Production of Extracellular Protein by *Trichosporon* sp. X—19

Pilot Plant Scale Submerged Culture of the Yeast and the Nutritive Values of Extracellular Protein Produced

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In the previous papers[1~4] we have selected a yeast strain that produces extracellular protein (ECP), *Trichosporon* sp.X—19, and reported the cultural conditions of the yeast for ECP production.

Many reports 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 et al. with bacteria.[5~11]

Comparing the protein production by yeast with that bacteria, the former may be more advantageous: First, some yeasts have been familiar as fermentative food stuffs, second, the separation process may be easier because of their large cell size.

In this paper we carried out a large scale batch cultivation of *Trichosporon* sp.X—19 using propagation tank, and studied nutritive value of the ECP produced.

We have found that *Trichosporon* sp.X—19, accumulates a considerable amount of protein in culture medium and surface of cells under acidic cultural condition of pH 3.0 and the protein was washed out easily with dilute alkaline solution.[1]

A large scale batch cultivation of *Trichosporon* sp.X—19 was carried out using 800 l propagation tank to obtain ECP for the nutritional estimation. The yeast was precultured in 20 l YM medium (yeast extract 0.3%, peptone 0.5%, and glucose 1.0%, pH 5.0) with jarfermentor for 28hr at 30°C. Five hundred l of Czapeck—Dox modified medium (L—arginine 0.211%, glucose 3.0%, K_2SO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.005%, KCl 0.05% and $FeSO_4$ 0.001%, pH 3.0) in 800 l propagation tank was inoculated with the seed culture and maintained at 30°C with aeration and agitation. The aeration and agitation conditions used were as follows: air flow rate; 100 l/min and revolution number of agitation; 150 rpm. The process of this fermentation is shown in Fig. 1.

Two serise of this fermentation gave 1,000 l of culture broth. A flow chart illustrating the isolation procedure of the ECP is presented in Fig. 2. The properties that the ECP is soluble in alkaline but not in acidic solution were used for this isolation process. Finally 1.38Kg of ECP

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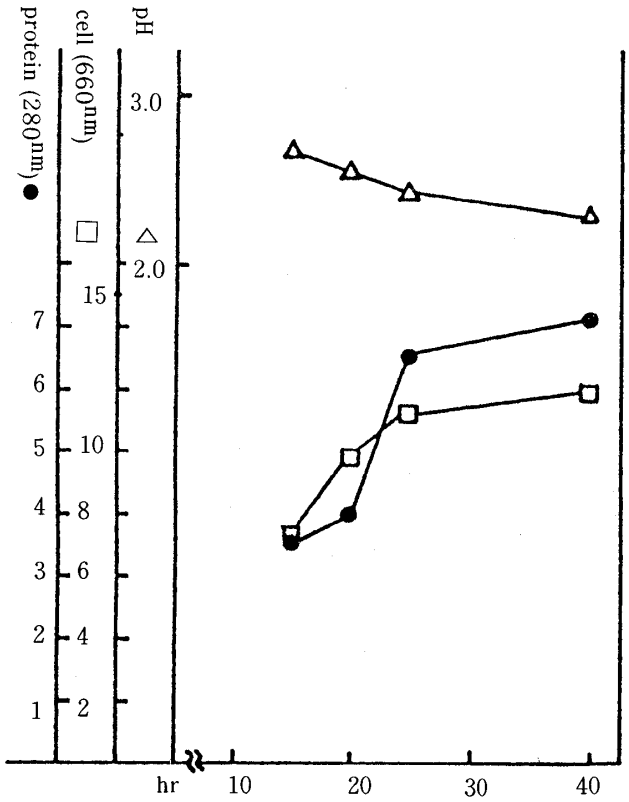

Fig.1. Cultural process of protein production by *Trichosporon* sp. X−19.

