

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

Maxime Toussaint, Cyril Bontemps, Arnaud Besserer, Laurence Hotel,
Philippe Gérardin, Pierre Leblond

► **To cite this version:**

Maxime Toussaint, Cyril Bontemps, Arnaud Besserer, Laurence Hotel, Philippe Gérardin, et al.. Whole-cell biosensor of cellobiose and application to wood decay detection. Journal of Biotechnology, Elsevier, 2016, 239, pp.39 - 46. 10.1016/j.jbiotec.2016.10.003 . hal-01655326

HAL Id: hal-01655326

<https://hal.univ-lorraine.fr/hal-01655326>

Submitted on 6 Dec 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Research article

2

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

4 Maxime Toussaint ^{1,2}

5 Cyril Bontemps ^{1,2*}

6 Arnaud Besserer ³

7 Hotel Laurence ^{1,2}

8 Philippe Gérardin ³

9 Pierre Leblond ^{1,2*}

10

11 ¹Laboratoire Dynamique des Génomes et Adaptation Microbienne, UMR 1128, Université de
12 Lorraine, Vandœuvre-lès-Nancy, F-54506, France

13 ²Laboratoire Dynamique des Génomes et Adaptation Microbienne, UMR 1128, INRA,
14 Vandœuvre-lès-Nancy, F-54506, France

15 ³Laboratoire d'Etudes et de Recherches sur le Matériau Bois, EA4370, Université de
16 Lorraine USC INRA, Faculté des Sciences et Technologies, 54506, Vandœuvre-lès-Nancy,
17 F-54506, France

18 **Correspondence**(*): Dr. Cyril Bontemps, Laboratoire Dynamique des Génomes et
19 Adaptation Microbienne, UMR 1128, INRA-Université de Lorraine, Faculté des Sciences et
20 Technologies-Campus Aiguillettes, 54506 Vandœuvre-lès-Nancy, France

21 Pr. Pierre Leblond, Laboratoire Dynamique des Génomes et
22 Adaptation Microbienne, UMR 1128, INRA-Université de Lorraine, Faculté des Sciences et
23 Technologies-Campus Aiguillettes, 54506 Vandœuvre-lès-Nancy, France

24 **E-mail** : cyril.bontemps@univ-lorraine.fr

25 pierre.leblond@univ-lorraine.fr

26

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

28

29

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 β -1,4 linked glucose units) or sometimes into
72 cellodextrines (generally from three to six β -1,4-linked glucose units) [7,8]. These cellulose
73 degradation by-products will be hydrolyzed later on into glucose by the action of β -
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 (β -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 COCL₂ 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).

114

115

116

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⁻¹ 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 10⁸-10⁹ spores/mL) of each biosensor on HT* plates supplemented
147 with 5 g.L⁻¹ 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 μ l
166 were mixed with 300 μ 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.

178

179

180

181

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 α 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 Φ 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 Φ 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⁻¹) 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 μM 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 μM 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 μM (Figure 3). An increasing signal was observed
276 in response to cellobiose concentration to reach a plateau from 80 μM . The stability of the
277 colorimetric reaction was assessed every two minutes for 1 h at 80 μM 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 μM 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 μ 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.

373

374
375

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/Cellooligosaccharide 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/cellotriose 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.

483
 484
 485
 486
 487
 488
 489
 490
 491
 492
 493
 494
 495
 496
 497
 498
 499
 500

TABLE

Table 1. Bacterial strains and vector used in this study

Strain or plasmid	Relevant characteristic(s)	Reference
<i>E. coli</i> DH5α	<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⁻ SCP2⁻</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⁻ SCP2⁻</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_{pUC}*: replicative origin in *E. coli*; Apra^R: 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⁻¹ 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 μ M to 15000 μ M. 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.

547

548

549 **Acknowledgement**

601 This work was funded by the Région Lorraine and the French National Research Agency
602 through the Laboratory of Excellence ARBRE (ANR-11- LABX-000-01).

