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Differentitation of Yeast Species by Techniques of Electrophoresis and Immunodiffusion

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단백질의 전기영동 패턴 및 항체 특성을 이용한 효모의 동정

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Abstract

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunodiffusion method were used for the species differentiation of yeasts, *Saccharomyces cerevisiae, Candida utils, Candida tropicalis,* and *Kleuyveromyces fragilis.* Comparing the electrophoretic patterns of soluble and membrane proteins, *Saccharomyces cerevisiae* was similar to *Candida utilis* but was different from *Candida tropicalis* and *Kleuyveromyces fragilis.* In immunochemical properties of soluble proteins, *Saccharomyces cerevisiae* was almost identical with *Candida utilis.* However, *Saccharomyces cerevisiae* or *Candida utilis* was quite different from *Candida tropicalis* and *Kleuyveromyces fragilis* in their immunoreactivities. In immunochemical properties of membrane proteins, almost the same results were obtained irrespective of four yeast species. By using SDS-PAGE and immunodiffusion methods, *Saccharomyces cerevisiae* and *Candida utilis* were difficult to differentiate but both species were easily differentiated from *Candida tropicalis* and *Kleuyveromyces fragilis.*

Introduction

Because yeast is rich in vitamins and essential amino acids required by man and higher animals, it has been of great commercial value as food, drug, and feed supplements. A great varieties of yeast are encountered in fermentation as well as many other processes and products. However, the species cultivated commercially belong to just 3 out of the 39 genera recognized, *Saccharomyces, Candia,* and *Kleuyveromyces.* Especially, for pharmaceutical purposes, the usage of yeast is legally restricted to the strain of *S. cerevisiae* [1]. Thus, it is necessary to identify the yeast species for the prevention of foreign yeast contaminations in the processes. Until now, it has been a artificial class based on comparative evaluation of morphological, cultural, physiological, and sexual characteristics to distinguish and identify species. These methods are time consuming and can be used only for the live yeasts. As alternative approaches for the detection of contaminating microorganisms in food stuffs, enzyme immunoassays[2-4], chemical method[5], High Pressure Liquid Chromatography[6], and DNA-DNA hybridization methods[7,8] were investigated.

As a preliminary experiments for the rapid identification of a live or dead yeast species, we tried a biochemical and immunochemical

methods. In this paper, first we compared the electrophoretic patterns of both the membrance and soluble proteins of yeasts. And for the rapid and unequivocal identification of the yeast species, immunodiffusion method was introduced.

Materials and Methods

Materials

Saccharomyces cerevisiae ATCC 7752 and *Candida tropicalis* ATCC 20115 were purchased from American Type Culture Collection (ATCC). *Kluyveromyces fragilis* SAFM 1010 was a stock at Byun's Lab and *Candida utilis* was from Jeil Universal Company. N,N,N,N'-tetramethylethylenediamine (TEMED) from Aldrich (Milwaukee, Wis. U.S.A.) and agarose from BRL (Gaithersburg, Md. U.S.A.). Acrylamide, N,N'-methylenebisacrylamide (MBA), SDS molecular weight markers, Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA) were purchased from Sigma Chemicals (St. Louis, Mo. U.S.A.).

Cultivation of yeasts

S. cerevisiae, *C. utilis*, *C. tropicalis* and *K. fragilis* were grown in YPD medium[9]. Four yeast species were grown in a shaking incubator ar 250 rpm 30℃, for 12 hr as a seed culture. Five ml of seed culture was used to inoculate a 2000 ml Erlenmeyer flask containing 400 ml medium and the cells were harvested after 24 hr cultivation at 30℃.

Isolation of soluble and membrance proteins

The isolation and extraction of soluble and membrane proteins from 4 yeast species were conducted by following the procedure described by Christensen and Cirillo[10], as shown in Fig. 1.

