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Regular paper

Improvement of ligninolytic properties by recombinant expression of glyoxal oxidase gene in hyper lignin-degrading fungus *Phanerochaete sordida* YK-624

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Running title: Ligninolytic improvement by expression of GLOX gene

Abstract

1

2 Glyoxal oxidase (GLOX) is a source of the extracellular H_2O_2 required for the

3 oxidation reactions catalyzed by the ligninolytic peroxidases. In the present study, the

4 GLOX-encoding gene (*glx*) of *Phanerochaete chrysosporium* was cloned, and *bee2*

5 promoter of *P. sordida* YK-624 was used to drive the expression of *glx*. The expression

6 plasmid was transformed into a *P. sordida* YK-624 uracil auxotrophic mutant (strain

7 UV-64), and 16 clones were obtained as GLOX-introducing transformants. These

8 transformants showed higher GLOX activities than wild-type *P. sordida* YK-624 and

9 control transformants harboring marker plasmid. RT-PCR analysis indicated that the

10 increased GLOX activity was associated with elevated recombinant *glx* expression.

11 Moreover, these transformants showed higher ligninolytic activity than control

12 transformants. These results suggest that the ligninolytic properties of white-rot fungi

13 can be improved by recombinant expression of *glx.*

14

15 **Introduction**

16

17 Lignin is one of the most abundant and recalcitrant natural polymers, consisting of
18 various nonphenolic phenylpropanoid units complexed into a three-dimensional
19 network. Therefore, lignin degradation is a key step for carbon recycling in forest
20 ecosystems, and the process is a central issue in lignocellulose-based industries (*e.g.*, in
21 paper pulp manufacture and production of bioethanol and other renewable chemicals).[1)]
22 In nature, white-rot fungi (members of the basidiomycete group) are able to degrade
23 recalcitrant lignin efficiently [2)]. The biodegradation process is initiated via a
24 one-electron oxidation of lignin units, a step that is mediated by extracellular heme
25 peroxidases (including lignin peroxidase (LiP), manganese peroxidase (MnP), and
26 versatile peroxidase), enzymes that are of keen interest as industrial biocatalysts.[3, 4)]
27 Activation of molecular oxygen to hydrogen peroxide by extracellular oxidases
28 represents a common step in the fungal decay strategy, as shown by biochemical [2, 5)] and
29 genomic [6, 7)] evidence. White-rot decay is based on peroxide activation of
30 high-redox-potential fungal heme peroxidases that depolymerize lignin, leaving a
31 cellulose-rich residue.[8)] The mechanism of enzymatic attack on lignin by these fungi has
32 been extensively investigated because of its biotechnological interest.[1, 9)]

33 The white-rot fungus *Phanerochaete chrysosporium* secretes three known classes

34 of extracellular enzymes under ligninolytic (secondary metabolic) conditions in defined

35 glucose medium: glyoxal oxidases (GLOXs), LiPs, and MnPs.[3] GLOX represented a

36 family of radical copper oxidases produced by filamentous fungi, mainly organisms

37 belonging to the group of white-rot fungi; the GLOX from *P. chrysosporium* is the most

38 extensively studied of this enzyme family. GLOX acts as a source of the extracellular

39 hydrogen peroxide that is required for the oxidations catalyzed by the LiPs and MnPs

40 involved in lignin degradation.[10, 11] The enzyme catalyzes the oxidation of aldehydes to

41 the corresponding carboxylic acids by coupling the reaction to the reduction of

42 dioxygen to H_2O_2. Although GLOX activity was detected under ligninolytic

43 conditions,[10, 11] there is no evidence for GLOX involved in lignin degradation.

44 The white-rot fungus *Phanerochaete sordida* YK-624, which was isolated from

45 rotted wood, exhibits greater ligninolytic activity and selectivity than either *P.*

46 *chrysosporium* or *Trametes versicolor*.[12] In our previous study, we demonstrated that *P.*

47 *sordida* YK-624 produces MnP [12, 13] and LiP [14, 15] as ligninolytic enzymes. Moreover,

48 we previously identified a protein (BUNA2) that accumulated to high levels in cultures

49 of *P. sordida* YK-624 under wood-rotting conditions. The promoter region of the

50 BUNA2-encoding gene, designated *bee2*, was successfully cloned and demonstrated to

51 be a useful regulator for the high expression of genes under ligninolytic conditions.[16)]

52 More recently, we have reported that the production of MnP was increased by the

53 recombinant expression of a 5-aminolevulinic acid synthase-encoding gene.[17)]

54 In the present study, we report the transformation of *P. sordida* YK-624 with a

55 recombinant *glx* gene under regulation of the *bee2* promoter, yielding elevated transcript

56 accumulation and GLOX activity under wood-rotting conditions. Moreover, we

57 demonstrate that these transformants exhibit improved ligninolytic properties.

