Effects of Glucose Concentration on Ethanol Fermentation of White-Rot Fungus Phanerochaete sordida YK-624 Under Aerobic Conditions

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Effects of glucose concentration on ethanol fermentation of white-rot fungus *Phanerochaete sordida* YK-624 under aerobic conditions

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Abstract

White-rot fungi are microorganisms capable of ethanol fermentation; however, the specific conditions activating ethanol fermentation are unclear in contrast to fermentation by yeasts. In this study, we investigated the conditions favoring ethanol fermentation by the white-rot fungus *Phanerochaete sordida* YK-624, which is able to produce ethanol from woody material. In aerobic stationary cultivation with various concentrations of glucose (0.8-33 g/l), the fungus produced ethanol in media containing an initial glucose concentration of 2.8 g/l or higher. The amount of glucose consumption, mycelial weight, and ethanol production on the second day of culture increased in a concentration-dependent manner at low glucose concentrations; however, these were saturated at high concentrations. Biomass yields (growth/glucose consumption) were decreased until the initial glucose concentration increased to 6.0 g/l, after which the biomass yields showed constant values at higher concentrations (12-33 g/l). On the other hand, ethanol yields increased with decreasing biomass yields. In short shaking cultivation using mycelial suspension, trace amounts of instantaneous aerobic ethanol production were observed with 1.1 and 2.1 g/l glucose, but the relative gene expression levels of key enzymes at the pyruvate branch point showed no significant differences between ethanol production and non-production conditions. From these experimental results, it appears that the white-rot fungus *P. sordida* YK-624 produces ethanol due to overflow in sugar metabolism under aerobic conditions, although *P. sordida* YK-624 prioritizes glucose utilization for respiratory growth.

1. Introduction

Ethanol fermentation to produce alcoholic beverages is one of the oldest and most important fermentation processes. In this biological process, alcohol-producing microorganisms convert sugars into ethanol and carbon dioxide. Generally, ethanol fermentation is considered to be an anaerobic process to obtain cellular energy in the absence of oxygen; however, some microorganisms are able to produce ethanol aerobically at high external sugar concentrations. This phenomenon is known as the Crabtree effect, and is considered to be caused by insufficient capacity for respiratory pyruvate metabolism [16].

White-rot fungi have an important position in forest ecosystems, because they degrade wood components, especially lignin. White-rot fungi are the only microorganisms that completely degrade lignin, secreting several oxidoreductases that together degrade it [17]. Lignin is a major component accounting for 20-30% of wood, giving it physical strength and stiffness [1, 18]. Lignin also protects plant polysaccharides from microbiological attack; therefore, lignin is an obstacle to the fermentation of lignocellulosic materials [17]. Accordingly, lignin removal is a key factor for success in lignocellulosic biorefineries. Recently, several reports have discussed the ethanol fermentation of lignocellulosic materials by white-rot fungi. Some white-rot fungi are able to produce ethanol from lignocellulose in single-pot cultivation [8, 10]. In particular, *Phlebia* sp. MG-60 is able to ferment several lignocellulosics to ethanol, and exhibits a higher ethanol conversion rate from wood after aerobic precultivation for sufficient delignification [8, 9]. Based on these observations, white-rot fungi are considered to be suitable for bioethanol production from woody biomass.

In laboratory-scale experiments related to ethanol fermentation by wood-rot fungi, special semi-aerobic conditions have often been used [9, 10, 12]. The fundamental operation for creating semi-aerobic conditions is sealing the culture vessel. Although low-oxygen conditions are created during respiration by wood-rot fungi, the

eventual oxygen concentration is influenced by fungal viability and culture conditions. Fungi are thought to grow using aerobic respiration during the period with sufficient remaining oxygen, and that ethanol fermentation begins upon reaching a specific low oxygen threshold. However, there is no experimental evidence for a strong link between change in oxygen concentration, fungal growth, and ethanol fermentation. In addition, some white-rot fungi are able to produce ethanol for 24-48 hours under semi-aerobic conditions, which is not long enough to consume the oxygen [9, 7]. Moreover, some reports have examined aerobic ethanol fermentation by wood-rot fungi [12-14].