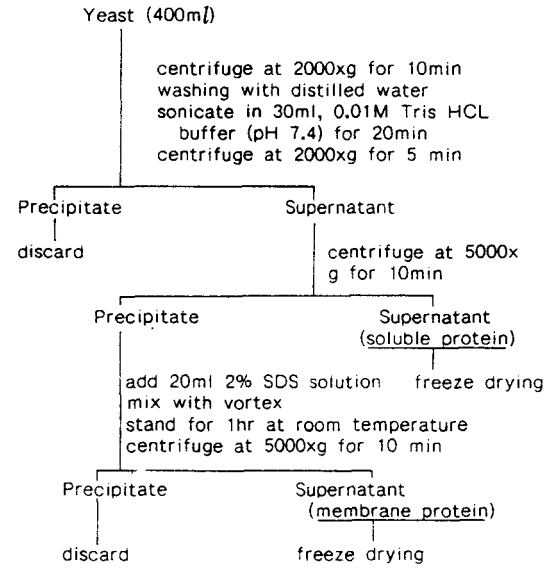

Fig. 1. Flow chart of preparations of soluble protein and membrane protein

SDS-polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) was done according to the methods of Dreyfuss *et al.*[11]. Molecular weight determination of protein bands was done by simultaneous electrophoresis of SDS molecular weight markers from Sigma Chemicals.

Ouchterlony double immunodiffusion

Antibodies were obtained according to the immunization protocols described by Johnston and Thorpe[12]. Antiserum to the soluble and membrane proteins of yeasts were raised in rabbits. For the determination of immunochemical identity of 4 yeast species, double immunodiffusion technique developed by Ouchterlony and Nilsson[13] was followed.

Results and Discussion

SDS-PAGE patterns of 4 yeast species

Polyacrylamide gel electrophoresis was carried

out with the soluble and membrane proteins of 4 yeast species, *S. cerevisiae*, *C. utilis*, *C. tropicalis* and *K. fragilis* in the presence of SDS. Fig. 2-A and B shows the result of the separation of soluble proteins and membrane proteins, respectively, on a 12.5% gel. Soluble protein composition of *S. cerevisiae* was similar to that of *C. utilis* but was different from those of *C. tropicalis* and *K. fragilis*. Most of the soluble proteins of *S. cerevisiae* and *C. utilis* had the low molecular weights of under 45,000 dalton. The soluble proteins of *C. tropicalis* showed more bands specially above the region of molecular weight of 66,000 dalton, when compared the SDS -PAGE patterns of the other yeast species. However, K. fragilis showed much less soluble protein bands as compared with the other yeast species (Fig. 2-A).

Membrane protein compositions of *S. cerevisiae* and *C. utilis* were almost identical. The protein band of molecular weight of about 46,000 dalton was shown in all 4 species of yeast. *S. cerevisiae* and *C. utilis* showed the distinguished protein band at molecular weight of 45,000 dalton. Yeast membrane proteins were separated into more polypeptides as compared with soluble proteins. Plasma membrane polypeptides of yeast have been resolved into approximately 20 polypeptides by one-dimensional SDS -PAGE[14]. In this study, more than 25 bands were separated in membrane protein of all yeast species.

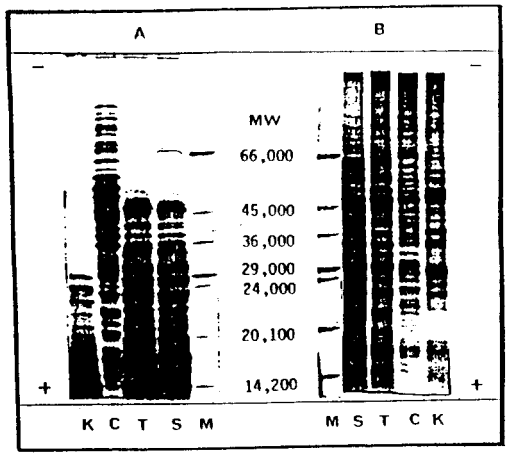

Fig. 2. SDS-polyacrylamide gel electrophoretic patterns of soluble protein(A) and memberane protein(B) S: S. cerevisiae, T: C. utilis, C: C. tropicalis, K: K. fragilis, and M: molecular weight markers

Ouchterlony double immunodiffusion

For the rapid and unequivocal identification of yeast species, double immunodiffusion method, developed by Ouchterlony and Nilsson[13] was used. Fig.3 shows the precipitin line formed between antiserm against *S. cerevisiae* soluble protein and various yeast soluble proteins. Antiserum obtained by immunization of *S. cerevisiae* soluble protein reacted with 3 species of yeast soluble proteins, *S. cerevisiae*, *C. utilis* and *K. fragilis* but did not react with *C. tropicalis* soluble protein. With antiserum of *S. cerevisiae* the soluble proteins of *S. cerevisiae* and *C. utilis* showed more precipitin lines than *C. tropicalis* and *K. fragilis* (Fig. 3-A and B).

