
- 474 [29] Thibessard, A., Haas, D., Gerbaud, C., Aigle, B., et al., Complete genome sequence of  
475 *Streptomyces ambofaciens* ATCC 23877, the spiramycin producer, *J. Biotechnol.* 214 (2015)  
476 117–118.  
477
- 478 [30] Rückert, C., Albersmeier, A., Busche, T., Jaenicke, S., et al., Complete genome  
479 sequence of *Streptomyces lividans* TK24, *J. Biotechnol.* 2015, *199*, 21–22.  
480
- 481 [31] Wilkinson, C.J., Hughes-Thomas, Z.A., Martin, C.J., et al., Increasing the efficiency of  
482 heterologous promoters in actinomycetes, *J. Mol. Microbiol. Biotechnol.* 2002, *4*, 417–426.



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**TABLE**

**Table 1.** Bacterial strains and vector used in this study

<b>Strain or plasmid</b>	<b>Relevant characteristic(s)</b>	<b>Reference</b>
<i>E. coli</i> DH5 $\alpha$	<i>supE44 ΔlacU169(Φ80lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-11 relA1</i>	[26]
<i>E. coli</i> S17.1	<i>recA pro hsdR RP4-2-Tc::Mu-Km::Tn7</i>	[27]
<i>S. coelicolor</i> KC900	<i>act1::KC900 hisA1 uraA1 strA1 pgf SCP1<sup>-</sup> SCP2<sup>-</sup></i>	[28]
<i>S. ambofaciens</i> ATCC 23877	Wild-type strain	[29]
<i>S. lividans</i> TK24	Wild-type strain	[30]
<i>S. coelicolor</i> M145	<i>SCP1<sup>-</sup> SCP2<sup>-</sup></i>	[15]
<i>Streptomyces</i> sp. S9N29	Environmental wild-type strain	[9]
<i>Streptomyces</i> sp. S4N27	Environmental wild-type strain	[9]
<i>Streptomyces</i> sp. S6N6	Environmental wild-type strain	[9]
<i>Streptomyces</i> sp. S2N2	Environmental wild-type strain	[9]
<i>Streptomyces</i> sp. S9N14	Environmental wild-type strain	[9]
<i>Streptomyces</i> sp. S1N3	Environmental wild-type strain	[9]
<i>Streptomyces</i> sp. S4N22	Environmental wild-type strain	[9]
pIB139	<i>oriT attP int aac(3)IV PermE*</i>	[31]

501

## 502 **Figure legends**

503 Figure 1: Design and construction flowchart of the biosensor

504 A) Schematic illustration of the cellobiose biosensor system. The biosensor construction  
505 consists in a *Streptomyces* conserved *cebR*-box sequence cloned between the constitutive  
506 promoter *PermE*\* and the reporter gene *xyIE*. Once integrated in a *Streptomyces* genome  
507 harboring an endogenous CebR able to recognize the *cebR*-box, this transcriptional  
508 repressor will bind it and prevents the *xyIE* expression. The presence of cellobiose alleviated  
509 the CebR binding on the *cebR*-box and enables the transcription of *xyIE*. In presence of  
510 catechol, the XylE protein will emit a yellow compound revealing the presence of cellobiose.  
511 B) Flowchart of the biosensor construction. I. For the construction of the biosensor, the  
512 reporter gene *xyIE* was amplified from *Streptomyces coelicolor* KC900 with the Biosens\_F  
513 and Biosens\_R primers (955 bp). The primer Biosens\_F was designed from 5' to 3' with a  
514 *Bam*HI site as a future cloning site in pIB139. The black box symbolizes the *cebR*-box, the  
515 light grey box a Ribosome Binding Site (RBS) and the white arrow the beginning of the *xyIE*  
516 gene. The reverse primer Biosens\_R has from 5' to 3', an *Eco*RI site as future cloning site  
517 with pIB139 and the white arrow the end of the *xyIE* gene. II. After amplification, the PCR  
518 product was cloned in the pIB139 plasmid that enables the integration of the construction into  
519 *Streptomyces* chromosome.

520 *PermE*\*: constitutive promoter; *ori<sub>pUC</sub>*: replicative origin in *E. coli*; Apra<sup>R</sup>: Apramycine gene  
521 resistance; *oriT*: transfert origin, Int: integrase gene; MCS: Multiple cloning site.