Phanerochaete sordida YK-624 is known to be an excellent lignin-degrading basidiomycete fungus [6] and can ferment ethanol from several monosaccharides under semi-aerobic conditions [19]. In addition, the fungus is able to produce ethanol from woody material after adjustment of culture conditions following aerobic cultivation for delignification [19]. It has even been shown that the fungus produces ethanol at early stages after sealing culture vessels, regardless of saccharide source, whether monosaccharides or woody biomass [19]. Based on these observations, we investigated the relationship between ethanol productivity of *P. sordida* YK-624 and culture conditions, such as oxygen and glucose concentration. The regulatory mechanism of ethanol fermentation was also examined by gene expression analyses of glycolytic, respiratory and fermentation systems.

2. Materials and Methods

2.1. Strain and fermentation conditions

P. sordida strain YK-624 (ATCC 90872), maintained on potato dextrose agar (PDA) at 4 ºC, was used in this study. Two mycelial discs (10 mm diameter), obtained from PDA plates incubated at 30 ºC for 3 days, were inoculated into 100-ml Erlenmeyer flasks containing 10 ml T-medium (12-33 g/l glucose, 10 g/l yeast extract, 10 g/l KH_2PO_4, 2 g/l $(NH_4)_2SO_4$, and 0.5 g/l $MgSO_4 \cdot 7H_2O$, pH 4.5) [12 ,19]. For semi-aerobic incubation, the flask was

immediately sealed with a rubber cap after inoculation. Other flasks were incubated under ambient atmospheric conditions (aerobic conditions). After incubation, mycelia were separated from the culture fluid and then PDA discs were removed. After incubation, pH of the culture fluid had dropped to 3.6-3.7 at day 6, in aerobic culture. The fluid was analyzed by HPLC to determine ethanol and glucose concentrations as described previously [19]. The recovered mycelia were dried at 105ºC and weighed. The oxygen concentration in the headspace of semi-aerobic cultures was measured by gas chromatography (GL Science GC-3200 chromatograph) on a Molecular Sieve 5A 30/60 packed column (3 m × 1.6 mm outer diameter) with a thermal conductivity detector, as described previously [11]. To evaluate the oxygen concentration in the culture fluid, other culture flasks were prepared separately, and resazurin (0.002%) was added to day 6 cultures. After 24 hours, the color of the culture medium was observed for evaluation of oxygen remaining in culture fluid.

For glucose-limited conditions, two mycelial pellets of *P. sordida* YK-624 were inoculated into 0.6 g/l glucose-containing T-medium, and were then incubated for 5 days. The mycelial mat was recovered and the PDA discs were removed, then the mat was washed with distilled water. Subsequently, the mat was homogenized in distilled water. Homogenate (0.2 ml, containing 1 mg mycelium) was inoculated into 10 ml glucose-limited T-medium (containing 1.3-6.0 g/l glucose), and was then incubated at 30ºC under ambient atmospheric conditions. Mycelial growth, glucose consumption, and ethanol production were measured as described above.

A mycelial disc was inoculated onto agar plates (1.5%) with T-medium containing 0-10 g/l glucose (T-agar plates), and hyphal elongation on the agar medium was measured.

2.2. Ethanol production, RNA extraction and transcriptional analysis in shaking culture containing low concentration of glucose