58

59 **Materials and Methods**

60

61 **Strains**

62 *P. sordida* strain YK-624 (ATCC 90872), uracil auxotrophic *P. sordida* strain

63 UV-64,[18)] and *P. chrysosporium* ME-446 (ATCC 34541) were used in this study. All

64 strains and transformants were maintained on potato dextrose agar (PDA) slants at 4 °C.

65

66 **Construction of a GLOX-encoding gene expression vector, co-transformation of**

67 **UV-64, and screening of regenerated clones**

68 Genomic DNA was isolated from *P. chrysosporium* ME-446 mycelium using

69 ISOPLANT II (Nippon Gene, Tokyo). Gene sequences for the GLOX-encoding gene

70 were obtained from the DOE Joint Genome Institute and used to design PCR primers.

71 The full-length *glx* (1907 bp) gene was amplified using the primer pair PcGLOX

72 F1-PcGLOX R1. The procedure for constructing the *glx* expression plasmid,

73 pBUNA2pro-GLOX, is described in Fig. 1. The plasmid pBUNA2pro, which was

Fig. 1

74 generated in our previous study,[16)] was used for the construction of the *glx* expression

75 plasmid. Primers GLOX F1 and Pc GLOX R1 were designed to amplify the *glx* gene

76 and to introduce a *Bgl* II site just before the first methionine codon and to add a *Bgl* II

77 site just after the stop codon, respectively (step 1). The amplified DNA fragment was

78 digested with *Bgl* II (New England Biolabs) and cloned into *Bgl* II digested

79 pBUNA2pro (step 2), yielding plasmid pBUNA2pro-GLOX (step 3).

80 pBUNA2pro-GLOX was sequenced to verify the absence of PCR errors.

81 UV-64 protoplasts were prepared by a standard technique using cellullases and

82 then co-transformed with p*PsURA5* and pBUNA2pro-GLOX using the PEG method.[18)]

83 The co-transformed clones (G strains) were screened by PCR, as described

84 previously,[19)] with the following modifications: primers PcGLOX F2 and PsGPD R1

85 were designed to amplify the *glx* gene fused with the *bee2* promoter.

86

87 **Enzyme assay**

88 *P. sordida* YK-624, G strains, and U strains (UV-64 protoplasts were
89 singly-transformed with p*PsURA5*, yielding transformants designated as U strains) were
90 incubated on PDA plates at 30 °C for 3 days, and 10-mm diameter disks then were
91 punched out from the growing edge of the mycelia using a sterile cork borer. For
92 determining the activities in the extracellular culture fluids, two mycelial disks from
93 each source plate were placed into a 100-mL Erlenmeyer flask containing 10 mL
94 nitrogen-limited Kirk medium,[20)] which then was statically incubated at 30 °C for 2, 4, 6,
95 and 8 days. Meanwhile, for detecting the activities in fungal-treated beech wood meals,
96 these strains were cultured in 50-mL Erlenmeyer flasks containing 0.5 g of
97 extractive-free beech wood meal (80-100 mesh) and 1.25 ml of distilled water, and
98 incubated at 30 °C for 5, 10, 15, and 20 days. Following the culture period, the method
99 described by Hirai *et al*. (1994) was modified for enzyme extraction. Briefly,
100 fungal-treated wood meal was homogenized with 25 mL of 50 mM malonate (pH 4.5)
101 or phosphate (pH 6.0) buffer containing 0.05% Tween 20 (Wako) using a Polytron
102 PT1200E (Kinematica, Canada) homogenizer for a total of 5 min (20 s blending with
103 10-min intervals) at 4 °C.

104 MnP activity was measured by monitoring the oxidation of 2,6-dimethoxyphenol

105 to coerulignone (extinction coefficient at 470 nm (ε470) = 49.6 mM^{-1} cm^{-1}).[21] The

106 reaction mixture (1 mL) contained 2,6-dimethoxyphenol (1 mM), $MnSO_4$ (1 mM), and

107 H_2O_2 (0.2 mM) in 50 mM malonate buffer (pH 4.5). GLOX activity was measured by

108 monitoring the oxidation of guaiacol to the tetramer (ε470 = 26.6 mM^{-1} cm^{-1}). The

109 reaction mixture (1 mL) contained guaiacol (1 mM), 0.08 nkat of horseradish

110 peroxidase, and 2-methylglyoxal (1 mM) in 50 mM phosphate buffer (pH 6.0). A

111 standard curve was constructed for horseradish peroxidase (Y axis: production of the

112 tetramer; X axis: H_2O_2 concentration) and used to calculate the production of H_2O_2 in

113 the reaction system. One katal (kat) was defined as the amount of enzyme producing 1

114 mol of product per second.