522

523 Figure 2: Illustration of the detection of the cellobiose by the biosensor

524 The S4N27 biosensor strain was grown for 2 days on HT\* plates without cellobiose in A) or  
525 with cellobiose at 5 g.L<sup>-1</sup> in B). After catechol addition the yellow color is only observed in  
526 presence of cellobiose.

527

528 Figure 3: Detection threshold of cellobiose by the biosensor.

529 The experiments were performed in HT\* medium complemented with increasing cellobiose  
530 concentrations ranging from 1.5 μM to 15000 μM. For each concentration, the results are  
531 expressed as the ratio of the *xyIE* activity measured in presence and in absence of cellobiose  
532 (background signal). An activity ratio of 1 (represented by the black horizontal line)  
533 corresponds to an activity equivalent to the background signal. Results for each  
534 concentration corresponded to three independent cultures. Error bars represent the standard

535 deviation. Significant differences of signal intensity between the different conditions and the  
536 background signal are represented by asterisks and were determined with the Student's *t*-  
537 test ( $P < 0.05$ ).

538

539 Figure 4: Detection of the wood degradation by a lignolytic fungus.

540 Wood leachates were obtained from a spruce wood stick infected for 7 weeks by *Poria*  
541 *placenta* (black box) and from a non-infected control (white box). The results correspond to  
542 five independent biosensor assays and are expressed as the relative expression with the  
543 non-infected condition. Thus, the activity ratio of the non-infected spruce wood condition has  
544 a value of one and corresponds to the background signal. The significant difference between  
545 the two experimental conditions was attested by a Student's *t*-test ( $P < 0.05$ ) and is  
546 symbolized by an asterisk.

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#### 549 **Acknowledgement**

601 This work was funded by the Région Lorraine and the French National Research Agency  
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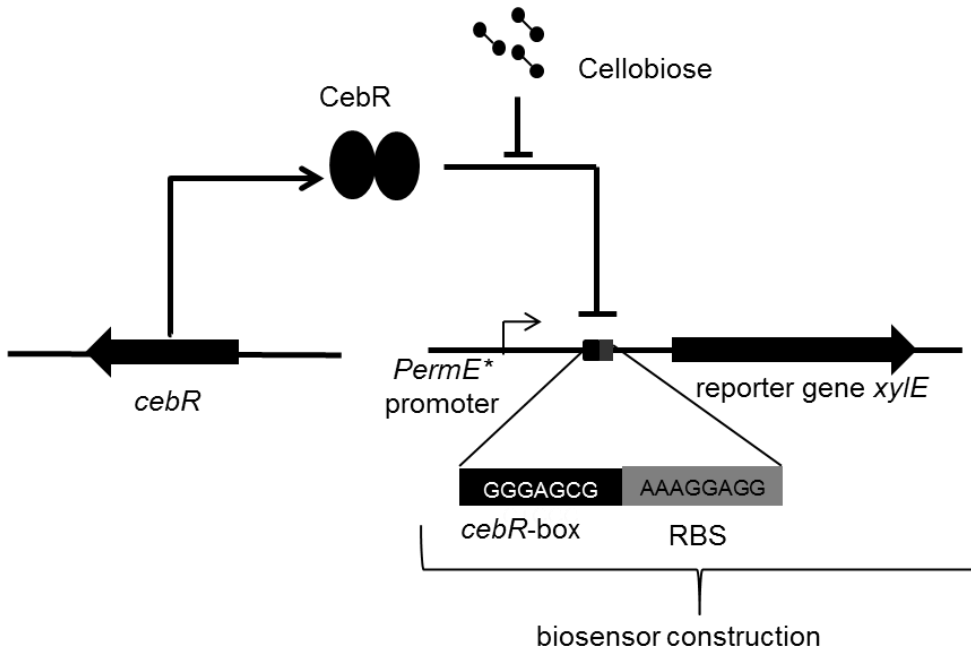
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#### 604 **Conflict of interest.**