A mycelial mat grown in glucose-free T-medium was prepared as described above, and the mat was recovered by filtration. The recovered mycelia were homogenized in fresh glucose-free T-medium. Various concentrations of glucose solution and homogenate were mixed (resulting in 22.7 ± 0.8 mg/5 ml mycelial homogenate), and were incubated at 30ºC, 150 rpm (rotary shaking mode, EYELA multi shaker MMS-3020). After 6 h of incubation, the mycelia were recovered by centrifugation (15,000 × *g* for 3 min) and stored at -80ºC until RNA extraction. The supernatant was used for analysis of ethanol concentrations. RNA extraction, and quantitative reverse-transcription PCR (qRT-PCR) were performed as described previously [20]. Total RNA extraction and reverse transcription were performed with a Qiagen RNeasy Mini Kit and a PrimeScript RT-PCR Kit (TaKaRa Bio Inc.) according to the respective manufacturer's protocol. Reaction conditions for qRT-PCR were as follows: preincubation, 95°C for 30 s; amplification, 40 cycles of 95°C for 10 s and 60°C for 30 s. The PCR reagent employed was TB Green Premix *Ex Taq* II (Tli RNase H Plus, TaKaRa Bio Inc.). Specific primers for the genes actin-1 (*act1*; CGGACCTCCTCGTCAAGAAC / TGGAGCTCCTGCTCGAAGTC), pyruvate decarboxylase (*pdc*; GGTCACGGAAATCTTTGGCG / CGTTCAGCTCGTTGCAGTTT), pyruvate dehydrogenase E3 subunit (*pdhE3*; GTCTCAAGACCGCCTGCATTG / GACGCCCTCGACTTCGATAC), pyruvate dehydrogenase kinase (*pdhk*; GAAACGAAGCTGCAAAGCCA / GAGAGCCACATGTTGACCGA), and glyceraldehyde 3-phosphate dehydrogenase (*gpd;* GCAGGAATCAACGGTTTCGG / TCGATGAAGGGGTCGTTGAC) were used in RT- and qRT-PCR. The sequences of *pdc* (accession number LC057149) and *gpd* (accession number AB285023) were determined as in previous reports [19, 21]. The *act1* and *pdhE3* full sequences and *pdhk* partial sequence were determined from cDNA sequences (accession number LC377777-LC377779) obtained from unpublished RNA sequencing results. The relative expression levels were calculated from threshold cycle (Cq) value differences from *act1* as a reference gene.

2.3 Statistical analysis

Data presented are the average of three replicate cultures in each experiment. One-way analysis of variance (ANOVA) was carried out to determine the significance of differences. Statistical analyses were performed using Tukey's method and Differences between means at a confidence level of 5% ($p < 0.05$) were considered statistically significant.

3. Results

After 2-day cultivation of *P. sordida* YK-624 in T-medium containing 20 g/l glucose under aerobic and semi-aerobic conditions, the oxygen remaining, glucose consumption, mycelial growth, and ethanol production were analyzed. Although 16.9% oxygen remained in the flask headspace under semi-aerobic culture, 0.51 g/l ethanol production was confirmed (Table 1). Even in aerobic cultures, *P. sordida* YK-624 produced 0.61 g/l ethanol. Glucose consumption and mycelial growth were significantly higher in aerobic cultures. In contrast, a significantly higher efficiency of ethanol conversion from glucose (percentage of theoretical maximum; %TM) was observed under semi-aerobic conditions (28.4 ± 0.9%TM) than under aerobic conditions (25.1 ± 0.3%TM). From these results, it was indicated that possibilities of the aerobic ethanol fermentation of *P. sordida* YK-624. And it was also suggested that the limitation of oxygen supplementation leads to improvement of ethanol fermentation efficiency resulting from suppression of glucose consumption and mycelial growth. Then, the relationship between aerobic ethanol fermentation and glucose concentration was investigated. *P. sordida* YK-624 was then cultivated with 12, 23, and 33 g/l glucose under aerobic conditions, and changes in ethanol production, glucose consumption, and mycelial growth were measured (Supplementary Figure 1a-c). The shape of the curves for mycelial growth and glucose consumption were almost the same in each medium until glucose was exhausted at day 4. Ethanol was produced under all these