115

116 **Determination of ligninolytic properties**

117 G strains, U strains, and *P. sordida* YK-624 were cultured in 50-mL Erlenmeyer

118 flasks containing 0.5 g of extractive-free beech wood meal (80-100 mesh) and 1.25 ml

119 of distilled water, which then were incubated at 30 °C for 28 days. After incubation,

120 weight loss, Klason lignin content, and acid-soluble lignin content of the fungal-treated

121 wood meal were determined, as previously described.[12] The selection factor (SF),

122 which is an indicator of ligninolytic selectivity, was calculated as follows: SF = lignin

123 loss/holocellulose loss. Holocellulose loss was calculated as follows: total weight loss –

124 lignin loss.

125

126 **Transcriptional analysis of strain G-22**

127 Strain G-22 was inoculated in liquid medium as described above and then statically

128 incubated at 30 °C for 2, 4, 6, and 8 days. The mycelia were then collected and stored at

129 -80 °C pending transcriptional analysis. One hundred milligrams of mycelial mat was

130 ground to a fine powder using a mortar and pestle under liquid nitrogen, and then total

131 RNA was isolated from the powder using an RNeasy Plant Mini kit (Invitrogen).

132 RT-PCR was performed using 200 ng of total RNA with a PrimeScript RT-PCR Kit

133 (TaKaRa Bio, Otsu) and the gene-specific primer sets PcGLOX F3-PsGPD R1 (to

134 amplify recombinant *glx*) and ActinF-ActinR (to amplify the actin-encoding gene, *act*).

135 PCR was performed for 28 cycles of template denaturation at 95 °C for 30 s, primer

136 annealing at 56 °C for 1 min, and DNA extension at 72 °C for 1 min using Ex Taq DNA

137 Polymerase (TaKaRa Bio).

138

139 **Nucleotide sequence accession number**

140 The nucleotide sequences of *glx* derived from *P. chrysosporium* have been

141 deposited in the DDBJ database (http://www.ddbj.nig.ac.jp/) under accession no.

142 AB910536.

143

144 **Results and Discussion**

145

146 **Isolation of recombinant *glx*-expressing transformants**

147 *P. chrysosporium* was cultured in Kirk liquid medium for 5 days, and then

148 genomic DNA was extracted from the mycelia. Based on the genome database of *P.*

149 *chrysosporium*, two primers were designed to amplify the genomic DNA encoding

150 GLOX. Genomic DNA of *glx* obtained by genome PCR was inserted between the *bee2*

151 promoter and *gpd* terminator by restriction enzyme digestion and ligation (Fig. 1). This

152 expression plasmid, pBUNA2pro-GLOX, was introduced into strain UV-64 together

153 with p*PsURA5* as a marker plasmid. The presence of the *bee2* promoter-*glx* fusion gene

154 in each uracil prototrophic clone was confirmed by PCR using genomic DNA as the

155 template (data not shown). We obtained 16 of these "G" strains that were

156 co-transformed with pBUNA2pro-GLOX and p*PsURA5*.

157

158 **GLOX production and ligninolytic properties of recombinant *glx*-expressing**

159 **transformants**

160 In our previous reports, *P. sordida* YK-624 produces only LiP and MnP as

161 ligninolytic enzymes,[12-15] and LiP activity has been hardly detected in the culture

162 containing Mn ion.[12] Therefore, we focused MnP among ligninolytic enzymes, and the

163 production of GLOX and MnP by G strains was compared with those by

164 singly-transformed (p*PsURA5* alone) "U" strains. The mean GLOX activity in the

165 culture medium inoculated with G strains for 5 days was 0.66 nkat/flask, whereas that in

Fig. 2

166 the culture medium of U strains was 0.19 nkat/flask (Fig. 2a). The mean MnP activity in

167 the culture medium inoculated with G strains was 8.51 nkat/flask, whereas that in the

168 culture media of U strains was 7.78 nkat/flask (Fig. 2b). These results indicate that G

169 strains showed higher GLOX activities than U strains, although MnP activities of G

170 strains did not apparently differ from those of U strains.

171 The effect of recombinant *glx* expression on the ligninolytic properties of G strains

Fig. 3

172 was investigated. G strains showed 1.08-fold higher ligninolytic activity (Fig. 3a) and

173 1.18-fold higher SF values than U strains (Fig. 3b). In particular, strain G-22 showed

174 1.22-fold higher ligninolytic activity and 1.25-fold higher SF values than U strains (data

175 not shown). These results indicated that the ligninolytic properties were improved by

176 the recombinant expression of *glx* using the *bee2* promoter, with strain G-22 exhibiting

177 the highest lignin degradation activity among the 16 screened co-transformants. In our
178 previous study, the promoter region of *bee2* was demonstrated to be a useful regulator
179 for the high expression of genes under ligninolytic conditions.[16] The results of the
180 present study are consistent with that previous work. The proposed role of GLOX in
181 lignin biodegradation is to generate H_2O_2 for LiP- and MnP-mediated reactions.[22]
182 Moreover, the identification of *glx* transcripts in decayed wood is consistent with a
183 close physiological connection between extracellular peroxidases and GLOX.[23] These
184 reports, together with our present results, suggest that GLOX plays an important role in
185 lignin degradation by white-rot fungi.