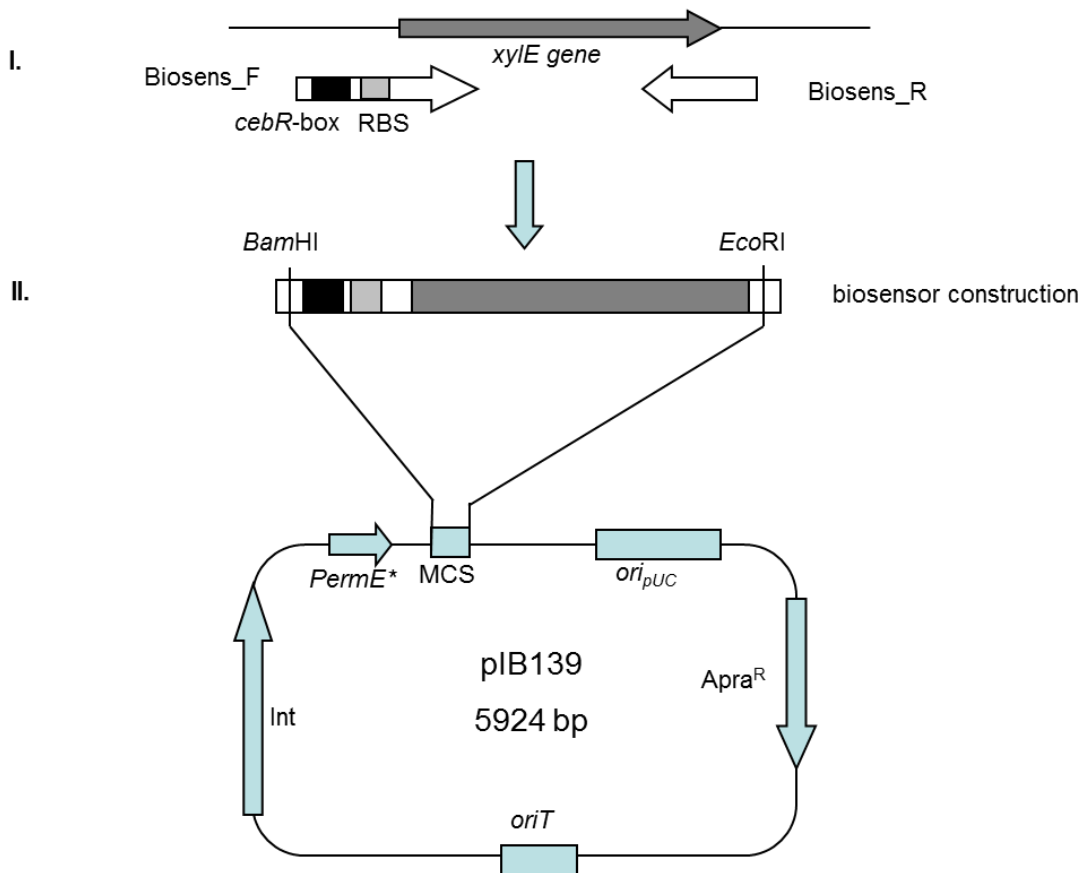
605 The authors declare no financial or commercial conflict of interest.

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A)



B)