105 culture conditions, and the maximum ethanol concentration after incubation in 12, 23, and 33 g/l glucose-containing
106 medium was 0.61 g/l (at day 2), 2.0 g/l (at day 4), and 2.4 g/l (at day 6), respectively. Ethanol production reached a
107 maximum before glucose exhaustion. After glucose was exhausted, ethanol was degraded quickly, and the mycelial
108 weight started to decrease. Generally, ethanol is oxidized to acetate via acetaldehyde in various organisms. After that,
109 acetate would converted into acetyl-CoA, then mineralized into water and carbon dioxide in TCA cycle. Accordingly,
110 there is a possibility that *P. soridida* YK-624 is able to utilize ethanol to survive under glucose-depleted condition.
111 We then investigated various properties of aerobic ethanol fermentation by *P. sordida* YK-624 under glucose-limited
112 conditions (0.8, 1.3, 2.8 and 6.0 g/l). To exclude the effects of glucose carrying over from the PDA used for
113 inoculation, mycelia were obtained after incubation in 0.6 g/l glucose medium for 5 days, which was a sufficient
114 incubation period for depletion of glucose. Aliquots of homogenate obtained from washed mycelia were inoculated
115 into glucose-limited media. The time courses of the mycelial growth, the glucose consumption, and the ethanol
116 production under glucose-limited conditions are shown in Supplementary Figure 1d-f. Ethanol production was
117 clearly observed in 2.8 and 6.0 g/l glucose after a 2-day incubation period (0.23 and 0.59 g/l ethanol, respectively),
118 whereas no ethanol peak was detected throughout the incubation period in 0.8 and 1.3 g/l glucose (Supplementary
119 Figure 1f). In addition, glucose and ethanol in cultures quickly disappeared, as in media with high glucose
120 concentrations. Figure 1 shows the glucose consumption, mycelial growth and ethanol production in day-2 cultures
121 containing respective glucose concentration. The mycelial dry weight, glucose consumption, and ethanol
122 concentration reached a maximum at 6.0 g/l glucose. At much higher glucose concentrations (11, 23, and 33 g/l), the
123 amount of glucose consumption and the ethanol production tended to decrease, so there is a possibility that the high
124 concentration of glucose had affected the fungal metabolism (e.g. responses to osmotic stress, Crabtree-effect).
125 Actually, glucose consumption and ethanol production of *P. sordida* YK-624 was clearly decreased in the culture

126 containing 55 g/l or more initial glucose concentration (data not shown). *P. sordida* YK-624 was able to grow in
127 glucose-free T-medium. Although an accurate value for mycelial growth dependent on glucose could not be
128 determined, the values calculated by dividing the amounts of mycelial growth by the glucose consumption in day-2
129 culture are shown as biomass yields in Fig. 2, together with %TM. Biomass yields at lower initial glucose
130 concentrations showed more than 1 g/g. On the other hand, the biomass yields decreased with increasing initial
131 glucose concentrations (from 0.8 to 6.0 g/l), and showed constant values at higher glucose concentrations. The
132 ethanol production efficiency (%TM) showed the opposite trend as biomass yield, increasing dependent on initial
133 glucose concentration at lower concentration ranges. However, when glucose concentration exceeded 6.0 g/l, %TM
134 was saturated. If such aerobic fermentation was resulting from Crabtree effect (which is a phenomenon that high
135 external glucose induces aerobic ethanol production rather than respiration growth [2]), aerobically mycelial growth
136 of *P. sordida* YK-624 should be repressed under high glucose concentration. Therefore, we examined the aerobically
137 mycelial elongation rates of *P. sordida* YK-624 by using agar mediums containing various concentration of glucose,
138 mycelial elongation on T-agar plates containing 0.05-10 g/l glucose did not show any differences (Supplementary
139 Figure 2). This result indicates that the aerobic growth of *P. sordida* YK-624 is not affected by glucose concentration
140 within this range.

141 Then, the possibility of the depletion of oxygen in the stationary culture medium by resulting from
142 covering the culture surface with mycelial mat, was inspected. In order to simply evaluate the oxygen concentration
143 in culture fluid, resazurin (0.002%) was added to the aerobic and semi-aerobic culture at day 6. Immediately, blue
144 color (resazurin) changed to pink color (resorufin) in both culture. Although aerobic culture maintained pink color
145 after an additional 24 hours of incubation, semi-aerobic culture became colorless. At this time, the oxygen content
146 in the headspace of the semi-aerobic culture was lower than 0.5%. With N_2 gas bubbling of the sealed aerobic culture,

147 the color of the culture was changed to colorless, and this color change was reversible, with semi-aerobic and N_2-
148 purged aerobic cultures becoming re-colorized under atmospheric air. These results indicate that the culture fluid had
149 not reached the anaerobic state during 6 days of aerobic cultivation. However, it was not able to realize that the state
150 of oxygen concentration at the inner part of mycelial mat by this method.