186 To confirm whether the improvement of the ligninolytic properties resulted from
187 an increase in GLOX production, GLOX and MnP activities produced in beech wood
188 meals inoculated with G-22 and wild type (WT) were determined. The MnP activity of
189 G-22 was similar to that of WT, and no drastic fluctuations were observed (Fig. 4b). In
190 contrast, higher GLOX activities were detected at 10 and 15 days in G-22, although
191 similar GLOX activities for each strain were detected at 5 days (Fig. 4a). These results
192 indicate that the improvement of the ligninolytic properties correlates with the higher
193 production of GLOX.

Fig. 4

194

195 **GLOX production and transcriptional analysis of G-22**

196 Transcriptional analysis was performed by RT-PCR to confirm whether the
197 increment of GLOX activity reflected increased accumulation of *glx* transcript.
198 Specifically, we analyzed a time course of GLOX activity in G-22 and WT. GLOX
199 activity in G-22 increased gradually after 4 days, remaining relatively stable through 8
200 days (Fig. 5a). GLOX activity in G-22 was higher than that in WT during the incubation.
201 The highest GLOX activity in G-22 was observed at 8 days, when activity was 7.2-fold
202 higher than that in WT.

Fig. 5

203 We next analyzed the transcription of recombinant *glx* in G-22 by RT-PCR (Fig.
204 5b). The analysis indicated that high levels of recombinant *glx* transcript were detected
205 after 4 to 8 days. These results suggest that the increase of GLOX activity in G-22 was
206 caused by *bee2*-driven expression of the recombinant *glx*.

207 To conclude, we found that the recombinant expression of *glx* under the control of
208 the *bee2* promoter is effective for the improvement of the ligninolytic properties in
209 white-rot fungi. We also provide the evidence that physiologic productivity of GLOX in
210 white-rot fungi is not enough under ligninolytic condition. In our next study, we
211 propose to investigate the molecular breeding of superior lignin-degrading fungi by the
212 simultaneous expression of *glx* and a MnP-encoding gene (*mnp4*). To our knowledge,

213 this is the first report to demonstrate that GLOX is involved in lignin degradation.

214

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216

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220

221 **Reference**

222 1. Martínez AT, Ruiz-Dueñas FJ, Martínez MJ, del Río JC, Gutiérrez A. Enzymatic

223 delignification of plant cell wall: from nature to mill. Curr. Opin. Biotechnol.

224 2009;20:348–357.

225 2. Martínez AT, Speranza M, Ruiz-Dueñas FJ, Ferreira P, Camarero S, Guillén F,

226 Martínez MJ, Gutiérrez A, del Río JC. Biodegradation of lignocellulosics:

227 microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int.

228 Microbiol. 2005;8:195–204.

229 3. Kirk TK, Farrell R. Enzymatic" combustion": the microbial degradation of lignin.

230 Annu. Rev. Microbiol. 1987;41:465–505.

231 4. Ruiz-Dueñas FJ, Martínez AT. Microbial degradation of lignin: how a bulky
232 recalcitrant polymer is efficiently recycled in nature and how we can take
233 advantage of this. Microb. Biotechnol. 2009;2:164–177.
234 5. Baldrian P, Valásková V. Degradation of cellulose by basidiomycetous fungi.
235 FEMS Microbiol. Rev. 2008;32:501–521.
236 6. Martinez D, Challacombe J, Morgenstern I *et al*. Genome, transcriptome, and
237 secretome analysis of wood decay fungus *Postia placenta* supports unique
238 mechanisms of lignocellulose conversion. Proc. Natl. Acad. Sci. USA.
239 2009;106:1954–1959.
240 7. Martinez D, Larrondo LF, Putnam N *et al*. Genome sequence of the lignocellulose
241 degrading fungus *Phanerochaete chrysosporium* strain RP78. Nat. Biotechnol.
242 2004;22:695–700.
243 8. Martínez AT, Rencoret J, Nieto L, Jiménez-Barbero J, Gutiérrez A, del Río JC.
244 Selective lignin and polysaccharide removal in natural fungal decay of wood as
245 evidenced by in situ structural analyses. Environ. Microbiol. 2011;13:96–107.
246 9. Hammel KE, Cullen D. Role of fungal peroxidases in biological ligninolysis. Curr.
247 Opin. Plant Biol. 2008;11:349–355.
248 10. Kersten PJ. Glyoxal oxidase of *Phanerochaete chrysosporium*: its characterization

249 and activation by lignin peroxidase. Proc. Natl. Acad. Sci. USA.