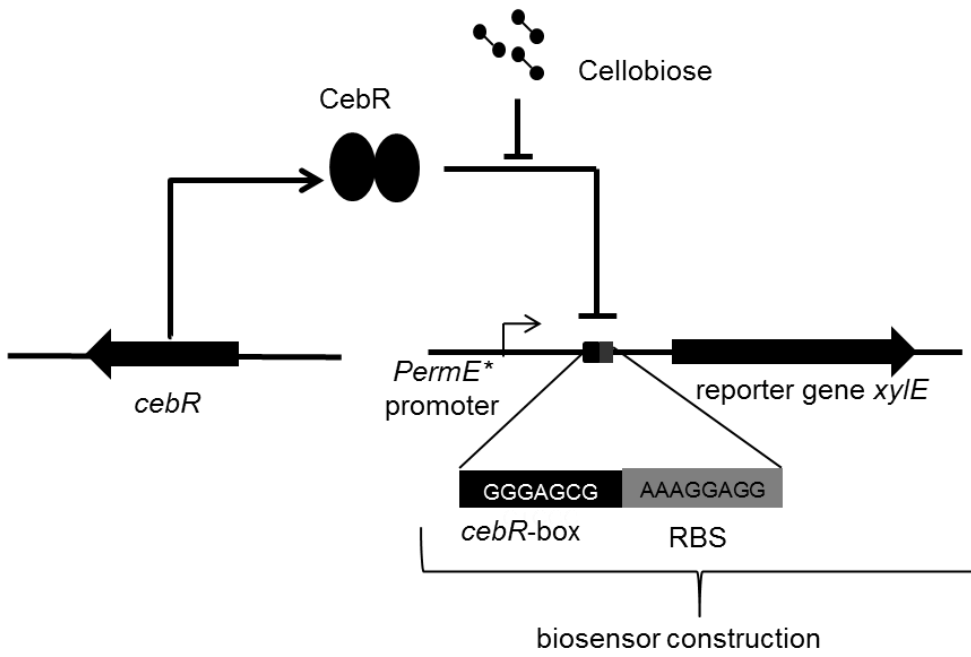
603

604 **Conflict of interest.**

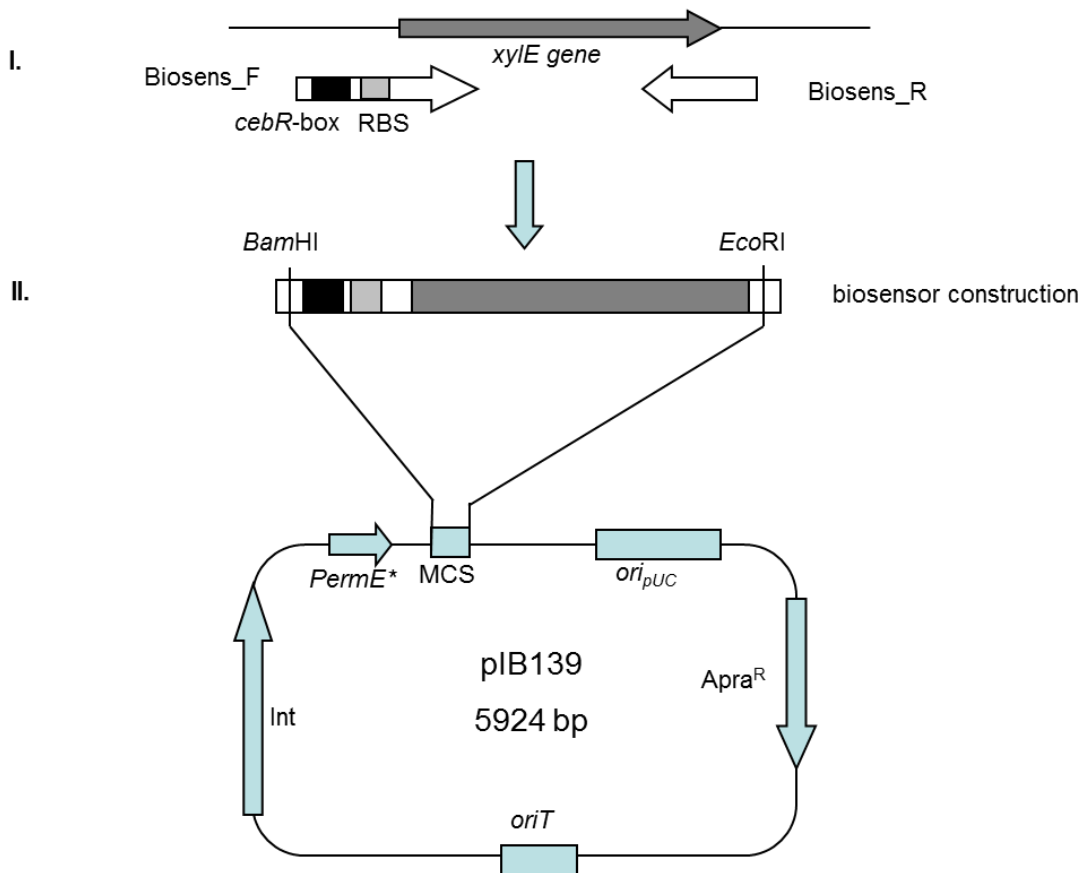
605 The authors declare no financial or commercial conflict of interest.