151 Therefore, in order to avoid osmotic stress and to avoid occurrence of hypoxic environment due to the
152 formation of fungal mycelium aggregation and growth, lower concentrations of glucose solution were added to the
153 high concentrations of mycelial suspension, and the mixtures were aerobically incubated with agitation for a short
154 time (150 rpm, 6 h, 30 ºC). At this time, the glucose concentrations in each suspension were set 0.15, 0.65, 1.1 and
155 2.1 g/l. As a result, trace amounts of ethanol production were confirmed when suspensions were incubated in 1.1 and
156 2.1 g/l glucose (Supplementary Figure 3a). In this incubation period, the amount of glucose scarcely decreased, and
157 no significant increases in biomass were observed. From these results, we concluded that *P. sordida* YK-624 is able
158 to produce ethanol aerobically from glucose in T-medium. Finally, total RNA was extracted from these suspensions
159 to synthesize cDNA, and expression analyses for key enzymes of respiratory and fermentation pathways were
160 performed. Relative expression levels of *gpd*, *pdc*, *pdhE3* and *pdhk* are shown in Supplementary Figure 3b. Gene
161 expression data showed no significant differences based on glucose concentration.

162

163 **4. Discussion**

164 In the present study, *P. sordida* YK-624, which can ferment ethanol on beech wood meal and in liquid
165 medium [19], was able to produce ethanol in T-medium containing 20 g/l glucose after a 2-day incubation period
166 under semi-aerobic conditions, which was too short for development of anaerobic conditions by respiration during
167 growth. Therefore, we suspected that the fungus is able to ferment ethanol under aerobic conditions. The oxygen

168 concentration in the headspace of 2-day semi-aerobic cultures was reduced to 16.9%, and a concentration that
169 indicates that the culture conditions were still aerobic. Therefore, the aerobic fermentation of ethanol by *P. sordida*
170 YK-624 was confirmed. In addition, a time course of ethanol production during stationary cultivation in glucose-
171 containing media was analyzed. Over time, *P. sordida* YK-624 accumulated ethanol in the stationary culture with
172 initial glucose concentration at 2.8 g/l or more during growth or until glucose was consumed (Supplementary Figure
173 1). However, no ethanol production was observed in 1.3 g/l glucose. It was not clear why ethanol was not detected,
174 but this was probably due to either less fermentation or to ethanol volatilization or decomposition. At this time, the
175 results of colorimetric assay using resazurin indicated that the solution of aerobic culture remained under aerobic
176 conditions during ethanol production. However, the possibility that the inside of the hyphal mat that formed with
177 mycelial growth is in an anaerobic state could not be excluded in this method. Based on these results, in order to
178 avoid mycelial aggregation and glucose exhaustion during cultivation, aerobically shaking incubation for short
179 periods using mycelial suspension was carried out. As a result, ethanol production was observed in suspension culture
180 including 1.1 and 2.1 g/l glucose; however, the suspension containing 0.65 g/l or less glucose did not produce a
181 detectable amount of ethanol under this experimental condition (Supplementary Figure 3). These results indicated
182 that *P. sordida* YK-624 is able to ferment ethanol from glucose aerobically when incubated with certain levels of
183 glucose, similarly to other wood-rot fungi that can produce ethanol [12-14]. In these reports, authors reported that
184 some of white rot fungi were able to aerobically produce ethanol from glucose and xylose in T-medium and, they
185 also mentioned that the efficiency of ethanol production was better under oxygen-limited condition than aerobic
186 condition. From these facts, they suggested to possibility of Crabtree effect. However, it was not shown the
187 experimental evidences of Crabtree effect in this reports (e.g. repression of respiration growth, change in gene
188 expression by high glucose level). Similarly, *P. sordida* YK-624 showed higher fermentation efficiency if oxygen

189 supply was limited (Table 1). Therefore, we investigated the glucose-concentration dependence of respiration growth
190 and gene expression of *P. sordida* YK-624.