250 1990;87:2936–2940.

251 11. Kersten PJ, Kirk TK. Involvement of a new enzyme, glyoxal oxidase, in

252 extracellular hydrogen peroxide production by *Phanerochaete chrysosporium.* J.

253 Bacteriol. 1987;169:2195–201.

254 12. Hirai H, Kondo R, Sakai K. Screening of lignin degrading fungi and their

255 ligninolytic enzyme activities during biological bleaching of kraft pulp. Mokzai

256 Gakkaishi. 1994;40:980–986.

257 13. Hirai H, Kondo R, Sakai K. Effect of metal ions on biological bleaching of kraft

258 pulp with *Phanerochaete sordid YK-624*. Mokuzai Gakkaishi. 1995;41:69–75.

259 14. Sugiura M, Hirai H, Nishida T. Purification and characterization of a novel lignin

260 peroxidase from white-rot fungus *Phanerochaete sordida* YK-624. FEMS

261 Microbiol. Lett. 2003;224:285–290.

262 15. Hirai H, Sugiura M, Kawai S, Nishida T. Characteristics of novel lignin

263 peroxidases produced by white-rot fungus *Phanerochaete sordida* YK-624. FEMS

264 Microbiol. Lett. 2005;246:19–24.

265 16. Sugiura T, Mori T, Kamei I, Hirai H, Kawagishi H, Kondo R. Improvement of

266 ligninolytic properties in the hyper lignindegrading fungus *Phanerochaete sordida*

267 YK-624 using a novel gene promoter. FEMS Microbiol. Lett. 2012;331:81–88.
268 17. Hirai H, Misumi K, Suzuki T, Kawagishi H. Improvement of manganese
269 peroxidase production by the hyper lignin-degrading fungus *Phanerochaete*
270 *sordida* YK-624 by recombinant expression of the 5-aminolevulinic acid synthase
271 gene. Curr. Microbiol. 2013;67:708–711.
272 18. Yamagishi K, Kimura T, Oita S, Sugiura T, Hirai H, Transformation by
273 complementation of a uracil auxotroph of the hyper lignin-degrading
274 basidiomycete *Phanerochaete sordida* YK-624. Appl. Microbiol. Biotechnol.
275 2007;76:1079–1091.
276 19. Sugiura T, Yamagishi K, Kimura T, Nishida T, Kawagishi H, Hirai H. Cloning
277 and homologous expression of novel lignin peroxidase genes in the white-rot
278 fungus *Phanerochaete sordida* YK-624. Biosci. Biotechnol. Biochem.
279 2009;73:1793–1798.
280 20. Tien M, Kirk TK. Lignin peroxidase of *Phanerochaete chrysosporium*. Methods
281 Enzymol. 1988;161:238–249.
282 21. Périé FH, Gold MH. Manganese regulation of manganese peroxidase expression
283 and lignin degradation by the white rot fungus *Dichomitus squalens*. Appl.
284 Environ. Microbiol. 1988;57:2240–2245.

285 22. Vanden Wymelenberg A, Sabat G, Mozuch M, Kersten PJ, Cullen D, Blanchette
286 RA. Structure, organization, and transcriptional regulation of a family of copper
287 radical oxidase genes in the lignin-degrading basidiomycete *Phanerochaete*
288 *chrysosporium*. Appl. Environ. Microbiol. 2006;72:4871–4877.
289 23. Janse BJH, Gaskell J, Akhtar M, Cullen D. Expression of *Phanerochaete*
290 *chrysosporium* genes encoding lignin peroxidases, manganese peroxidases, and
291 glyoxal oxidase in wood. Appl. Environ. Microbiol. 1998;64:3536–3538.

Fig. 1 Plasmid map of the *glx* expression plasmid pBUNA2pro-GLOX.

Fig. 2 GLOX (a) and MnP (b) activities in the extracellular culture fluids inoculated with U strains and G strains for 5 days.

Fig. 3 Ligninolytic properties of U strains and G strains. Lignin degradation (a) and selection factor (b) by each strain for 28 days.

Fig. 4 GLOX (a) and MnP (b) activities detected in fungal-treated beech wood meals. G-22 (triangles) and WT (squares).

Fig. 5 Production of GLOX and expression of *glx* by strain G-22. (a) Time course of GLOX activity in strain G-22 (triangles) and WT (squares). (b) RT-PCR analysis of *glx* in strain G-22. The expression of actin was used as an internal control.