When antiserum against *C. utilis* soluble protein was loaded at the center well, the precipitin lines formed was almost identical with Fig.3. When antiserum against *C. tropicalis* soluble protein was loaded at the center well, precipitin line was observed only between the center well and the wells containing *C. tropicalis* soluble protein. These results indicated that immunochemical properties of *S. cerevisiae* and *C. utilis* were very similar and these were clearly distinguished from *C. tropicalis* or *K. fragilis*. Immunochemical similarity of *S. cerevisiae* and *C. utilis* is in agreement with the result of SDS-PAGE.

According to our results described above, *S. cerevisiae*, *C. tropicalis* and *K. fragilis* can be easily distinguished from each other by using only one agar plate with antiserum against *S. cerevisiae*. We arranged the template as shown in Fig.4: center well, antiserum against *S. cerevisiae* soluble protein: well 1, *S. cerevisiae*; well

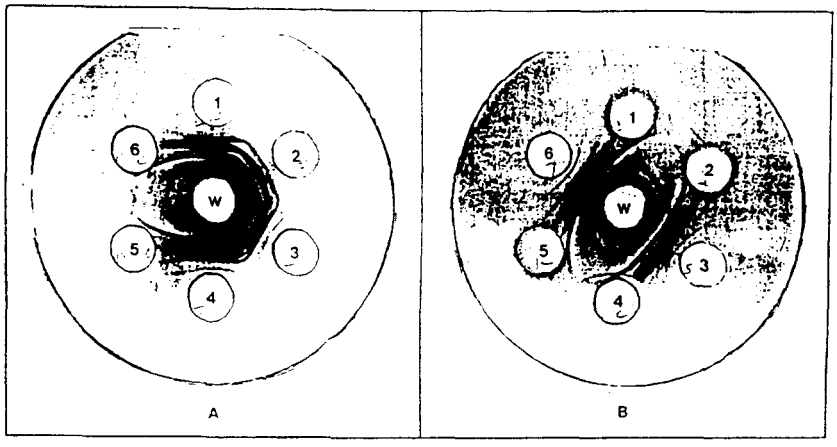

Fig. 3. Precipitation patterns between antiserum against *S. cerevisiae* soluble protein. All the other wells contained 100 mcl of the soluble proteins (6 mg/ ml) indicated

(A); 1 and 2: *S. cerevisiae*, 3 and 4: *C. utilis*, 5 and 6: *C tropicalis*

(B); 1, 2 and 5: *K. fragilis*, 3: *S. cerevisiae*, 4: *C. tropicalis*, 6: *C. utilis*