191 The Crabtree effect is a phenomenon in aerobic alcoholic fermentation that depends on glucose
192 concentration, and the phenomenon is observed in yeasts of several *Saccharomycetaceae* lineages [2, 5]. The
193 Crabtree effect has been divided into two types, long-term and short-term. The long-term effect is defined as aerobic
194 fermentation under steady-state culture, whereas the short-term effect describes aerobic fermentation that responds
195 to addition of excess sugar to sugar-limited aerobic cultures. Postma *et al.* [15] has reported that *Saccharomyces*
196 *cerevisiae* showed a decrease in cell yield (mass ratio of biomass to glucose) resulting from repression of respiration
197 and increased ethanol fermentation, a long-term Crabtree effect. The short-term Crabtree effect has been explained
198 as an overflow in sugar metabolism due to activation of key glycolytic enzymes, limited respiration capacity, and
199 feedback regulation of respiration-associated enzymes [2, 4]. The resulting excess pyruvate is converted to ethanol
200 for the production of ATP for cell growth by PDC and alcohol dehydrogenase. In yeast showing the Crabtree effect,
201 the expression of the PDC gene and PDC activity are upregulated, and the expression of the gene for the E3 subunit
202 of PDH is downregulated. In addition, PDH activity is inhibited by overexpressed PDHK. In pyruvate metabolism,
203 PDC and PDH catalyze key enzymatic reactions at the pyruvate branch point [3]. As shown in Fig. 2, the biomass
204 yield in each initial glucose concentration for *P. sordida* YK-624 decreased until 6.0 g/l. This indicates that the
205 mycelial growth was caused by both glucose and yeast extract in T-medium, and also that the glucose-dependency
206 of mycelial growth increased with glucose concentration. On the other hand, the glucose utilization for growth was
207 probably saturated at 0.6 g/l or more, because there was no significance in the amount of mycelial growth. Although
208 we could not estimate the amount of mycelial growth derived from glucose in liquid culture, the equivalent hyphal
209 elongation rates were observed on the agar medium containing different concentrations of glucose (Supplementary

Figure 2). Based on these results, it was suggested that there is a limit to the amount of glucose available for growth under each culture condition. This speculation is also supported by the fact that the amount of glucose consumption was constant in the liquid cultures containing with high initial glucose concentrations (Fig. 1). In addition, in the comparison of gene expression levels of key enzymes at the pyruvate branch point under ethanol-produced and non-produced condition, there was almost no difference in relative expression of *pdc*, *pdhE3* and *phdk* (Supplementary Figure 3). As the glucose concentration was independent on these gene expression levels in respiration/fermentation, it is clear that aerobic ethanol fermentation by *P, sordida* YK-624 does not depend on the Crabtree effect.

In this study, we demonstrated that the white-rot fungus *P. sordida* YK-624 is able to aerobically produce ethanol, and its regulatory mechanism is different from that of Crabtree-positive yeasts. Based on the experimental results, (i) the expression of key enzymes at the pyruvate branch point were not dependent on glucose concentration, (ii) high glucose concentration did not improve mycelial growth, and (iii) ethanol production was greatly increased in the range of glucose concentrations at which mycelial growth was saturated. These observations suggest that glucose and/or its metabolites are utilized for ethanol production as a result of overflow of respiration catabolism. On the other hand, the amount of glucose consumption and the ethanol production has also reached steady state in higher glucose concentrations. Therefore, it is possible that enhanced glucose uptake and fermentation-related enzymes lead to improved aerobic ethanol fermentation by *P. sordida* YK-624.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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295

296 **Figure captions**

297 **Fig. 1.** Mycelial growth (black circles), glucose consumption (gray rhombi) and ethanol production (white circles)
298 in second-day aerobic cultures of *P. sordida* YK-624 incubated in media containing various initial
299 concentrations of glucose. Values are presented as means ± standard deviation of triplicate cultures. Values
300 with the same letters/symbols on each graph were not statistically different ($p < 0.05$).

301 **Fig. 2.** Biomass yield (mycelial growth / glucose consumption ratio) and %TM (% of theoretical maximum of
302 ethanol) from second-day aerobic cultures of *P. sordida* YK-624 incubated in media containing various initial
303 concentrations of glucose. Gray bars and open circles indicated biomass yields and %TM, respectively. Values
304 are presented as means ± standard deviation of triplicate samples. Values with the same letters were not
305 statistically different ($p < 0.05$).

306

Table 1. Ethanol production, glucose consumption and mycelial growth after 2-day cultivation of *P. sordida* YK-624 in T-medium containing 2% glucose under semi-aerobic/aerobic condition.

culture condition	ethanol production (g/l)	glucose consumption (g/l)	mycelial growth (g/l)	oxygen remaining (%)	%TM
semi-aerobic	0.51 ± 0.03	3.54 ± 0.16	3.06 ± 0.16	16.9 ± 0.8	28.4 ± 0.9
aerobic	0.61 ± 0.01*	4.71 ± 0.12*	3.56 ± 0.11	n.d.	25.2 ±0.3*

Values are means ± standard deviation. * indicates significant difference from the respective value of semi-aerobic culture.

n.d., not determined.

