Improved β-glucan yield using an Aureobasidium pullulans M-2 mutant strain in a 200-L pilot scale fermentor targeting industrial mass production

Improved β-Glucan Yield Using an *Aureobasidium pullulans* M-2 Mutant Strain in a 200-L Pilot Scale Fermentor Targeting Industrial Mass Production

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Abstract β-(1→3)-D-glucans with β-(1→6)-glycosidic linked branches are known to be immune activation agents and are incorporated in anti-cancer drugs and health-promoting supplements. β-Glucan concentration was 9.2 g/L in a 200-L pilot scale fermentor using mutant strain *Aureobasidium pullulans* M-2, which was from an imperfect fungal strain belonging to *A. pullulans* M-1. The culture broth of *A. pullulans* M-2 had a faint yellow color, whereas that of the wild-type had an intense dark green color caused by the accumulation of melanin-like pigments. β-Glucan produced by *A. pullulans* M-2 was identified as a polysaccharide of D-glucose monomers linked by β-(1→3, 1→6)-glycosidic bonds through GC/MS and NMR analysis. When a conventional medium was used in the culture of *A. pullulans* M-2 in a 3-L jar fermentor, β-glucan concentration was 1.4-fold that produced by the wild-type. However, when medium optimized by statistical experimental design was used with dissolved oxygen at 10%, the β-glucan concentration was 9.9 g/L with yield of 0.52 (g β-glucan/g consumed sucrose), which was 2.9-fold that of the wild-type. This level of productivity was reproduced when the fermentation was scaled up 200-L. The industrial production of high β-glucan without melanin-like pigments is highly expected as a health-promoting supplement or functional food.

Keywords: *Aureobasidium pullulans*; β-glucan; black yeast; functional food; mutant

1. Introduction

β-Glucans are polysaccharides of D-glucose monomers linked by β-glycosidic bonds. Various types of β-glucan occur in wild organisms as storage molecules of saccharides that are an energy source and as structural molecules in cell walls. The β-glucan contained in mushrooms such as *Agaricus bisporus* [1] and *Pleurotaceae citrinopileatus*, which is a Chinese mushroom called Yuhuangmo [2], is also found in barley, oats and maize [3–5], and the cell walls of brewery yeast [6]. These β-glucans are considered non-digestible carbohydrates and classified as pathogen-associated molecular patterns, which can be recognized by pattern recognition receptors (PRRs). The effects of β-glucans on humans are considered to be mediated via PRRs such as dectin-1 and Toll-like receptors (TLRs) that are located at the surface of microbes and trigger various kinds of immune responses [7]. When PRRs are stimulated the expression of a cytokine gene is upregulated, which causes subsequent stimulation of cell-mediated immunity [8, 9]. This means that β-glucan increases phagocytosis activity and nitric oxide production and inflammatory responses in vivo and in vitro [10]. The production of inflammatory cytokines enhances various physiological activities such as immunopotentiation, anti-tumor activity, and cancer cell proliferation suppression that maintain or promote good health [11–14]. Thus, applications of the β-glucans, not only as food additives and functional foods, but also as medical, pharmaceutical, and cosmetic raw materials are increasing year by year.

However, the supply of β-glucan cannot currently meet the demand because of low

productivity and high cost [15, 16]. Commercial β-glucan is extracted from the yeast cell wall and purified, which is associated with a high manufacturing cost. Moreover, some allergens originating from the yeast may contaminate the β-glucan product. It has been found that β-glucan is produced by a microorganism belonging to *Aureobasidium* sp. (commonly known as "black yeast"), which is an imperfect fungus widespread in nature, that exhibits a function equivalent, or superior, to that of the β-glucan derived from mushrooms [14, 16].

The present study aims to improve the production of β-glucan using strain improvement by UV mutation of the wild-type *A. pullulans* M-1. The culture medium of the mutant strain was optimized to improve β-glucan productivity and scaled up to 200-L to target industrial mass production. The chemical structure of the β-glucan produced by this method was characterized by chemical analysis.

2. Materials and Methods

2.1. Microorganism and mutation

The parent strain, *Aureobasidium pullulans* M-1 (Accession No. FERM BP-08615 deposited in the International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan), was cultured on a PDA (potato dextrose agar, Difco Laboratories Inc., Franklin lakes, NJ, USA) slant for 7 d. PDA is composed (per liter) of 4 g of potato extract, 20 g of dextrose, and 15 g of agar (pH 5.6). Colonies were suspended in 10 mL of phosphate buffered saline (PBS) and adjusted to a cell concentration of ~10^6 colony forming units (CFU)/mL. Aliquots (200 μL) of this cell suspension were applied to a PDA

plate and the microbial cells were exposed to a 20-W UV lamp at a distance of 60 cm for 10 min. Under these conditions the cell survival rate was 0.1%. After UV exposure, the plates were subjected to static culture at 25°C for 4 d. The colonies that formed on the plates were cultured at 4°C for 3 d to induce chlamydospore formation.

2.2. Selection of a high β-glucan producer

The first selection was done by visual observation and comparatively large colonies were selected. The second selection was carried in flask culture. Production medium (40 mL), composed (per liter) of 12 g of sucrose, 2 g rice of bran, 0.83 g of sodium ascorbate, and 0.23 g ascorbic acid, was placed in a 300-mL Erlenmeyer flask and sterilized at 121°C for 20 min. The first selected colony was inoculated and cultured at 24.5°C in a rotary shaker at 150 rpm. The productivity of β-glucan of the mutant strain was confirmed using a 3-L fermentor (Bioneer-N300-3L, Marubishi Co. Ltd., Tokyo, Japan) with an aeration rate and agitation rate of 0.5 vvm and 200 rpm, respectively.