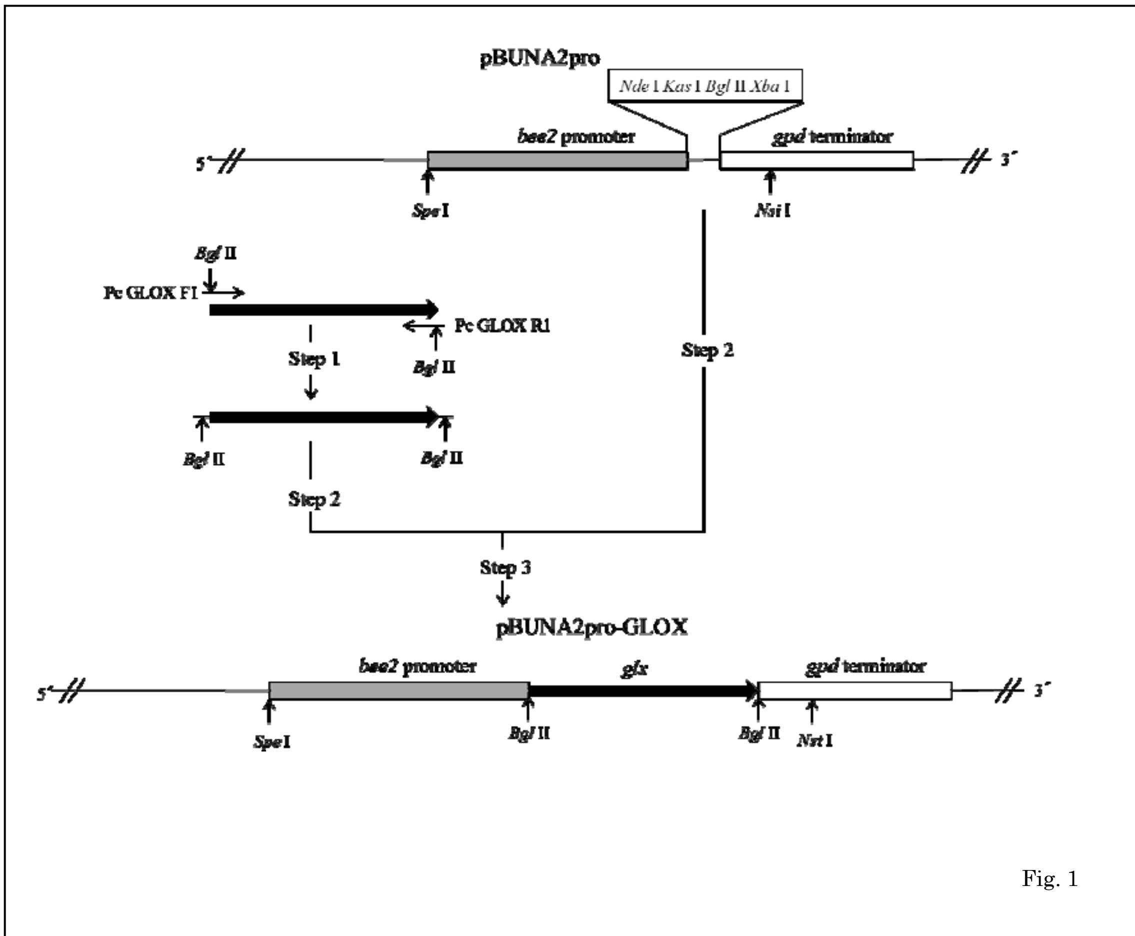
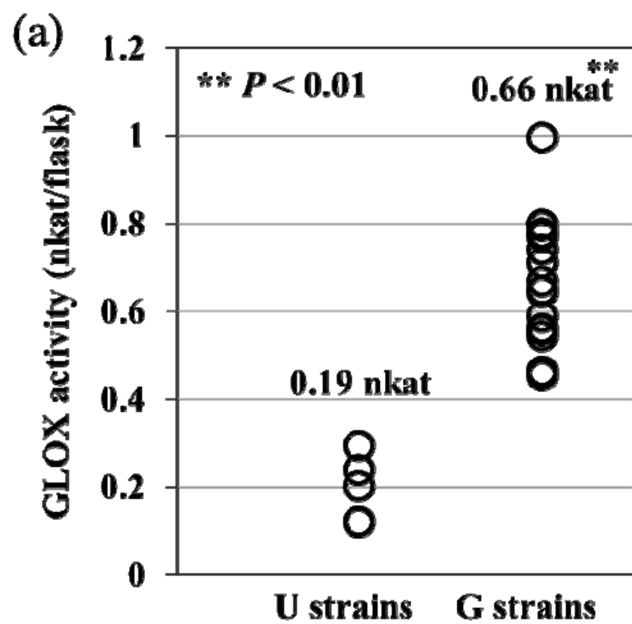


