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Full Length Research Paper

Corncob is the optimum carbon resource for the growth of *Tremella aurantialba*

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The minimum medium without carbon source was used to investigate the effects of five kinds of hexoses, four kinds of pentose, four kinds of bi-saccharide and three kinds of polysaccharides on *Tremella aurantialba* growth. The result demonstrated that (1) The fittest carbon source of *T. aurantialba* was the five-carbon aldose in all the tested monosaccharides; (2) Polysaccharides linked with -1,4-glycosyl bond is fitter for the growth of *T. aurantialba* than that linked with -1,4-glycosyl bond (3) Corncob, which contained abundant poly-xyloses linked with -1,4-glycosyl bond, is the fittest for growth of *T. aurantialba*. The reaction system of -1,4-glycosidase was employed to study the reason that the polysaccharides linked in -1,4-glycosyl bond is fitter for growth of *T. aurantialba*. The result showed that *T. aurantialba* broth can inhibit the activity of -1,4-glycosidase produced by *T. aurantialba*.

Key words: *Tremella aurantialba*, glycosyl bond, carbon source, aldose, ketose.

INTRODUCTION

Tremella aurantialba, extensively used in Chinese medicine (inhabits in wood). During the last 15 - 20 years, studies on *Tremella mensenterica* polysaccharides from fruit body, mycelium, and the crude extract of broth have been carried out in China and Japan. It is reported (Xiong and Yu, 2000) that the composition of *T. mensenterica* decoction remarkably relaxes the tracheal smooth muscle and is a kind of anti-asthmatic. The report also shows that orally taking or subcutaneous injection of crude extract of *T. aurantialba* to mice can increase macro-phages, enhance phagocytic function, adjust the effects on specific immunocompetence of mice, and increase mice nonspecific immunocompetence (Li et al., 2000). The fermented products from *T. aurantialba* can remar-kably prolong the clotting time of the thrombogen and increase the volume of meninges' blood flow (Liu et al., 2003). The polysaccharides from fruit bodies of *T. aurantialba* exhibited remarkable hypoglycemic activity in normal mice and two diabetic mouse models; strep-tozotocin-induced diabetes and genetic diabetes, following intraperitoneal administration (Kiho et al., 1995).

The polysaccharides from mycelia of *T. aurantialba* were reported to prevent and cure diet-induced hyperlipidemia (Wang et al., 2002). Zhang et al. (2004) reported that the polysaccharides of mycelia could decrease the blood sugar content. It is favour to increase the yield of mycelia in the fermentation of *T. aurantialba*. Via comparing with different kinds of hextoses, pentoses, bi-saccharides and polysaccharides, the different kinds of carbon source was found to affect significantly the growth of *T. aurantialba*. According to their structure's discrepancy, it was deduced that corncob is the fittest for the growth of *T. aurantialba*. The paper presented reported on the deducing process.

MATERIALS AND METHODS

Chemicals

α-D-glucosidase and 4-Nitrophenyl-α-D-glucopyranoside used in this experiment were purchased from Sigma. Albumin bovine was made in National Medical Chemical Reagent Co. Ltd, AB-8 macropores resin was purchased from Chemical plant of Nankai University. All other chemicals used in the test were of analytical grade.

Submerged incubation and fermentation

T. aurantialba, kindly presented by Weikjing Qu, a professor of East China Normal University, and grown in PDA medium at 28°C, was used in this study. The fungus was grown in 35 g solid seed

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medium containing 15 g bran and 20 ml water. Solid seed was incubated at 25°C for 7 days. During the culturing course, the flask was shaken once per day from the third to the fifth day.

The solid seed aforementioned was inoculated in 100 ml seed medium containing 2% sucrose, 1% corn powder, 0.5 % peptone. The medium was sterilized under 0.1 MPa for 30 min. The seed was incubated at 27°C in a rotary shaker (Shanghai Pharmaceutical Industrial Academe, China) at 150 rpm for 2 days. 10 ml seed culture was inoculated in 100 ml fermentation medium and fermentation was carried out under the same condition for 7 days. The fermentation medium contained 4% carbon source, 0.4% peptone, 1% corn powder, 1% bran, 0.15% KH_2PO_4, and 0.075% $MgSO_4$.

