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Optimization of an Extracellular Dextranase Production from *Lipomyces starkeyi* KCTC 17343 and Analysis of Its Dextran Hydrolysates

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We optimized dextranase culture conditions by batch fermentation using *Lipomyces starkeyi* KCTC 17343. Furthermore, dextranase was purified by an ultra-membrane, and then dextran hydrolyzates were characterized. Cell growth and dextranase production varied depending on the initial culture pH and temperature. The conditions of optimal dextranase production were met in a pH range of 4-5 and temperature between 25-30°C. At optimal fermentation conditions, total enzyme activity and specific enzyme activity were about 4.85 IU/ml and 0.79 IU/g cells, respectively. The specific growth rate was examined to be 0.076 hr^{-1}. The production of dextranase in culture broth was very stably maintained after mid-log phase of growth. The enzyme hydrolyzed dextran into DP (degree of polymerization) 2 to 8 oligodextran series. Analysis of the composition of hydrolysates suggested that the enzyme produced is an endo-dextranase.

Key words : *Lipomyces starkeyi*, dextranase, dextran, fermentation, oligodextran

Introduction

Dextran is a generic term for a bacterial exopolysaccharide synthesized from sucrose and composed of chains of D-glucose units connected by α-1,6 glycosidic linkages. Dextran can be branched by α-1,2, α-1,3, or α-1,4-linkages with various degrees, depending on the strains of *Leuconostocs* [2,16]. Dextrans have been used for more than 50 years as plasma volume expanders. Recently, dextrans have been investigated for delivery of drugs and proteins, primary to increase the longevity of therapeutic agents in the circulation [14]. The size of most dextrans that could be applied for medical use ranges from 5 to 100 kDa. The dextran, however, usually synthesized by *Leuconostocs* has much higher molecular weight of 5×10^7 to 10^9 and different types of branch were formed dependant on species [1,3,8,9]. Therefore, special techniques must be used to producel the lower molecular weight dextrans.

Lipomyces starkeyi, ascosporogeneous yeast, produces dextranase which selectively hydrolyzes the α-(1→6) glycosidic bond present in dextrans to produce oligodextrans [10,11]. Mostly the commercial dextranases have been produced from fungi, either *Penicillium* sp. or *Chaetomium* sp [5,13]. However, the dextranases of fungi are non-food grade because they contain various antibiotics and toxic metabolites that are not removed completely. Under circumstance like this, *L. starkeyi* dextranase has been studied for food related application because it is not known to produce antibiotics or toxic metabolites. *L. starkeyi* dextranase hydrolyzed high molecular weight of dextran to a controlled-size [9]. And also, the dextranase can block formation of dental plaque which is composed of salivary glycoproteins and sticky glucose polymers [15].

In this paper, we optimized the dextranase production with another strain *L. starkeyi* KCTC 17343 in the fermentation process. With partially purified enzyme, the pattern of dextran hydrolyzates was examined and compared with the previous reports.

Materials and Methods

Organism and culture conditions

The ascosporogenic yeast *Lipomyces starkeyi* KCTC 17343

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was purchased from a Biological Resources Center (Daejeon, Korea). *L. starkeyi* was maintained on agar slants containing mineral medium (MM) with 0.5% yeast extracts (Y) and 2% (w/v) soluble starch as a carbon source. Mineral medium (MM) consisted of 3.0 g KH_2PO_4, 0.01 g $FeSO_4 \cdot H_2O$, 0.01 g $MnSO_4 \cdot 4H_2O$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g NaCl, 0.05 g $CaCl_2$ per 1 liter deionized water. The medium pH was adjusted to 4.5 prior to sterilization. Working cultures were prepared by incubating the culture in 50 ml of pH 4.5 MMY (pH4.5) containing 1% (w/v) sucrose or soluble starch in 250 ml-shake flasks. Cultures were incubated on a shaker at 30°C and 200 rpm. The organism was sub-cultured biweekly.

Optimization of dextranase production

The effects of culture pH and temperature on dextanase production by *L. starkeyi* KCTC 17343 were determined by incubating the strain at different pH values and at various temperature conditions, respectively. All cultures were incubated in MMY medium containing 2% (w/v) soluble starch with a working volume of 50 ml in a shaking incubator (SI-300R, Jeio Tech, Daejeon, Korea). The strain was inoculated with 2.0% (v/v) of working volume and the growth was monitored at absorbance of 660 nm by a spectrophotometer. The biomass (g/l) was obtained by the standard curve plotted by comparing the absorbance and dry cell weight. Dextranase activity was measured as described in the method of Konig and Day [10]. And then, the culture supernatant was reacted with 5% (w/v) T-500 dextran (Sigma, MO, USA) for 20 min in 100 mM sodium acetate, pH 5.2. Activities were determined from increase rate of reducing sugar measured by DNS method [10]. One unit (IU) of dextranase was defined as the amount of enzyme which liberates 1 μmole of isomaltose equivalents in one minute. Specific enzyme production was expressed as IU/g dry weight cells.

