On the Production of Extracellular Protein by Yeast

Screening and Identification of Yeast Strain that Produce Extracellular Protein and Its Cultural Condition

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Many studies concerning intercellular protein production by microorganisms, especially by yeast, have been reported. However, few studies concerning extracellular protein production are as yet known and we have only found the reports by Udaka with bacteria.[1~7)]

Comparing the protein production by yeasts with that bacteria, the former may be more advantageous in the following points, i. e., some yeasts have been familiar as fermentative food stuffs, and the separation process may be easier because of their larger cell size.

Therefore, we attempted to obtain yeast strains which produce extracellular protein. Two hundred and ninety strains of yeast were employed. These strains included one hundred and twenty–one strains of identified yeast, i. e., twelve strains of *Saccharomyces*, five strains of *Debaryomyces*, twenty–nine strains of *Pichia*, two strains of *Petasospora*, forty–seven strains of *Hansenula*, eighteen strains of *Candida*, three strains of *Mycotorula* and five strains of *Rhodotorula*, and one hundred and sixty–nine strains of unidentified yeast isolated from various samples.

These yeasts were aerobically cultured in various media at 29 °C and the quantities of extracellular protein accumulated in cultured media were estimated. However, we could detect no protein production in any culture of yeast at pH 6. But in acidic cultivation (pH 3), three strains were found to accumulate over 50 μg of protein /ml in media. Among these three strains, X–19 strain, which had been isolated from soil as a xylose utilizer by Akaki, one of the authors, was most effective in protein production.[8~11)]

Morphological and physiological characteristics of X–19 strain were studied. The experimental results are shown in Fig. 1, Fig. 2, Fig. 3 and Table 1. It was proved that this strain belonged to the genus *Trichosporon* and named *Trichosporon* sp. X–19. However, concerning the species we could find no description fitting its characteristics in "The Yeast" edited by Lodder.[12)]

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We found the fact that the accumulation of extracellular protein by *Trichosporon* sp. X−19 occured in a relatively narrow pH range near 3 as shown in Fig. 4. Moreover, it was observed that yeast cells formed in this acidic cultural condition accumulated a fairly large amount of protein which was washed out with diluted alkaline. After the cultivation of the yeast, cells were separated from the culture media by centrifugation (12,000 rpm, 5 min, below 15 °C), and suspended in 25 ml of 0.05 N NaOH at room temperature. Five minutes later, the suspension was centrifuged (12,000 rpm, 5 min, 15 °C) to remove the yeast cells. A transparent supernatant was obtained by this procedure and the protein in the solution was estimated by Micro−Biuret method.[13)]

The effect of the alkaline treatment described above on the yeast cells was tested, and the experimental results showed that the treatment scarcely affected the survival rates of yeasts.

The use of acidic condition for yeast cultivation has the great advantage of repressing bacterial growth. Our experimental results suggest that the surface structure of yeast cell may change with the cultural condition (acidic or neutral) and that the accumulation of protein may be enhanced in the adaptation process of yeast to an abnormally acidic condition.

Composition of culture medium fitted for extracellular protein production by *Trichosporon* sp. X−19 was studied on carbon and nitrogen sources, it turned out that Czapeck−Dox modified medium (glucose 3%, glycine 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.05% and $FeSO_4$ 0.001%) gave better productivity of protein by the yeast than YM medium. Further investigation on nitrogen sources of culture medium enabled replacement of glycine by $NH_4H_2PO_4$, and this could indicate a commertial feasibility of protein production by *Trichosporon* sp. X−19 with the synthetic medium. Effects of amino acids on protein production by the yeast was also studied and it was shown that alanine, arginine, glutamic acid, serine, glutamine and asparagine gave better production than glycine, whereas aspartic acid gave quite poor production in contrast to the largest growth of the yeast. Moreover, it turned out that arginine had different properties on its protein production by the yeast because of its independency of pH of culture medium (between pH 3 and 6) unlike pH dependency of the other amino acids. Arginine also gave the best protein production. However, regardless of replacement of organic nitrogen sources in culture medium by inorganic substances, protein produced gave the same patterns on gel electrophoresis. The fact showed these protein to be identical.

In the following paper, we are going to report on the large scale cultivation of *Trichosporon* sp. X−19 using propagation tank and nutritive values of the extracellular protein produced by the yeast.

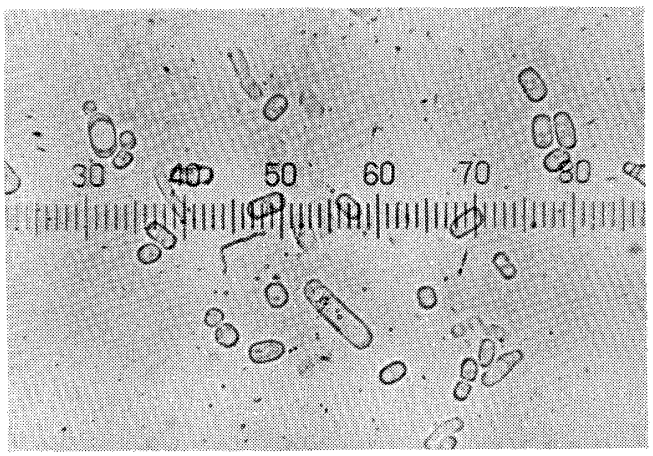

Fig. 1. X-19 strain.
After 3 days at 25° C in glucose-yeast extract-peptone water
One division shows 2.5μ.