Isolation of mycelia

The broth was filtered using 40-mesh stainless sieve and the mycelium was obtained. The mycelium was washed using water, dried to constant weight and then weighed.

Inhibitory ratio on -1,4-glucosidase broth of *T. aurantialba*

The inhibitory activity on -1,4-glucosidase was determined using 4-Nitrophenyl-α-D- glucopyranoside as the substrate. The reaction mixture contained 0.3 ml broth of *T. aurantialba*, 50 μl enzyme solution in which 1 mg/ml bovine serum protein and 0.53 μ/ml α-D-glucosidase were dissolved, and 50 μl 0.116 mol/L 4-nitrophenyl-α-D-glucopyranoside in 1.7 ml 0.2 mol/L of pH 6.8 phosphate buffer solution. After incubation for 20 min at 37°C, the reaction was stopped by adding 10 ml 0.1M Na_2CO_3. Water instead of sample solution was used as blank. UV absorbance was measured at the wavelength of 400 nm. The ratio of inhibition was calculated as shown in the following equation:

$$I = \frac{X - Y}{X} \times 100\%$$

Where I is inhibition rate, X = absorbency of the blank, and Y = absorbency of the sample.

Statistical analysis

The analyses were made at least in quintic replication and results presented were expressed as mean ± S.D. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). P-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Effect of different hexose on growth of *T. aurantialba*

Five kinds of hexose were employed in the present test. They were glucose, mannose, altrose, galactose and fructose. The result is as shown in Table 1. Table 1 demonstrated that all tested aldoses had nearly the same dry weight of mycelia (glucose: 2.01 ± 0.25; mannose: 1.96 ± 0.35; altrose: 1.85 ± 0.29; galactose: 1.94 ± 0.27) and were not significantly different from each other.

But when compared with ketoses (fructose), their dry weights of mycelia were significantly higher than that of fructose (1.53 ± 0.18) ($p < 0.01$) . The results proved that the more optimum carbon of *T. aurantialba* was aldoses and not ketose.

Table 1. Effect of different hextose on dry weight of *T. aurantialba.*

Kind of hextose	The dry weight of mycelium (g)
Glucose	2.01 ± 0.25
Mannose	1.96 ± 0.35
Altrose	1.85 ± 0.29
Galactose	1.94 ± 0.27
Fructose	1.53 ± 0.18**

Values represent the dry weight of mycelia in 100mL medium and were expressed as mean ±S.D. *p 0.05 (compared to glucose, mannose, altrose and galactose). **p 0.01 (compared to glucose, mannose, altrose and galactose).

Effect of different pentose on growth of *T. aurantialba*

Four kinds of pentoses were employed in the test. They were lyxose, xylose, ribose and xylulose. The result is shown in Table 2. Table 2 demonstrated that all tested aldose had nearly the same dry weight of mycelia (lyxose: 2.41 ± 0.23; xylose: 2.53 ± 0.17; ribose: 2.48 ± 0.27) and there were no significantly difference between them. But when compared with ketose (xylulose), their dry mycelia weights were significant higher than that of xylulose (1.98 ± 0.26) ($p < 0.01$). The results proved that the more optimum carbon of *T. aurantialba* was aldoses and not ketose.

Pentose is more optimum carbon source within mono-saccharide

When Table 1 was compared with Table 2, the conclusion was obviously gotten: (1) that dry weights using the five- carbon aldose as carbon source were higher than the use of six -carbon aldose as source; (2) that dry weight using the five- carbon ketose as carbon source was higher than the use of six-carbon ketose as source; (3) the five-carbon aldose was the most optimum carbon source within all tested mono-saccharide.

Effect of different bi-saccharides on growth of *T. aurantialba*

Four kinds of bi-saccharides employed in the test were maltose, sucrose, lactose and cellobiose. The result is as shown in Table 2. Table 3 demonstrated that maltose, lactose and cellose had nearly the same dry weight of mycelia (2.32 ± 0.18; 2.38 ± 0.19 and 2.41 ± 0.23, respectively) and were not significantly different from each other. But the dry weights of mycelia using sucrose

Table 2. Effect of different pentose on dry weight of *T. aurantialba.*

Kind of hextose	The dry weight of mycelium (g)
Lyxose	2.41 ± 0.23
Xylose	2.53 ± 0.17
Ribose	2.48 ± 0.27
Xylulose	1.98 ± 0.26**

Values represent the dry weight of mycelia in 100mL medium and were expressed as mean ± S.D. *p 0.05 (compared to lyxose, xylose and ribose). **p 0.01 (compared to lyxose, xylose and ribose).