Mass production of dextranase

At optima culture conditions, *L. starkeyi* dextranase was produced by batch fermentation in a 10 L fermenter (Semitel, BioTechnology Innovation, Dae-jeon, Korea). The medium with working volume 6 L was composed of MMY containing 2% (w/v) gelatinized soluble starch. The strain was inoculated with 10% (v/v) of the working volume. The pH was adjusted to 5.0 prior to sterilization. The aeration rate was set at 1.0 vvm and temperature was maintained at 30°C.

Analysis of dextran hydrolyzates

T-500 Dextran hydrolyzates were separated using an ion-exchange column, Aminex HPX-42A (Bio-Rad, Hercules, CA). Samples, 0.1% (w/v), were dissolved in deionized water and the pH was adjusted to 6.5. Fifty ul of sample was used for injection and column temperature was maintained at 85°C. The deionized water was used to elute hydrolysates and elution rate was fixed to 0.4 ml per min. And also, a 410 Differential Refractometer (Millipore Corporation, MA, USA) was used for detection of hydrolysates. Dextrans hydrolyzates were also analyzed by thin layer chromatography on E. Merck plates 0.25 mm of silica gel 60-Kieselguhr. The solvents as two ascents were used as follows : methylacetate, ethylacetate, propanol, and water in proportions of 85:20:50:85, respectively. The plates were air dried and spraied with the reagent, 5% (v/v) H_2SO_4 in ethanol containing 0.3% (w/v) alpha-naphthol. And then, the plates were heated to 100°C for 10 to 20 min for development. The oligodextrans were analyzed by comparing their chromatographic behavior with those of the standards.

Results and Discussion

The growth rate and dextranase production of *L. starkeyi* KCTC 17343 varied depending on the initial culture pH. As can be seen in Table 1, the dextranase production was directly in proportion to bacterial growth. As the culture pH increased from 2 to 5, the growth also increased. At pH 5, specific growth rate was highest and then dramatically decreased at pH 6. Total dextranase production followed the same pattern. Dextranase yields and specific productions at pH 3 were over 3 times lower than those at pH 4 to 5. At previous research, *Lipomyces* strains have been reported to have optimal growth at around 5.0 [11]. Interestingly, the optimum pH of dextranase fermentation is similar to that of recombinant dextranase that is expressed in *Pichia pastoris* [11,12]. And also, the growth rate and dextranase production of *L. starkeyi* KCTC 17343 varied depending on the initial culture temperature (Table 2). As culture temperature increased from 15 to 30°C, cell mass and specific growth rate also increased. At 30°C of culture temperature, the specific growth rate was highest and then decreased at 35°C. Dextranase yields at culture temperature 20 to 30°C were over 7 times higher than that at 15°C. It is consistent that *Lipomyces* strains have shown

Table 1. The effects of initial culture pHs on the cell growth and dextranase production from *Lipomyces starkeyi* KCTC 17343

Culture pH	Cell mass (g/l)	Specific growth rate (hr^{-1})	Doubling time (hr)	Enzyme activity (IU/ml)	Specific enzyme activity (IU/g)
2	4.02	0.041	16.90	0.19	0.05
3	4.51	0.049	14.14	1.25	0.28
4	5.42	0.065	10.66	3.51	0.65
5	5.55	0.074	9.36	4.02	0.72
6	3.65	0.032	21.66	2.12	0.58

All cultures were incubated in a shaking incubator with culture conditions of 30°C and 200 rpm.

Table 2. The effect of initial culture temperatures on the cell growth and dextranase production by *Lipomyces starkeyi* KCTC 17343

Culture temperature (°C)	Cell mass (g/l)	Specific growth rate (hr^{-1})	Doubling time (hr)	Enzyme activity (IU/ml)	Specific enzyme activity (IU/g)
15	1.51	0.025	27.72	0.05	0.03
20	3.49	0.047	14.74	3.43	0.98
25	5.42	0.073	9.49	4.57	0.84
30	5.54	0.076	9.12	4.35	0.79
35	2.42	0.028	24.75	2.1	0.87

All cultures were incubated in a shaking incubator with culture conditions of pH 5.0 and 200 rpm.

optimal dextranase production around at 25 to 30°C as reported in previous study [10,11]. As mentioned above, the optimum pH of dextranase fermentation is similar to that of enzyme activity.