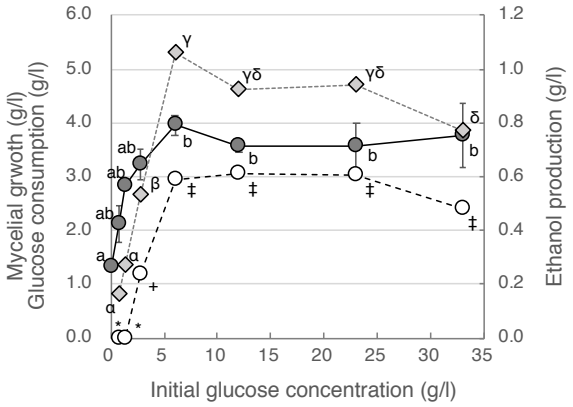

Mycelial grwoth (g/l)
Glucose consumption (g/l)
Ethanol production (g/l)
Initial glucose concentration (g/l)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
γ
γδ
γδ
δ
b
b
b
b
ab
ab
ab
a
β
α
α
‡
‡
‡
‡
+
*
*

Fig. 1.

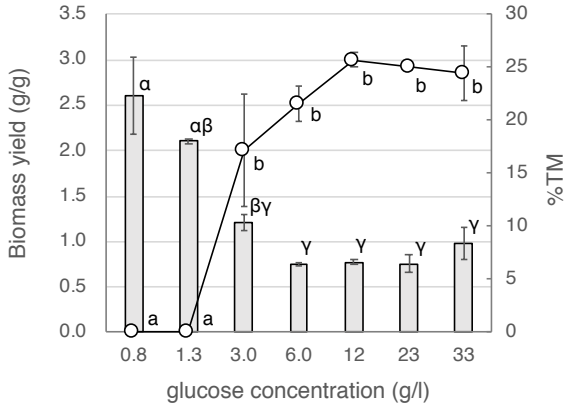
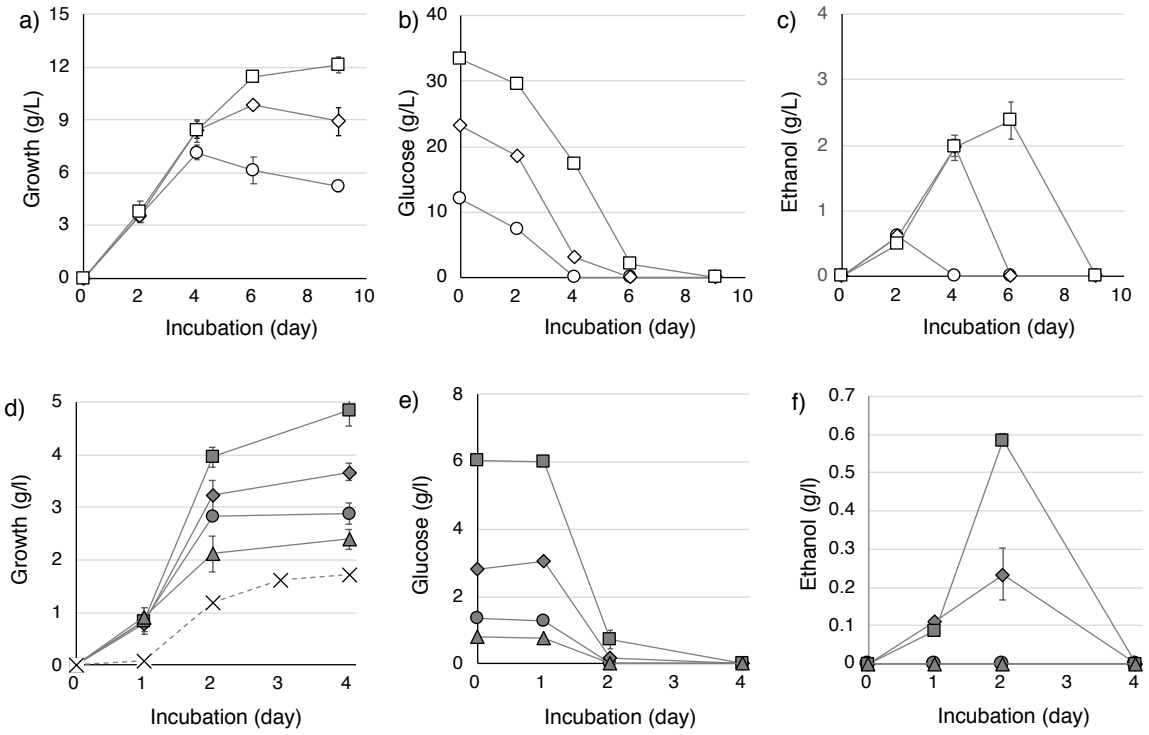
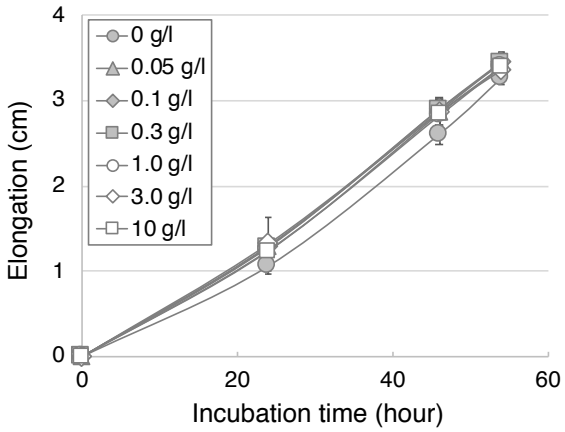