Fig. 1



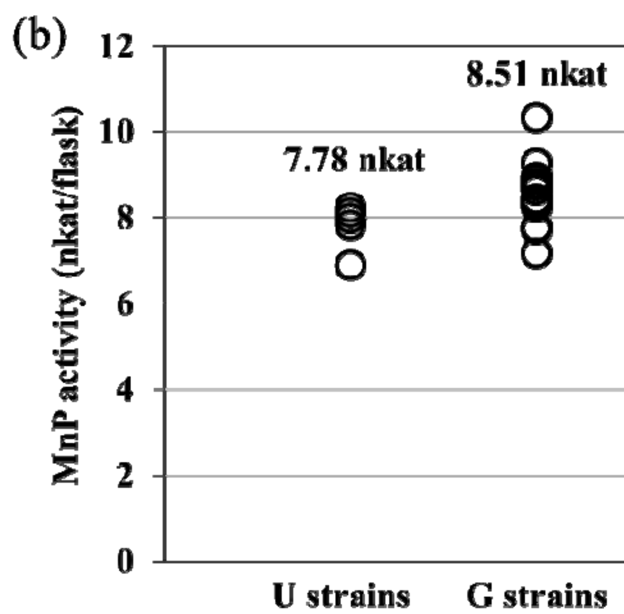
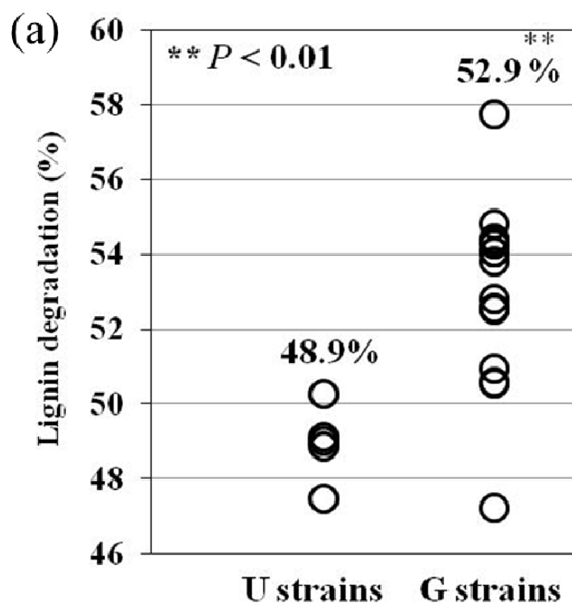