2.3. Gene sequence analysis

The ITS-5.8S and 28S rRNA-D1/D2 regions coding rRNA of the high β-glucan producer were investigated to confirm the strain species and its morphological and physiological properties according to previous reports [17, 18].

2.4. GC and EI-GC/MASS analysis

The sugar linkages of the β-glucan were analyzed according to the method described by

Anumura *et al*. [18]. Briefly, 100 g of sample were used for β-glucan extraction. Extracted β-glucan (9 mg) was completely methylated by 100 mg of powdered NaOH and 0.6 mL of methyl iodide followed by hydrolysis to form methylated monosaccharide. The methylated monosaccharide was hydrogenated and acetylated to partially methylated alditol acetate. The resulting alditol acetate was dissolved in chloroform and its chemical structure was analyzed by GC (HP5890 series II, Hewlett-Packard, Palo Alto, CA, USA) and EI-GC/MASS (JMS DX-303, JEOL, Tokyo, Japan) [19].

2.5. NMR spectroscopy

Purified sample was dissolved in dimethyl Sulfoxide-d6 (DMSO-d_6) and then heated at 80°C for 30 min, followed by H-D exchange by adding deuterium water. ^{13}C NMR spectra were recorded in DMSO-d_6 at room temperature using a Varian INOVA 500 NMR spectrometer (Varian, Palo Alto, CA, USA) operating at 125.7 MHz. Purified sample was dissolved in DMSO-d_6 and then heated at 100°C for 1 h, and ^{1}H NMR NMR spectra were recorded using an INOVA 600 NMR spectrometer at 599.5 MHz [20].

2.6. Enzymatic analysis

Purified sample (14.5 mg) was digested by 7.82 mg of cellulase I containing endo β-(1→3)-glucanase (Wako Pure Chem., Osaka, Japan) or 5.34 mg of cellulase II containing β-(1→3)-glucanase (Seikagaku Co., Tokyo, Japan) at 40°C for 16–24 h in acetate buffer (pH 4.5). The degradation product was analyzed by HPLC (LC-20A, Shimadzu, Kyoto, Japan) with a TSKgel Sugar AXG column (15 cm × 4.6 mm I.D.) (Tosoh Bioscience LLC, Tokyo,

130 Japan). The eluent was 0.5 M potassium borate buffer (pH 8.7) at 70°C and the eluent flow
131 rate was 0.4 mL/min. Excitation and emission wavelengths were 320 and 430 nm,
132 respectively.

133 **2.7. Optimization of β-glucan production in flask culture and confirmation of**
134 **productivity in the 3- and 200-L fermentors**

135 Box Behnken experimental design [21–23] was used to optimize the sucrose, rice bran, and
136 sodium ascorbate concentrations in flask cultures. Statistical analysis was performed using
137 STATISTICA (StatSoft Inc., OK, USA), and the regression of the parameters was solved by
138 Mathcad 2001 Professional (Mathsoft Engineering and Education Inc., Cambridge, MA,
139 USA). To confirm the possibility of industrial scale production of β-glucan by the mutant
140 strain, 3-L fermentor (MDL300, Marubishi Co. Ltd., Tokyo, Japan) and 200-L stirred-tank
141 reactors (Komatsugawa Chem. Eng. Co. Ltd., Tokyo, Japan) with working volumes of 2 L and
142 120 L, respectively, were used for β-glucan production. An optimized medium was used and
143 the dissolved oxygen concentration (DO) was controlled at approximately 0, 10, and 30% by
144 a DO controller with an agitation rate in the region of 100 to 150 rpm under a constant
145 aeration rate of 0.5 vvm. Culture temperature was controlled at 24.5°C. During cultivation,
146 the cell morphology was observed using an optical microscope (BX51, Olympus, Tokyo,
147 Japan).

148 **2.8. Measurement of β-glucan**

149 A portion **(**100 g) of the culture broth was centrifuged at 190 × g, and the resulting

supernatant was precipitated by adding 70% ethanol. The ethanol precipitate that contained high-molecular-weight substances was separated by centrifugation and digested with α-amylase, protease, and amyloglucosidase. The enzyme-treated solution was diluted with a four-fold volume of 95% ethanol to allow precipitation of β-glucan. The resultant 95% ethanol precipitate moiety was subjected to air-drying overnight and further air-drying at 105°C for 3 h before being weighed. This gave the weight of the β-glucan content in the culture broth.

3. Results and Discussion

3.1. Screening of the high β-glucan producer

A. pullulans M-1 cells were UV-irradiated at 0.1% of survival rate and 400 colonies of comparatively large sizes compared to the wild-type were selected; this was followed by a second selection. The colonies selected in the second screening described above were inoculated into 300-mL Erlenmeyer flasks containing 40 mL of the production medium and cultured for 7 d. Among the 400 colonies, a colony producing 5 g/L of β-glucan was selected and designated as *A. pullulans* M-2 (Accession No. FERM BP-08615 deposited in the International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology).