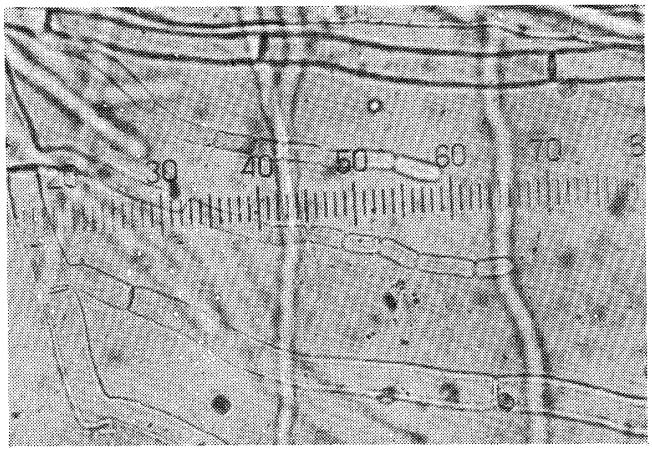

Fig. 2. X-19 strain.
Dalmau plate culture on corn meal agar
One division shows 2.5μ.

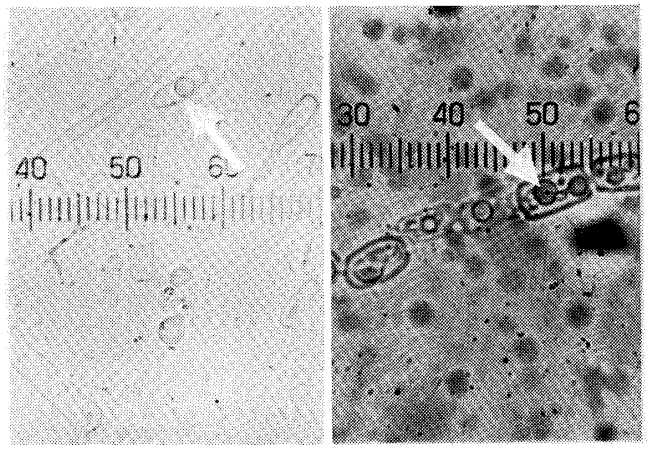

Fig. 3. Endospore-like granules (indicated by arrows) of X-19 strain.
After 1 month in glucose-yeast extract-peptone water
One division shows 2.5μ (left) and 1 μ (right).

Table. 1 Physiological Properties of Strain No. X-19.

Fermentation: Negative.			
Assimilation of carbon compounds:			
Glucose	+	D-Ribose	−
Galactose	+	L-Rhamnose	−
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	−
Cellobiose	−	Ribitol	weak
Trehalose	−	Galactitol	−
Lactose	−	D-Mannitol	+
Melibiose	−	D-Glucitol	+
Raffinose	weak	α-Methyl-D-glucoside	−
Melezitose	weak	Salicin	−
Inulin	+	DL-Lactic acid	+
Soluble starch	−	Succinic acid	+
D-Xylose	+	Citric acid	+
L-Arabinose	−	Inositol	−
D-Arabinose	weak		
Assimilation of nitrogen compounds:			
Potassium nitrate	−		
Sodium nitrite	−		
Growth in vitamin-free medium: Positive.			
Growth on 50%(w/w) glucose-yeast extract agar: Negative.			
Maximum temperature of growth: 39° C.			
Starch formation: Negative.			
Hydrolysis of urea: Positive (weak)			

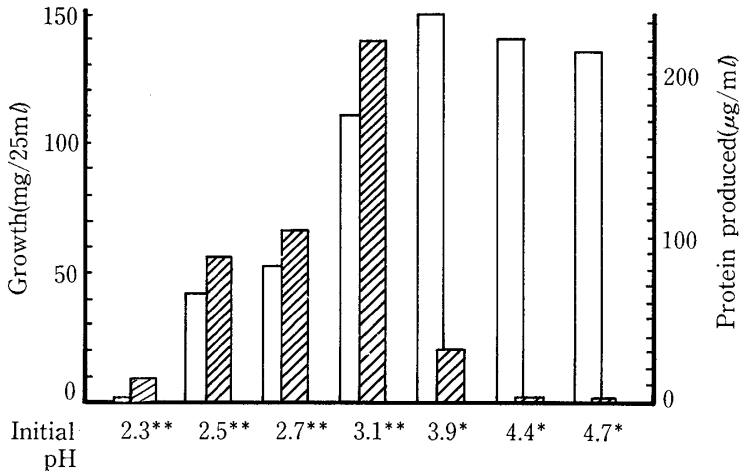

Fig.4. **Effect of Initial pH of Culture Medium on Protein Production by X-19 Strain. Culture medium: YM medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1.0% glucose); Culture period: *, 1 day; **, 4 days; □, growth of yeast (mg of dry celis per 25ml culture medium); ▨, protein produced in the culture supernatant.**

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