606

A)



B)



A



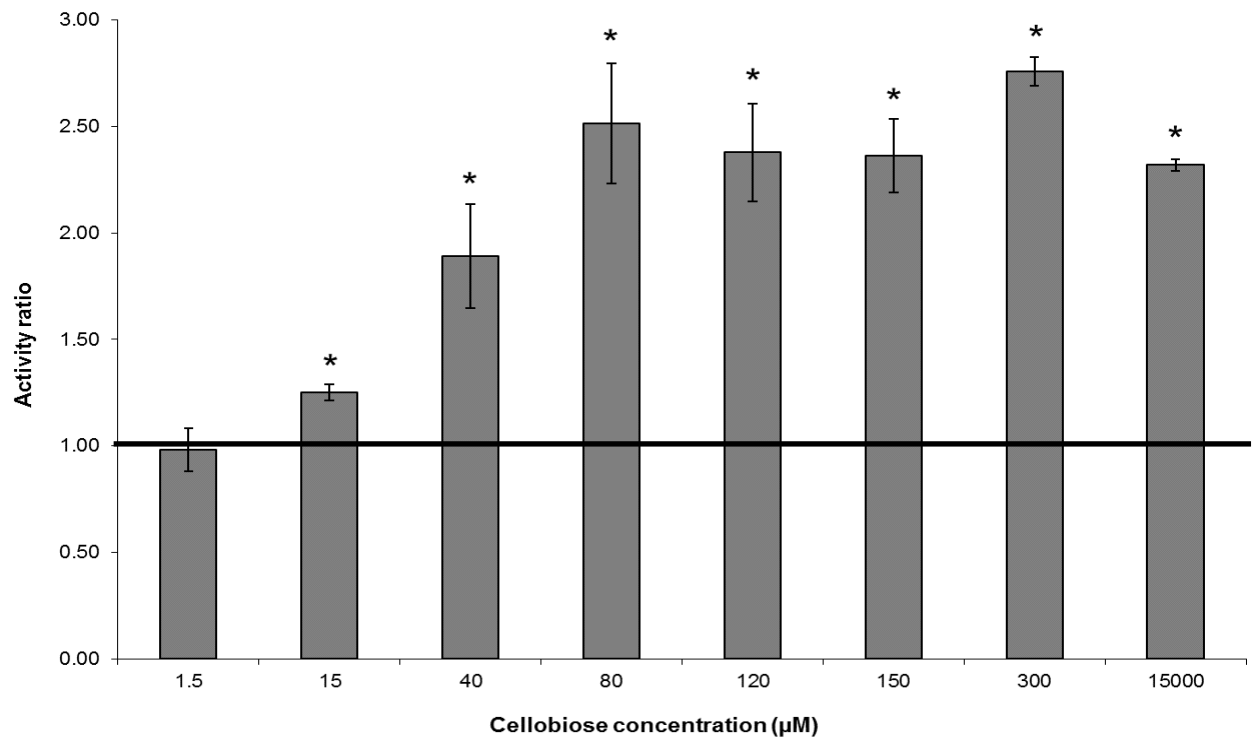
- cellobiose

B



+ cellobiose

611 Figure 3



612 **Figure 4**



613