3.2. Identification of *A. pullulans* M-1 and M-2

Molecular phylogenetic trees for the base sequence of the ribosomal RNA gene regions,

ITS-5.8S and 28SrRNA-D1/D2, were created by the neighbor-joining method using the top 20 sequences among the analogous sequences detected in an international DNA database. Comparison of ITS-5.8S and 28SrR sequences shows that the mutant strain classically defined as *A. pullulans* (Fig. 1) and belonging to an imperfect fungus *A. pullulans* is the same species as the parent strain *A. pullulans* M-1.

3.3 Comparison of the morphology of the parent strain with that of *A. pullulans* M-2 and their *β*-glucan productivities

When *A. pullulans* M-2 was cultured on PDA plate medium for 1 week, the shape of the vegetative hypha and the pattern of conidium formation were similar to those of the parent strain (Fig. 1A-1 and B-1) although the colony colors differed. The culture broth of *A. pullulans* M-1 had a dark green color, which is typical for *A. pullulans* (Fig. 1A-2), whereas *A. pullulans* M-2 exhibited a faint yellow color (Fig. 1B-2), suggesting that the M-2 strain does not accumulate any melanin-like pigment. The color of this product is very important to its commodity value because consumers prefer a transparent product rather than a dark one. This indicates that the β-glucan produced by *A. pullulans* M-2 is suitable for commercial product.

The β-glucan productivity of *A. pillulans* M2 was compared to that of the parent strain using a 3-L jar fermentor. The β-glucan concentration of *A. pillulans* M2 was 5.0 g/L at a culture time of 144 h, while that of the parent strain was 3.5 g/L (Fig. 2C).

3.4. Chemical structure of the β-glucan produced by *A. pullulans* M-2

188 The ^{1}H and ^{13}C NMR spectra of the β-glucan produced by *A. pullulans* M-2 are shown in Fig.
189 3. The ^{1}H signals at 4.5 ppm were assigned to the β-(1→3)-linkage (Fig. 3A). The ^{13}C signals
190 at 103, 86 , 68, and 61 ppm were assigned to the C-1 carbons of the β-(1→3; 1→6)-linkages,
191 C-3 carbons of the β-(1→3)-linkages, branched C-6 carbons (CH_2-O-R), and unbranched C-6
192 carbons (CH_2-OH), respectively (Fig. 3B).
193 The β-glucans produced by *A. pullulans* M-1 were detected by GC and EI-GC/MASS
194 after three consecutive methylations and their ratios are shown in Table 1. The ratio of
195 branched to total glucose residues was higher than 60%, indicating that the β-glucans
196 produced by *A. pullulans* M-2 were highly branched polysaccharides. Furthermore, from the
197 HPLC analysis, the enzymatic digestion products were glucose and gentibiose (data not
198 shown), suggesting the glucose in the glucan molecule could be assumed to be the β-anomer.
199 From these chemical and enzymatic analyses, a possible chemical structure of the β-glucan
200 produced by *A. pullulans* M-2 was derived and is given in Fig. 4.

201 **3.5. Improvement of the β-glucan productivity**

202 Independent variables corresponding to the sucrose (x_1), rice bran (x_2), and sodium ascorbate
203 (x_3) concentrations were varied according to the Box-Behnken design, and the β-glucan
204 concentrations at 120 and 144 h were recorded as responses and the statistical analyses are
205 shown in Tables 2 and 3, respectively. The effect of the sodium ascorbate concentration (x_3)
206 was negligible in this model, because its p-value was higher than 0.05. According to the
207 analysis of variance, the responses were generated by a non-linear quadratic model described

as follows,

$$Z = -1.77\times10^{-1}x_1+1.19\times10^{-2}x_1^2+3.60\times10^{-1}x_2+3.60\times10^{-1}x_2^2+3.79\times10^{-2}$$

The correlation coefficient of this model is 0.89. Optimized sucrose and rice bran concentrations were calculated to be 20 and 4.5 g/L, respectively. Using this optimized medium, a β-glucan production level was expected to be 10.1 g/L.

3.6. Scale-up of β-glucan production in a 200-L fermentor

To confirm the possibility of industrial scale production of β-glucan by *A. pullulans* M-2, β-glucan production using the optimized medium was carried out in a 3-L jar fermentor with a controlled dissolved oxygen level (DO) during the production phase. When the DO level was controlled at approximately 0% (Fig. 5A-1), the β-glucan concentration was 3.5 g/L (Fig. 5A-2). However, when the DO level was controlled at 10 or 30%, the β-glucan concentrations were 9.9 and 9.0 g/L, respectively. The β-glucan yields, based on consumed sucrose at DO levels of 0, 10, and 30%, were 0.28, 0.52, and 0.49 (g β-glucan/g consumed sucrose), respectively. This indicates that the 10% DO level in the production phase was optimal for β-glucan production. This result was scaled-up to the 200-L fermentor with the optimized medium. The DO level was decreased gradually and controlled at 10% from the culture time of 50 h. The reducing sugar concentration decreased gradually and was 2 g/L at the culture time of 144 h. The β-glucan concentration increased with decreasing reducing sugar concentration and reached 9.2 g/L with a β-glucan yield of 0.51 (g β-glucan/g sucrose) at 144 h (Fig. 5B), which was similar to that of the 3-L jar fermentor, suggesting that β-glucan

228 production in a 200-L stirred tank reactor was reproducible.