Biomass yield (g/g)
%TM
glucose concentration (g/l)
0.8
1.3
3.0
6.0
12
23
33
α
αβ
βγ
γ
a
b

Fig. 2.

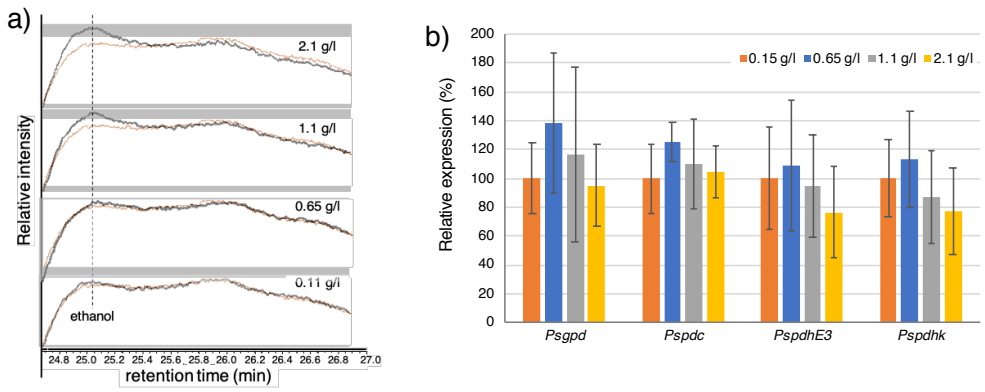


Supplementary Figure 1 | Time courses of growth (a, d), glucose consumption (b, e), and ethanol concentration (c, f) in aerobic cultures of *P. sordida* YK-624 containing an initial glucose concentration of 33 g/l (open square), 23 g/l (open rhombi), 11 g/l (open circle), 6.0 g/l (filled squares), 2.8 (filled rhombi), 1.3 g/l (filled circles), 0.8 g/l (filled triangle) and 0 g/l (crosses). Values are presented as means ± standard deviation of triplicate samples.



Supplementary Figure 2 | Hyphal elongation on T-agar plate containing various concentration of glucose.

Values are presented as means ± standard deviation of triplicate samples.



Supplementary Figure 3 | a) Ethanol production after short shaking cultivation with *P. sordida* YK-624 mycelial homogenate containing with 0.11, 0.65, 1.1 and 2.1 g/l glucose, and b) relative gene expression levels of key enzymes at pyruvate brunch point. a) Black lines indicate enlarged HPLC chromatograms of culture fluid obtained from homogenates containing various concentrations of glucose after 6 h of shaking cultivation, and red lines represent chromatograms of culture fluid without glucose. Dashed lines show retention time of ethanol. b) Actin gene was used as a reference gene. Relative expression was expressed as $2^{-\Delta\Delta Cq}$ calculated from the Cq values of each gene in cultures. Relative expression levels of each gene are expressed based on expression at 0.15 g/l being 100%. Values are presented as means ± standard deviation of triplicate cultures.