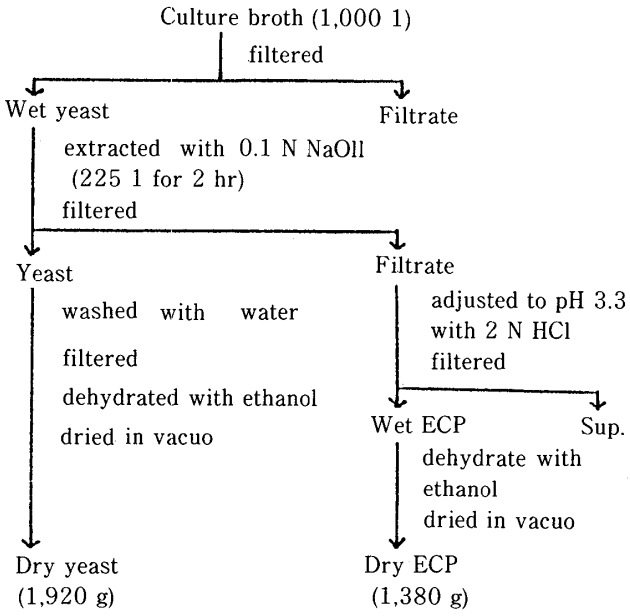

Fig.2. Procedure scheme of protein(ECP) preparation from culture broth of *Trichosporon* sp. X−19.

and 1.92Kg of dry yeast were prepared from the broth with the procedure shown in Fig. 2.

Crude protein (N× 6.25), diethyl ether extractable material (crude fat), crude ash and moisture contents of the ECP were determined by the general procedure described in the AOAC.[12]

Total amino acid content of the ECP was determined by colum chromatograph according to Spackmann et al. using an amino acid analyzer (JLC–3BC) after a 24–hr hydrolysis in 6N–HCl at 110°C.[13] For the determination of cystine, the sample was first oxidized with performic acid before acid hydrolysis.[14] Tryptophan was determined colorimetrically following the procedure of Spies and Chambers.[15] Nucleic acids of the ECP were extracted by the modified Schmidt–Thaunhauser–Schneider method and then DNA and RNA contents were determined by the optical absorption at a wavelength of 260nm and the orcinol method, respectively.[16]

As indicated in Table 1, this ECP preparation contained relatively higher amount of crude protein although it included 16% of nucleic acids. In the present experimens, however, the ECP was used as a protein source without removing nucleic acids.

A total amino acid analysis of the ECP is shown in Table 2. It was shown that except for sulfur–containing amino acids essential amino acid are present in adequate quantities. The protein nutritional quality can be estimated from the amino acid composition. Therefore, the contents of the essential amino acids of the ECP were compared with FAO/WHO provisional recommended pattern (FAO/WHO, 1973). As shown in Table 3, the amino acid score of the ECP was calucu-lated to be 45 and sulfur–containing amino acids was the first–limiting amino acid. Yeast cell are also frequently reported to be deficient in this amino acid.[17]

Table 1. Proximate composition of the ECP (%).

Moisture	8.1
Crude protein (N × 6.25)	80.0*
Crude ash	4.0
Crude fat	trace

*Contained nucleic acids (DNA 0.4% and RNA 15.6%).

Table 2. Amino acid composition of the ECP.

Amino acid	g/N 16g
Threonine	3.82
Valine	4.06
Methionine	0.83
Leucine	5.90
Isoleucine	3.84
Phenylalanine	3.61
Lysine	6.14
Tryptophan	5.81
Aspartic acid	7.37
Serine	3.25
Glutamic acid	7.49
Proline	2.88
Alanine	4.62
Cystine	1.39
Tyrosine	2.97

Table 3. Comparison of essential amino acid pattern of the ECP with that of FAO/WHO (1973) (mg–amino acid/g–N).

Amino acid	FAO/WHO pattern (1973)	ECP	Ratio to FAO/WHO pattern (1973)
Ile	250	261	104
Leu	440	401	91
Lys	340	417	123
Met+Cys	220	99	45
Phe+Tyr	380	514	135
Thr	250	259	104
Trp	60	395	658
Val	310	276	89

The nutritive values of the ECP were estimated by both in vitro digestion tests and rat feeding trials.

Digestion test was carried out by two enzymes, pepsin and trypsin. Fig. 3 shows the in vitro digestibilities of the ECP by the two kinks of enzyme. These in vitro digestion tests showed that