229 β-Glucan production levels observed in this present study were compared to
230 production levels reported in related studies (Table 4). Sucrose was used as a carbon source.
231 As a nitrogen source, $NaNO_3$ and $(NH_4)_2$ were used as inorganic nitrogen sources; otherwise
232 yeast extract or rice bran were used as organic nitrogen sources. Typical *A. pullulans* strains
233 produced 4.0 g/L of β-glucan at minimum yield, regardless of whether inorganic or organic
234 nitrogen sources were used. The productivity of β-glucan using the mutant strain *A. pullulans*
235 M-2 was 1.4-fold higher than that of *A. pullulans* M-1. Moreover, when the optimized
236 medium was used, the β-glucan yield and productivity were 2.9 times higher than that of the
237 wild-type. Based on the scale up criteria, such as agitation power per volume in the 200-L
238 stirred tank reactor, the β-glucan yield and productivity were maintained in a 10-kL stirred
239 tank reactor (data not shown) targeting for commercial production.

240 Recently, this β-glucan has become commercially valuable as a supplement for
241 boosting immunity against the common cold, flu, allergies, high cholesterol, diabetes, and
242 cancer. In addition, β-glucan is applied to the skin for dermatitis, eczema, wrinkles, bedsores,
243 wounds, burns, diabetic ulcers, and radiation burns. This β-glucan produced by the black
244 yeast mutant is colorless and does not contain melanin-like pigments, and is very suitable for
245 industrial level production.

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Figure Legends

Fig. 1. Phylogenetic tree of *A. pullulans* M-1 and M-2. ITS-5.8S (A) and 28SrRNA-D1/D2 (B) regions coding rRNA gene from genomic DNA of *A. pullulans* M-1 and M-2 were analyzed, respectively.

Fig. 2. Morphological properties and β-glucan production in flask culture. The shape of the colony on PDA plate: *A. pullulans* M-1 (A-1) and M-2 (B-1). Bars in A-1 and B-1 denote 1 cm. Appearance of culture broth of flask culture: *A. pullulans* M-1 (A-2) and M-2 (B-2). Mycelial morphology of β-glucan producers: *A. pullulans* M-1 (A-3) and M-2 (B-3). Bars in A-3 and B-3 denote 20 μm. Time courses of β-glucan production of *A. pullulans* M-1 and M-2 in 3-L jar fermentor (C). Open and closed circles in C denote *A. pullulans* M-1 and M-2, respectively.

Fig. 3. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of β-(1→3,1→6)-glucan produced by *A. pullulans* M-2.

Fig. 4. Estimated chemical structure of β-(1→3,1→6)-glucan produced by *A. pullulans* M-2.

Fig. 5. Optimal DO level in a 3-L jar fermentor (A) and scale up of β-glucan production to a 200-L pilot scale fermentor with a working volume of 120 L (B) in the culture of *A. pullulans* M-2. Optimized medium, composed of 20 g/L of sucrose, 4.5 g/L of rice bran, and 0.5 g/L of sodium ascorbate, was used. Dotted, solid, and thick lines in A-1 denote DO level controlled at approximately 0, 10, and 30%, respectively. Triangles, circles, and squares in A-1 denote DO level controlled at approximately 0, 10, and 30%, respectively. Closed and open symbols

344 in A-2 indicate β-glucan and residual sugar concentration, respectively. Dotted line and open
345 and closed circles in B denote DO level (%), residual sugar, and β-glucan concentrations,
346 respectively.

Table 1. GC and EI-GC/MASS analysis of methylated β-glucan

Glycoside linkage	Ratio of linkage type (%)
Non-reducing end of glucose	39.1
→3 glucose 1→	24.6
→3, 6 glucose 1→	36.3