A



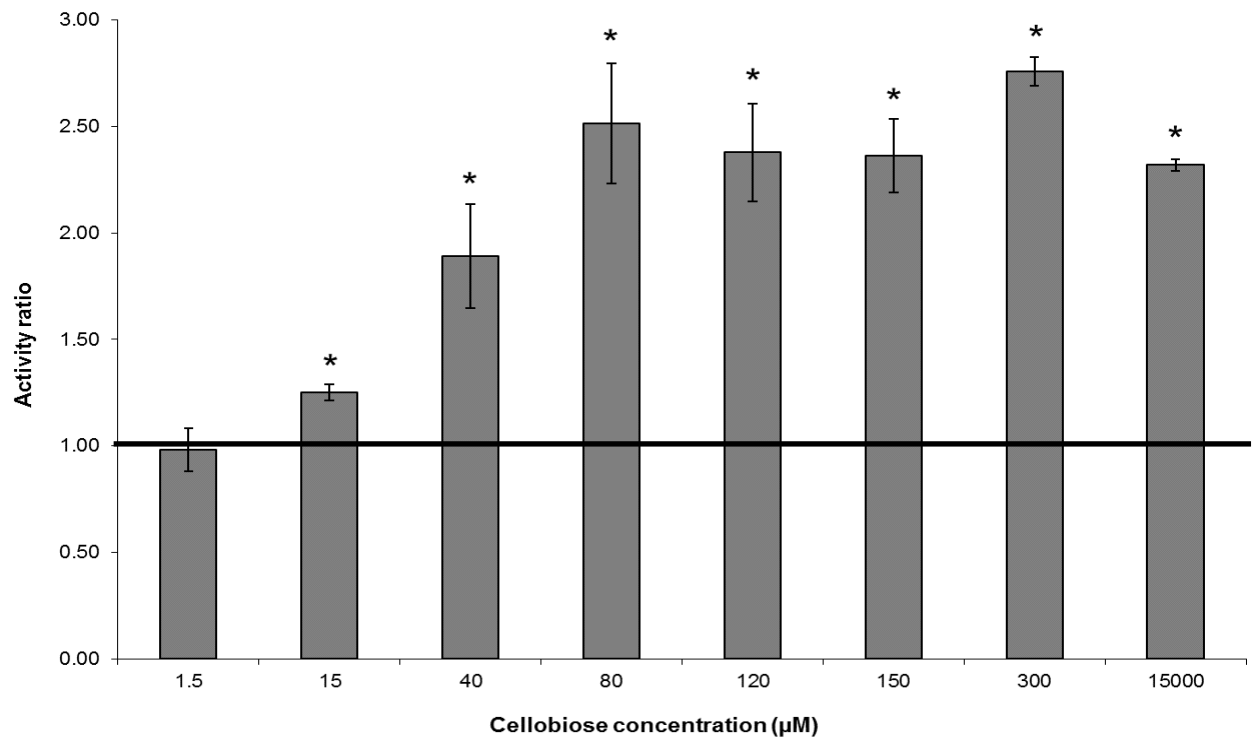
- cellobiose

B

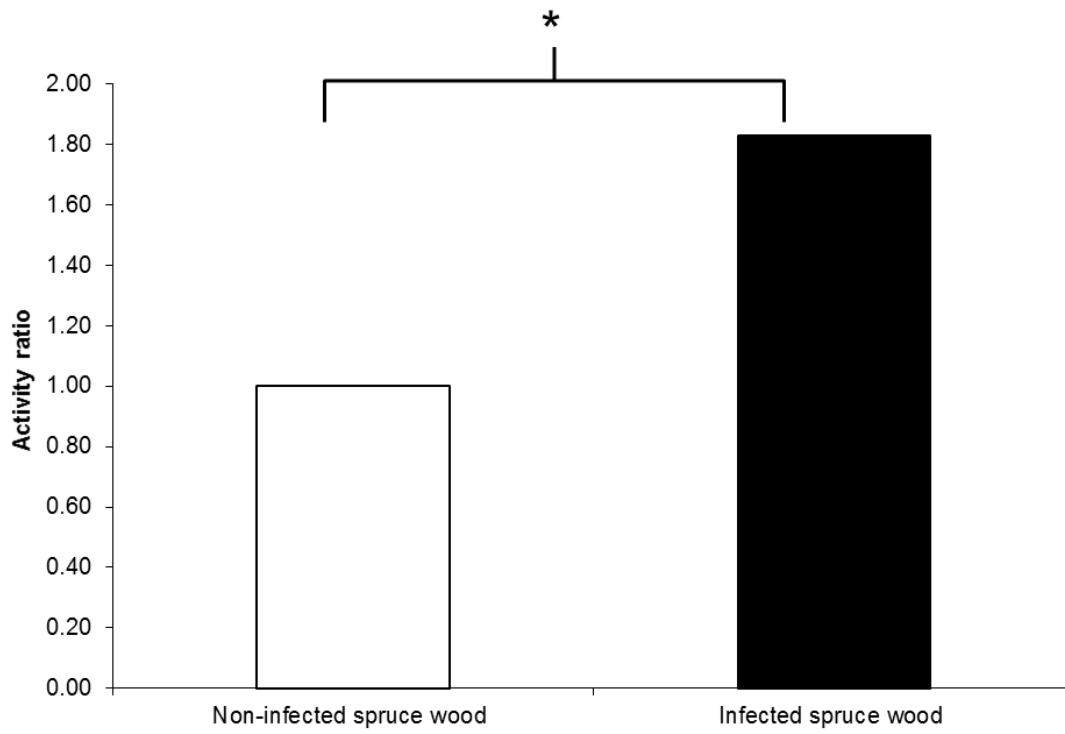


+ cellobiose

611 Figure 3



612 **Figure 4**



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