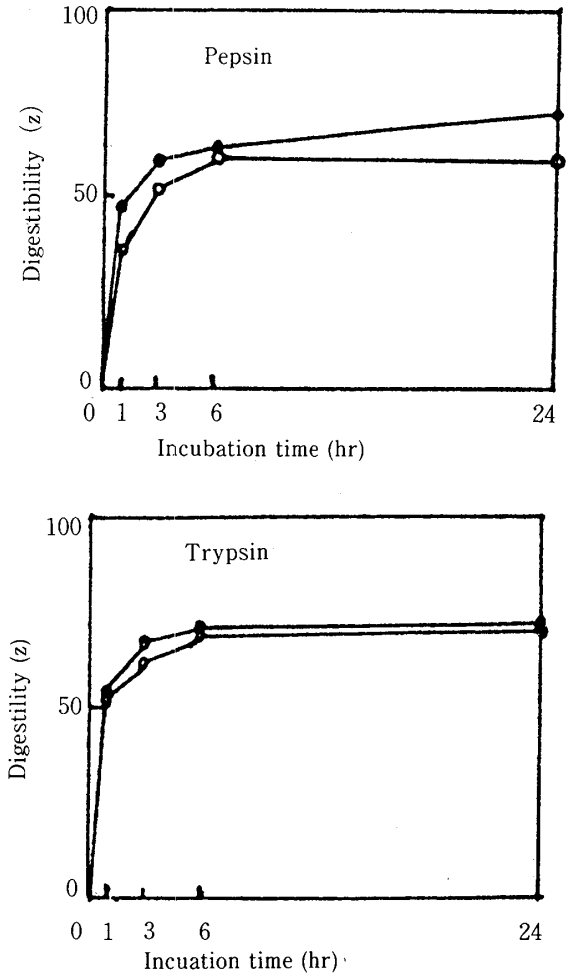

Fig.3. Digestibilities of ECP by pepsin and trypsin.
○ ECP, ● casein

the ECP was a highly digestible protein although the digestibility by pepsin was somewhat lower than that of casein as a reference protein. In addition, the digestibilities by both enzymes reached the maximum values within a fairly short time, 6 hr. These results indicate that the ECP has an excellent quality with regard to digestibility.

In the rat feeding trials, growth rate, protein efficiency ratio (PER), true digestibility (TD), biological value (BV), and net protein utilization (NPU) were estimated with 3 weeks old male rats of the Wister strain. The rats were given the ECP–containing diets at 10 and 25% protein level supplemented 0.5% L–methionine. The growth curves at 10% protein level are shown in Fig. 4. The diet of ECP supplemented methionine is superior than casein and the diet at 25% level gives almost same results as 10% level. PER values of ECP at 10 and 25% protein levels were 0.32 and 0.90, respectively, and were much lower than those of casein. By adding 0.5% of L–methionine to ECP containing diets, however, PER values increased to the value almost equal to those of casein. Though TD of ECP was comparable to that of casein, BV and NPU were much lower than those of casein as shown in Table 4. However, both BV and NPU became similar to those of casein by the supplementation of methionine as in the case of PER.

TD showed no significant difference among the 3 diet groups and the values were extremely

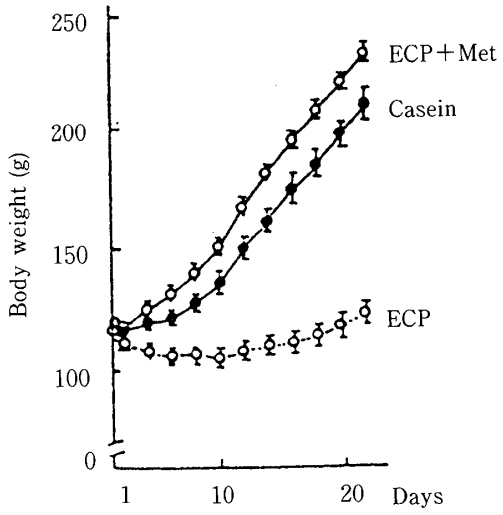

Fig.4. Growth curves of rats.
Each plot represents the mean ± S.D.

Table 4. Nutritonal values of the ECP

Diet	PER	TD	BV	NPU
ECP	0.32 ± 0.16^{a}	92.8 ± 2.1^{a}	42.2 ± 6.0^{a}	39.2 ± 5.6^{a}
ECP+Met	2.84 ± 0.25^{b}	94.3 ± 1.3^{a}	77.1 ± 1.3^{b}	72.4 ± 0.6^{b}
Casein	2.68 ± 0.17^{b}	96.8 ± 1.5^{a}	71.5 ± 2.2^{c}	69.6 ± 1.5^{b}

Each value is the mean±standard deviation (n=5).
a, b, c : Values in the same vertical column not sharing common superscripts are significantly different ($p<0.05$).

higher. These results, with in vitro digestibility data, shown that the ECP is a highly digestible protein. BV and NPU of the ECP were much lower, but improved by the supplementation with methionine to the values comparable to those of casein.

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