Table 2. Box-Behnken medium design and runs

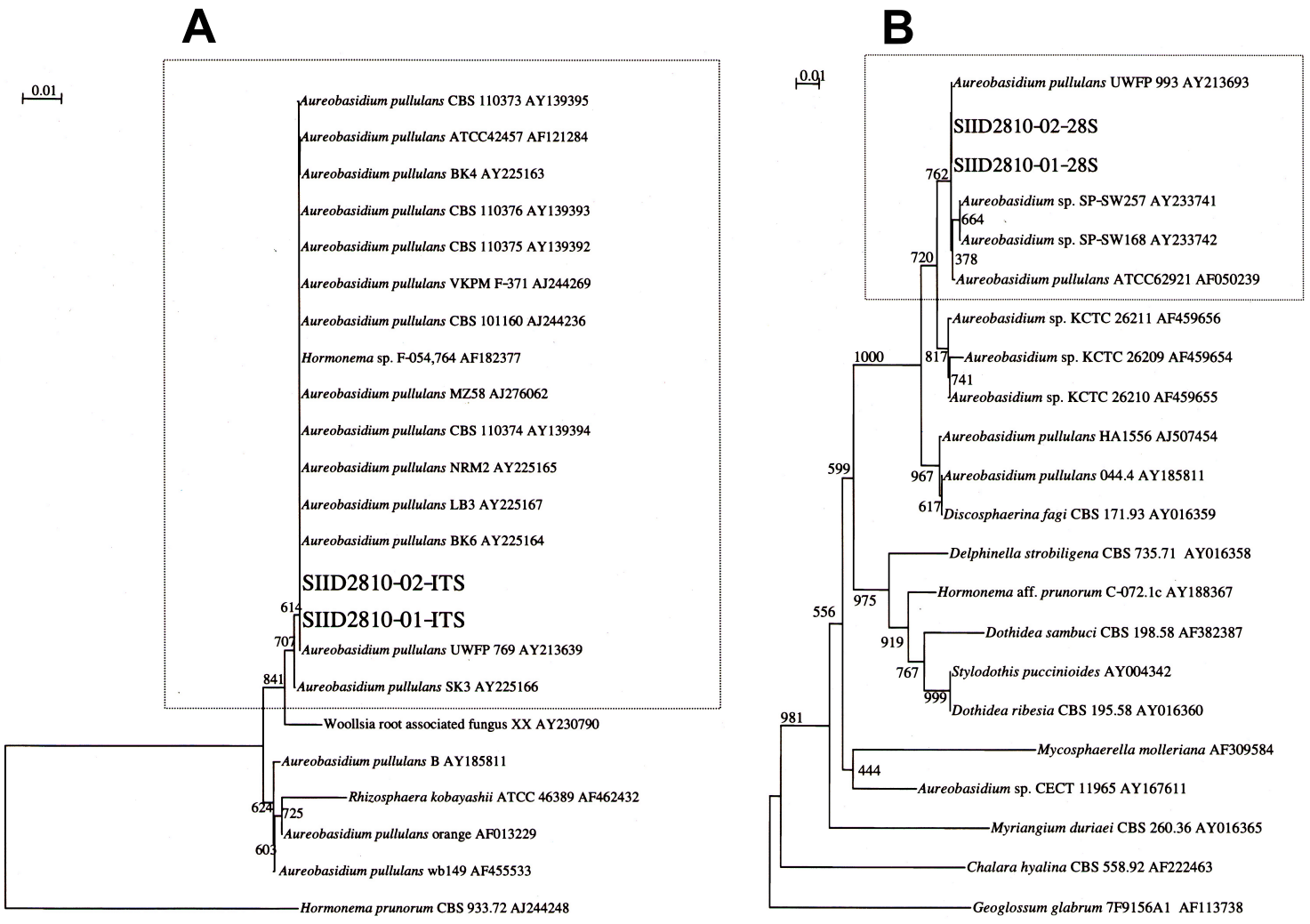
Medium composition (g/L)			β-glucan concentration (g/L)	
Sucrose	Rice bran	Sodium ascorbate	At 120 h	At 144 h
10	1	1.5	3.63	3.65
20	1	1.5	4.33	3.71
10	1	1.5	4.73	4.62
20	5	1.5	8.87	8.44
10	3	0.5	5.50	4.96
20	3	0.5	8.89	9.17
10	3	2.5	4.92	4.52
20	3	2.5	8.36	7.41
15	1	0.5	2.97	4.30
15	5	0.5	6.94	6.46
15	1	2.5	2.72	2.85
15	5	2.5	6.43	5.72
15	3	1.5	6.99	6.14
15	3	1.5	6.82	6.44
15	3	1.5	7.02	6.32
15	2	0.8	6.85	6.52

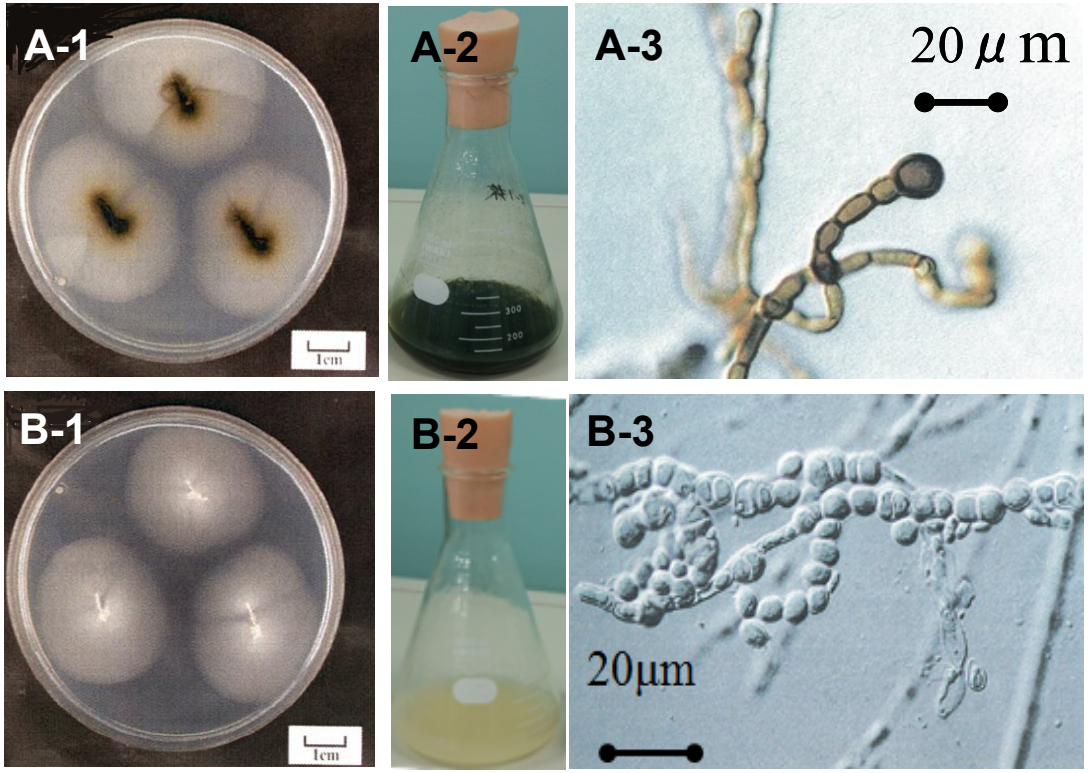
Table 3. Statistical analysis of the flask cultures