Table 3. Effect of different bi-saccharides on growth of *T. aurantialba.*

Kind of bi-saccharide	The dry weight of mycelium (g)
Maltose	2.32 ± 0.18
Sucrose	2.06 ± 0.17**
Lactose	2.38 ± 0.19
Cellobiose	2.41 ± 0.23

*p 0.05 (compared to maltose, lactose and cellobiose). **p 0.01 (compared to maltose, lactose and cellobiose).

as carbon source (2.06 ± 0.17) were less than the use of other tested bi-saccharides as carbon source, which was highly significant ($p < 0.01$). The molecule of sucrose linked one molecule of aldose with ketose, and other three kind of molecular were made of two molecule of aldose. The result proved that the more optimum carbon of *T. aurantialba* was aldoses and not ketose again.

Effect of different poly-saccharides on growth of *T. aurantialba*

Three kinds of polysaccharides were used in the test. They were starch, dextrin and cellose. The result is shown in Table 4. Table 4 demonstrated that the dry weight of *T. aurantialba* using starch and dextrin as carbon source (1.47 ± 0.35 and 1.41 ± 0.28, respectively) were less than that using cellose as carbon source (1.83 ± 0.25) ($p < 0.01$). The result demonstrated that the polysaccharides linked with -1,4-glycosyl bond is fitter for growth of *T. aurantialba* than that linked with -1,4-glycoside bond

Corncob was the optimum carbon for growth *of T. aurantialba*

Effect of diverse carbon sources on *T. aurantialba* growth showed significant difference. Among tested carbon sources, five-carbon aldose is fitter for the growth of *T.*

Table 4. Effect of different polysaccharides on growth of *T. aurantialba.*

Kind of polysaccharide	The dry weight of mycelium (g)
Starch	1.47 ± 0.35
Dextrin	1.41 ± 0.28
Cellose	1.83 ± 0.25

*p 0.05 (compared to starch and dextrin). **p 0.01 (compared to starch and dextrin).

aurantialba than hextose or five-carbon ketose and the polysaccharides linked with -1,4-glycosyl bond is fitter for *T. aurantialba* growth than that linked with -1,4-glycosyl bond.

Glucose is metabolized via the Embden-Meyerhof-Parnas pathway (EMP) and xylose via the hexose phosphate shunt. In the test, the five-carbon aldose as carbon source produces more mycelia. The result provides the proof that the ingredients with the inhibitory activity on the Embden-Meyerhof- Parnas pathway (EMP) exist in the *T. aurantialba* broth or there is lack of the key enzyme of the Embden-Meyerhof-Parnas pathway in *T. aurantialba*.

Polysaccharides linked with -1,4-glycosyl bond was fitter for *T. aurantialba* growth than that linked with -1,4-glycosyl bond in theory. It is possible that the ingredients with the inhibitory activity on -1,4-glycosidase exist in the broth of *T. aurantialba*.

The analysis of inhibitory effect on -1,4-glucosidase broth of *T. aurantialba* demonstrated that 0.3 ml broth resulted to 47.5% inhibitory ratio. The analysis of remnant dextrin, starch and cellulose in the broth showed that unused starch or dextrin was not less than 2.3%, however, the unused cellose was less than 0.5%. These tests proved that the broth of *T. aurantialba* can inhibit -1,4-glycosidase. It is the inhibitory activity that decreases the utilization ration of starch and dextrin in the fermentation of *T. aurantialba*.

In conclusion, the five-carbon aldose is the fittest carbon source of *T. aurantialba*. During the growing and metabolizing process, *T. aurantialba* can produce the ingredients that can inhibit -1,4-glycosidase and decrease the utilization ratio of starch and dextrin, which result in cellose being fitter for the growth of *T. aurantialba* than starch and dextrin. Corncob contained more than 80% poly-xylose linked with -1,4-glycosyl bond, so, it is reasonable to deduce that the corncob is the fittest carbon for the growth of *T. aurantialba*.

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