Fig. 2



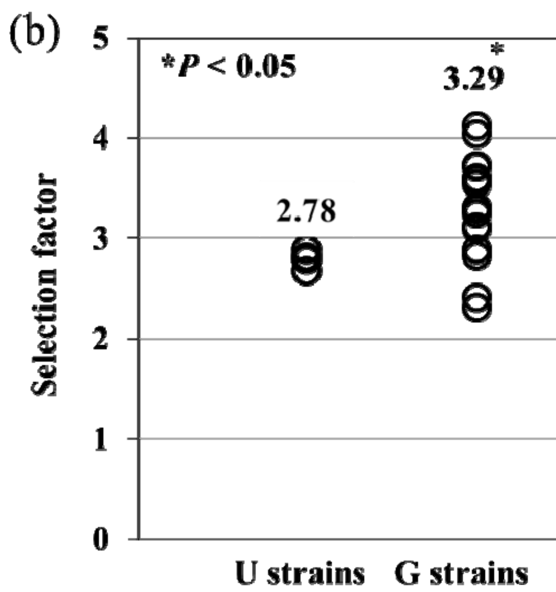


Fig. 3

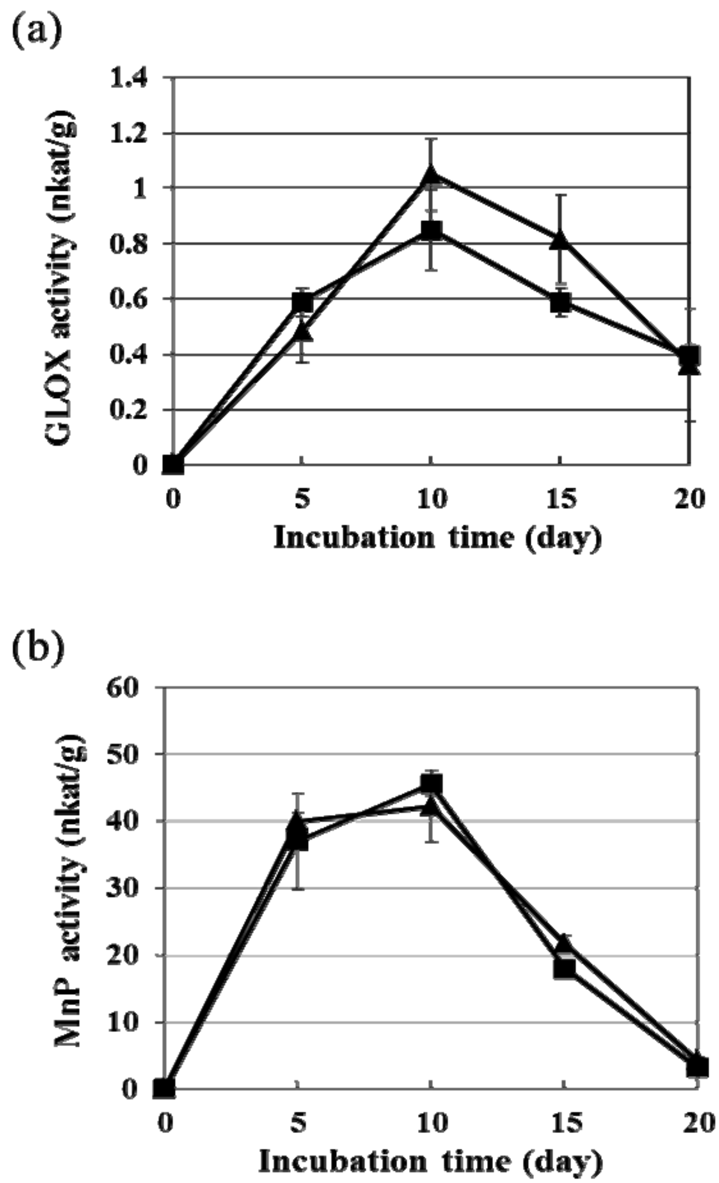
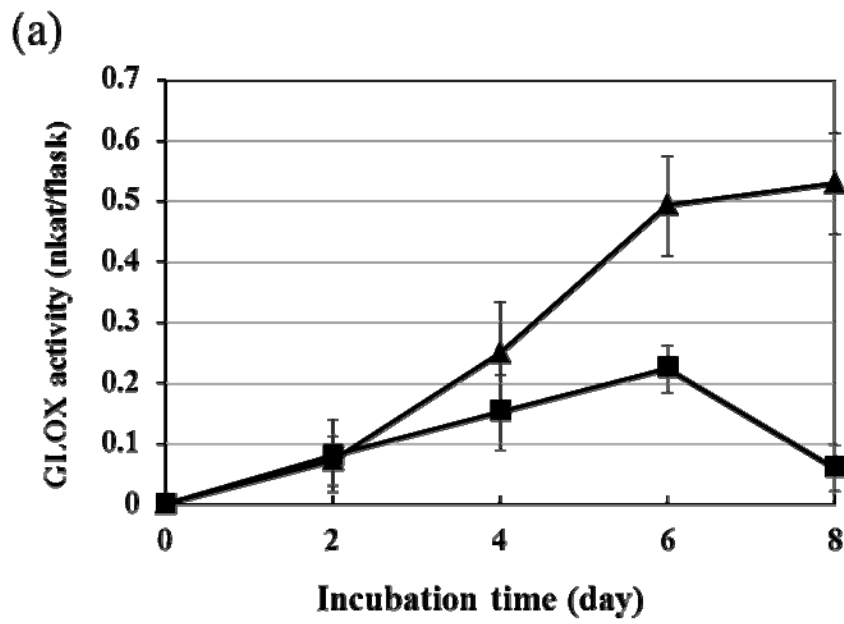


Fig. 4



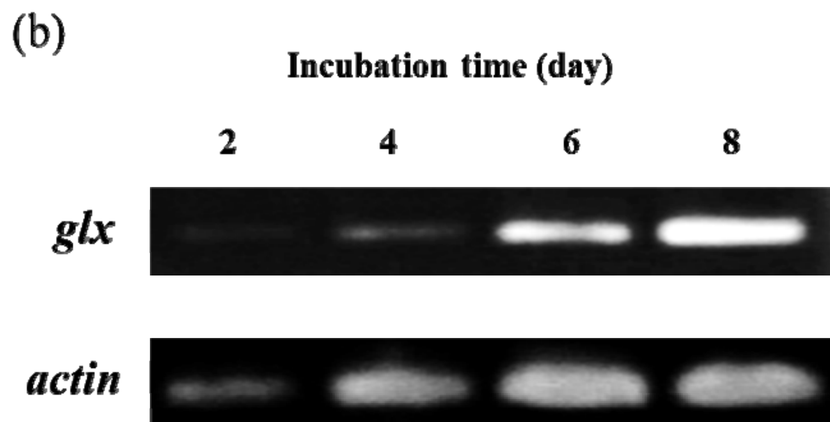


Fig. 5