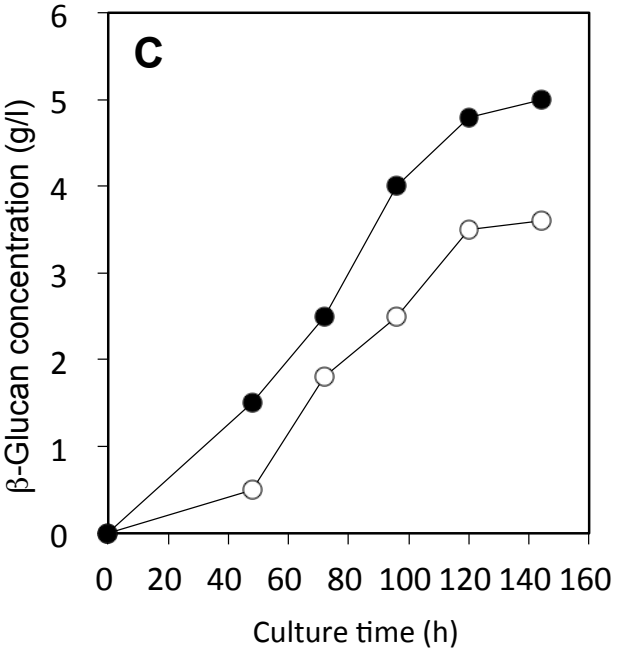
Variable	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Sucrose (%)	15.226	2	7.633	11.103	0.004
Rice bran (%)	22.001	2	11.001	16.000	0.002
Sodium ascorbate (%)	2.417	2	1.209	1.758	0.233
Error	5.500	8	0.688		

Table 4. Comparison of β-glucan productivities in various *Aureobasidium* strains

Strain	Culture	Medium	Yield (g/L)	Time (h)	Productivity (g/L/h)	Reference
A. pullulans	Flask	Sucrose $NaNO_3$	2.8	96	2.9×10^{-2}	15
A. pullulans	Flask	Sucrose $(NH_4)_2$	4.0	72	5.6×10^{-2}	14
A. pullulans MS-822 (KCTC1179BP)	50-L fermentor	Sucrose Yeast extract	2.5	168	1.5×10^{-2}	23
A. pullulans M-1	3-L fermentor	Sucrose Rice bran	3.5	144	2.4×10^{-2}	This work
A. pullulans M-2	3-L fermentor	Sucrose Rice bran	5.0	144	3.5×10^{-2}	This work
A. pullulans M-2	3-L fermentor	Optimized medium	9.9	144	6.9×10^{-2}	This work
A. pullulans M-2	200-L fermentor	Optimized medium	9.2	144	6.4×10^{-2}	This work


A
0.01
Aureobasidium pullulans CBS 110373 AY139395
Aureobasidium pullulans ATCC42457 AF121284
Aureobasidium pullulans BK4 AY225163
Aureobasidium pullulans CBS 110376 AY139393
Aureobasidium pullulans CBS 110375 AY139392
Aureobasidium pullulans VKPM F-371 AJ244269
Aureobasidium pullulans CBS 101160 AJ244236
Hormonema sp. F-054,764 AF182377
Aureobasidium pullulans MZ58 AJ276062
Aureobasidium pullulans CBS 110374 AY139394
Aureobasidium pullulans NRM2 AY225165
Aureobasidium pullulans LB3 AY225167
Aureobasidium pullulans BK6 AY225164
SIID2810-02-ITS
614
SIID2810-01-ITS
707
Aureobasidium pullulans UWFP 769 AY213639
841
Aureobasidium pullulans SK3 AY225166
Woollsia root associated fungus XX AY230790
Aureobasidium pullulans B AY185811
Rhizosphaera kobayashii ATCC 46389 AF462432
624
725
Aureobasidium pullulans orange AF013229
603
Aureobasidium pullulans wb149 AF455533
Hormonema prunorum CBS 933.72 AJ244248
B
0.01
Aureobasidium pullulans UWFP 993 AY213693
SIID2810-02-28S
SIID2810-01-28S
762
Aureobasidium sp. SP-SW257 AY233741
664
Aureobasidium sp. SP-SW168 AY233742
720
378
Aureobasidium pullulans ATCC62921 AF050239
Aureobasidium sp. KCTC 26211 AF459656
1000
817
Aureobasidium sp. KCTC 26209 AF459654
741
Aureobasidium sp. KCTC 26210 AF459655
Aureobasidium pullulans HA1556 AJ507454
599
967
Aureobasidium pullulans 044.4 AY185811
617
Discosphaerina fagi CBS 171.93 AY016359
Delphinella strobiligena CBS 735.71 AY016358
975
Hormonema aff. prunorum C-072.1c AY188367
556
919
Dothidea sambuci CBS 198.58 AF382387
767
Stylodothis puccinioides AY004342
999
Dothidea ribesia CBS 195.58 AY016360
981
Mycosphaerella molleriana AF309584
444
Aureobasidium sp. CECT 11965 AY167611
Myriangium duriaei CBS 260.36 AY016365
Chalara hyalina CBS 558.92 AF222463
Geoglossum glabrum 7F9156A1 AF113738