At the optimal fermentation conditions, kinetics of dextranase production by *L. starkeyi* KCTC 17343 was examined as can be seen in Fig. 1. Bacterial growth and dextranase production were investigated until the growth reached to a stationery phase. The maximal dextranase production was achieved at the early stage of growth. At this point, the specific growth rate and specific enzyme activity were 0.047 hr^{-1} and 0.18 IU/g cells, respectively. Interestingly, the total activity of dextranase in culture broth maintained very stable after mid-log phase of growth. After fermentation, crude dextranase was obtained after removing sugar and medium residues by ultra-membrane system. Typical chromatograms of HPLC and TLC of dextran hydrolyzates by HPLC and TLC were illustrated in Fig. 2. Enzymes which hydrolyze α-D-(1→6) glycosidic linkages are classified in two types: endodextranase (E.C. 3.2.1.68) and pullulanase (E.C. 3.2.1. 41). However, an extracellular endodextranase was observed from *Lipomyces starkeyi* [18]. Generally, Endo-dextranases act randomly on the internal part of dextran linkage (α, 1→6) and produce the isomaltooligosaccharides [15]. This enzyme do not cleave the α-(1→4) and β-(1→4) linkage. Until now, endodextranase produced from *Lipomyces starkeyi* ATCC 20825 has been extensively studied. It hydrolyzes the dextran to

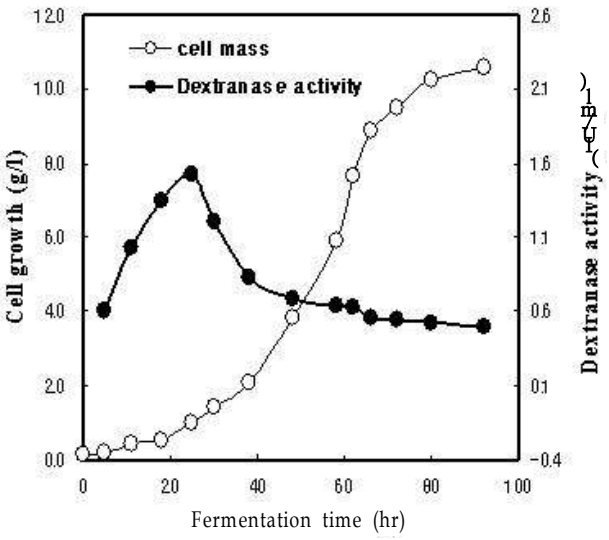

Fig. 1. Kinetics of growth and dextranase production by *Lipomyces starkeyi* KCTC 17343. Fermentation was performed with MMY containing 2% (w/v) starch at 30°C and pH 5.0.

glucose and isomaltotetrose through isomaltooligosaccharides [10-11]. In our studies in Fig. 2, peak numbers from 2 to 8 or more indicated the mixture of isomaltoglucooligosaccharides. Analysis of the composition of hydrolysates suggested that the enzyme produced is an endo-dextranase.

In conclusion, we optimized a simple batch fermentation to produce dextranase and investigated kinetics of enzyme production from *L. starkeyi* KCTC 17343. Through further study, we will confirmed whether the partially purified

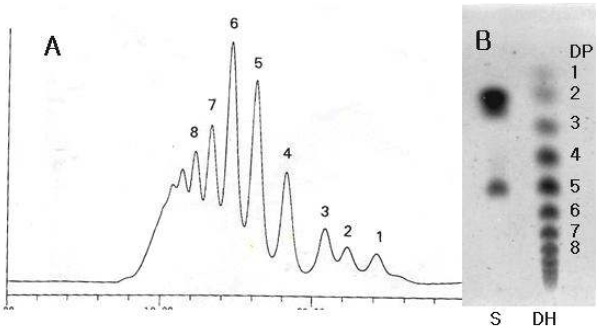

Fig. 2. Chromatograms of dextran hydrolysates analyzed by HPLC (A) and TLC (B). The numbers indicate the oligodextans ranged from DP 1 to 8. S represents standard oligodextrans DP 2 (above) and DP 5 (below). DH represents the hydrolyzates of dextran (T500) separated by TLC.

dextranse hydrolyze dextran into oligodextran series or not. And also, we expect that such a fermentation process could be applied for production of dextranase with good cost economics.

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초록 : *Lipomyces starkeyi* KCTC 17343에 의한 extracellular dextranase 최적생산과 덱스트란 hydrolysates 분석

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본 연구는 *Lipomyces starkeyi* KCTC 17343에 의한 dextranase 최적 생산 조건을 확립하고 dextran에 대한 효소 분해 특성을 규명하였다. 균주의 성장과 dextranase생산은 발효초기 pH와 온도에 따라 다르며 최적 pH는 4-5, 최적온도는 25-30°C의 범위에서 결정이 되었다. 최적 발효조건에서의 dextranase 생산은 total enzyme activity가 4.85 IU/ml으로 나타났다. 이때의 발효균주의 specific growth rate는 0.076h^{-1}이었다. 발효 중 dextranase의 활성은 발효 정상기에서도 안정성을 유지하였다. Dextranase에 의한 dextran을 가수분해 결과, 가수분해물의 구성은 DP2 to 8에 이르는 올리고 덱스트란으로 이루어졌다.