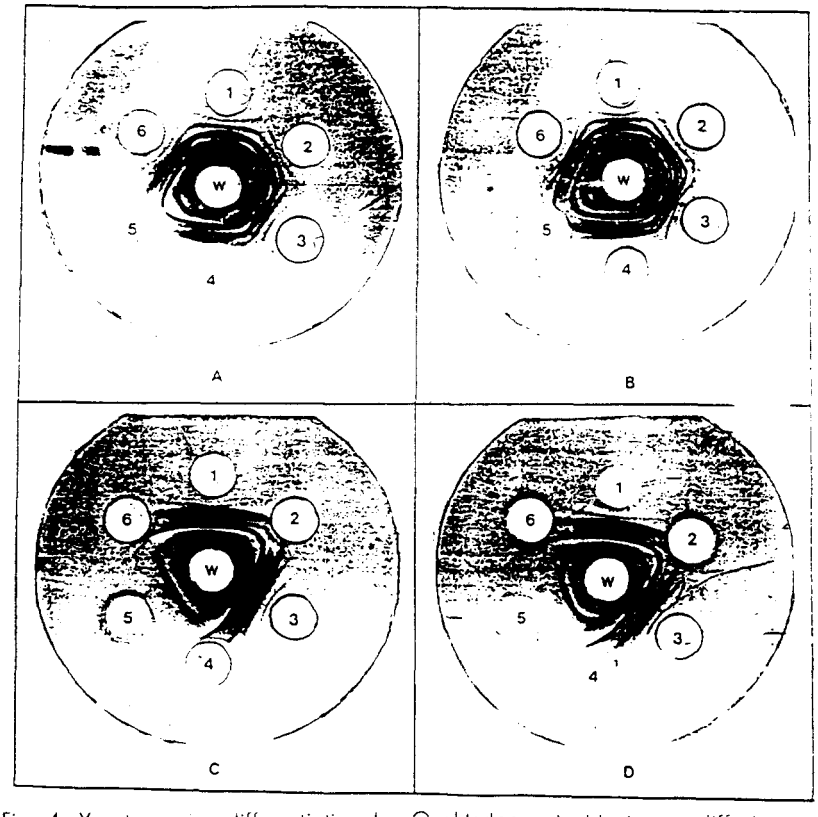

Fig. 4. Yeast species differentiation by Ouchterlony double immunodiffusion

Center well(W) contained 200 mcl of antiserum against *S. cerevisiae* soluble protein. All the other wells contained 100 mcl of the soluble proteins

1: *S. cerevisiae*, 3: *C. utilis*,, 5: *K. fragilis* (A); 2, 4 and 6: *S. cerevisiae*, (B); 2, 4 and 6: *C. utilis* (C); 2, 4 and 6: *C. tropicalis*, (D); 2, 4 and 6: *K. fragilis*.

3, *C. utilis*; well 5, *K. fragilis*; and well 2, 4, 6, soluble protein of testing yeast species. Fig.4-A and B are the results of double immunodiffusion when the testing soluble protein were *S. cerevisiae* and *C. utilis*, respectively. Precipitin lines formed were almost identical. Fig.4-C and D are the results of double immunodiffusion when the testing soluble proteins were *C. tropicalis* and *K. fragilis* respectively. The major difference of Fig.4-C and D is precipitin line formed between center well and well 2, 4, 6. Antiserum to *S. cerevisiae* soluble protein reacted with *K. fragilis* but did not react with *C. tropicalis* soluble protein. Precipitin lines formed between antiserum, obtained by immunization of various yeast membrane protein, and various yeast membrane protein were all the same.

Therefore, it is concluded that *S. cerevisiae* and *C. utilis* were difficult to distinguish but these 2 species were easily differentiated from *C. tropicalis* or *K. fragilis* by both SDS-PAGE and Ouchterlony double immunodiffusion technique.

Acknowledgement

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요 약

본 연구는 효모를 동정하는 방법으로서 각 효모의 단백질 조성 차이와 이들 단백질의 면역학적 특성에 의한 새로운 동정법을 개발하고자 하였다. *S. cerevisiae*, C. utilis, *C. tropicalis*, *K. fragilis* 의 4가지 효모를 배양하여 세포를 파괴시킨 다음 가용성 단백질과 막 단백질을 분리 추출하였다. 4종 효모의 가용성 단백질과 막 단백질의 조성은 SDS-polyacrylamide gel electrophoresis를 실시 비교하였다. *S. cerevisiae* 와 *C. utilis* 는 전기영동 pattern 상 유사하였고 이들은 쉽게 *C. tropicalis*, *K. fragilis* 로부터 구별이 가능하였다. 또한 4종 효모의 가용성 단백질과 막 단백질을 토끼에게 주사하여 각각에 대응하는 항체를 만든 후 Ouchterlony double immunodiffusion을 실시하여 형성된 precipitin line에 의한 효모의 동정을 수행하였다. 면역학적으로도 *S. cerevisiae* 와 *C. utilis* 의 유사성이 증명되었고 이들은 *C. tropicalis*, *K. fragilis* 와 상이함이 관찰되었다.

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