A-1
1cm
A-2
A-3
20 μ m
B-1
1cm
B-2
B-3
20μm


C
β-Glucan concentration (g/l)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
Culture time (h)

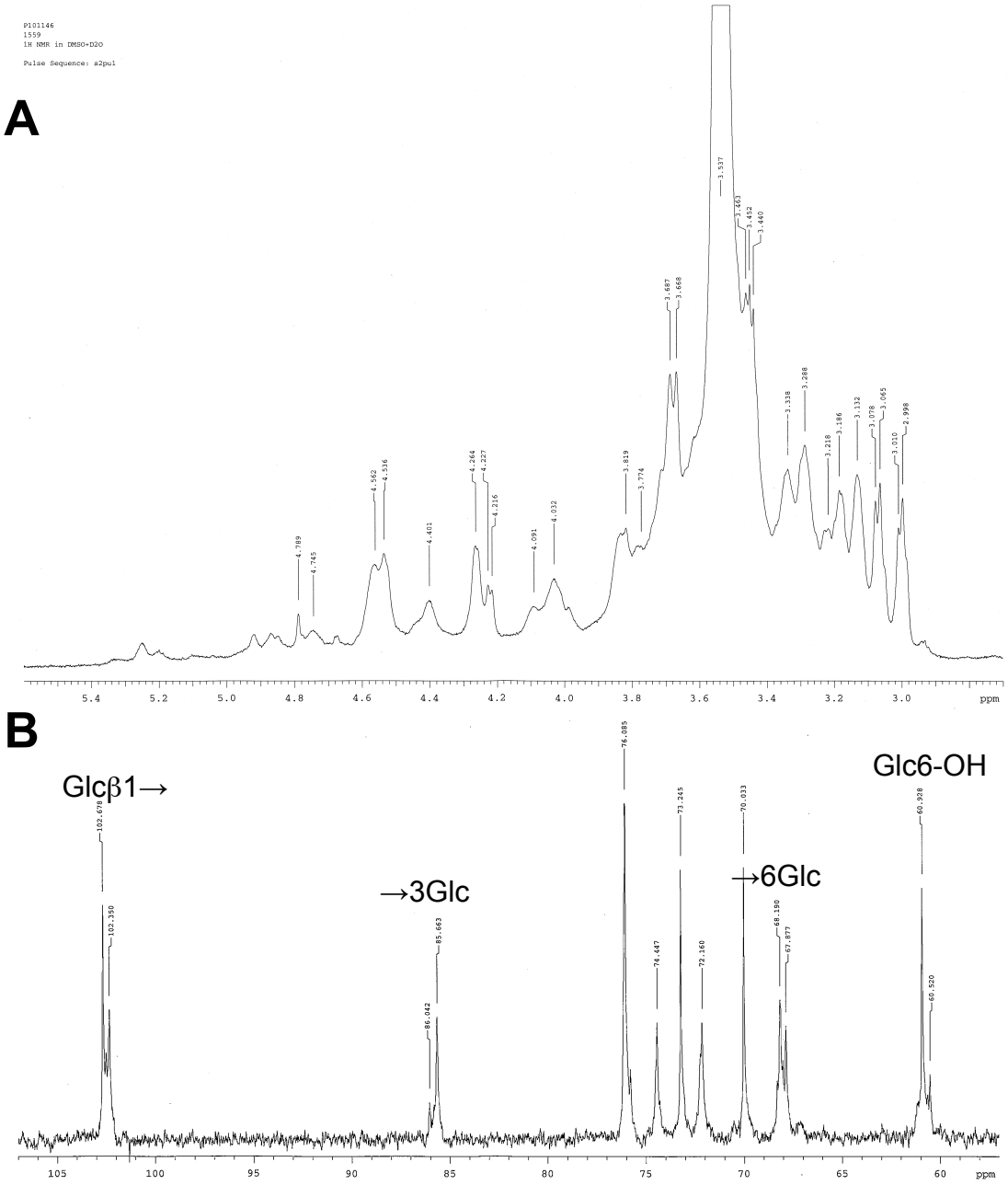
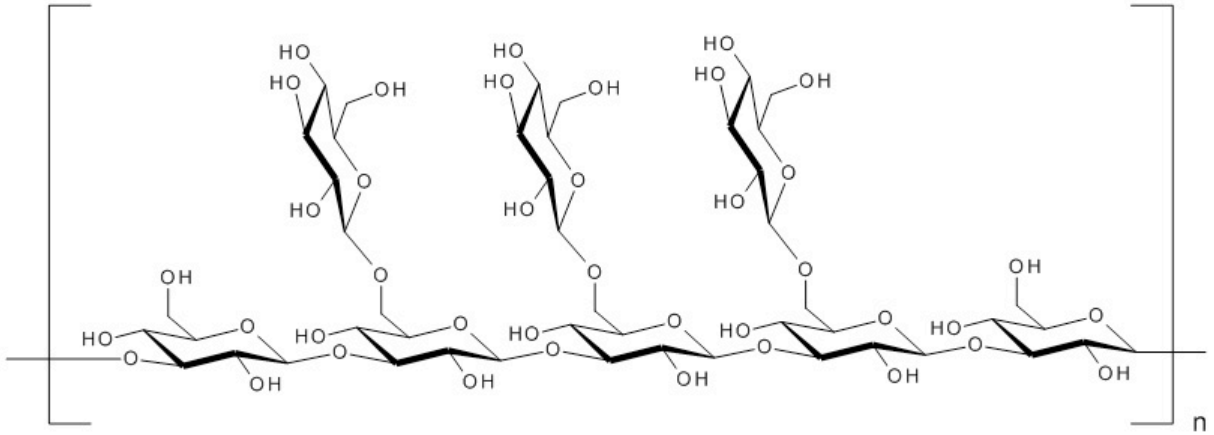
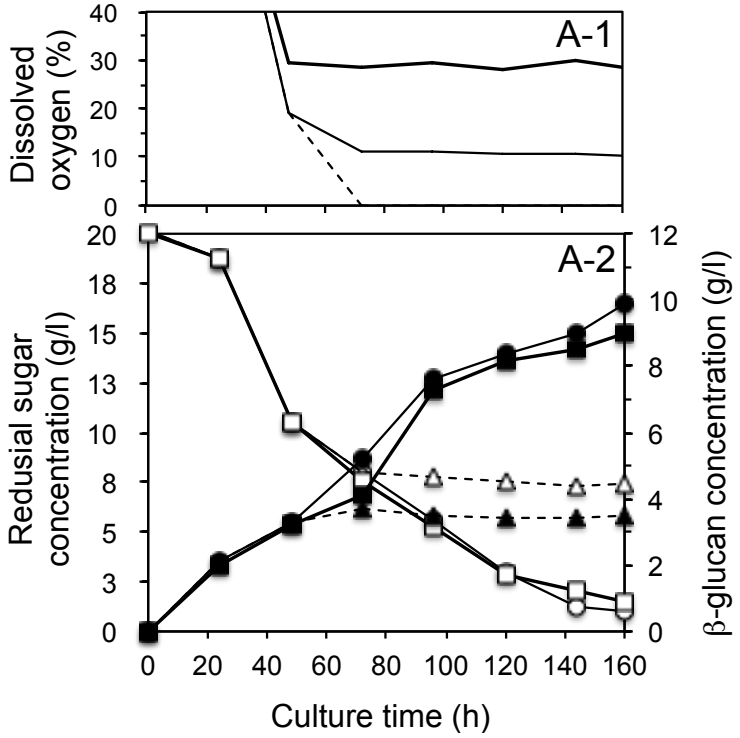
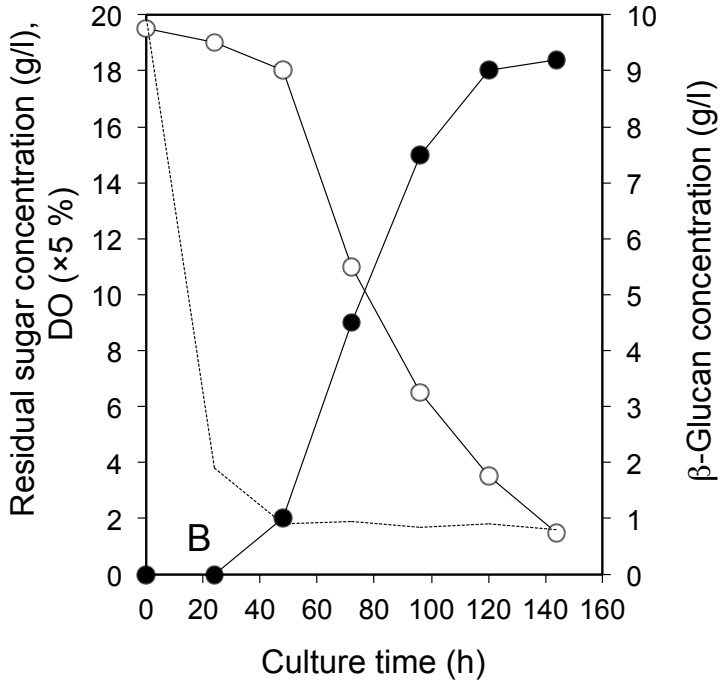

P101146
1559
1H NMR in DMSO+D2O
Pulse Sequence: s2pul
A
4.789
4.745
4.562
4.536
4.401
4.264
4.227
4.216
4.091
4.032
3.819
3.774
3.687
3.668
3.537
3.463
3.452
3.440
3.338
3.288
3.218
3.186
3.132
3.078
3.065
3.010
2.998
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
ppm
B
Glcβ1→
→3Glc
→6Glc
Glc6-OH
102.678
102.350
86.042
85.663
76.085
74.447
73.245
72.160
70.033
68.190
67.877
60.928
60.520
105
100
95
90
85
80
75
70
65
60
ppm

Fig. 4, Moriya et al.




A-1
Dissolved oxygen (%)
40
30
20
10
0
A-2
Redusial sugar concentration (g/l)
β-glucan concentration (g/l)
20
18
15
13
10
8
5
3
0
12
10
8
6
4
2
0
0 20 40 60 80 100 120 140 160
Culture time (h)


Residual sugar concentration (g/l), DO (×5 %)
β-Glucan concentration (g/l)
B
20
18
16
14
12
10
8
6
4
2
0
10
9
8
7
6
5
4
3
2
1
0
0 20 40 60 80 100 120 140 160
Culture time (h)