Short Communication

PRODUCTION OF AN ANTISERUM WHICH EFFECTIVELY PICKS UP *TRICHOSPORON BEIGELII* BY USING ITS EXTRACT OBTAINED MECHANICALLY OR AUTOLYSATE

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Strains of *Trichosporon beigelii* have been isolated from various materials. KING and JONG (1) pointed out that it would be clarified that such a cosmopolitan yeast comprised several taxa when suitable examination techniques were adopted. YAMADA et al. (2) examined the ubiquinone system of *T. beigelii* and showed that there were strains of Q9 and Q10.

Though serological methods are effective for discrimination of some yeasts, SEELIGER and SCHRÖTER (3) and TORHEIM (4) reported that it was difficult to get a high-titred antiserum that specifically agglutinated *T. beigelii*.

The authors also experienced that rabbits which were injected with killed cells after an ordinary schedule of immunization fell in a critical condition before an appreciable amount of agglutinating antibody appeared in blood and therefore it was considered that it might be impossible to apply usual methods to *T. beigelii*.

Antigenicity of some serum-proteins of warm-blooded animal is species-specific and the specificity is useful for discrimination of species. On the other hand, a rabbit antiserum against hen egg white albumin cross-reacted with duck egg white albumin, but anti-hen serum precipitated lesser amount of duck albumin. The amount of the precipitate of homologous protein is expected to give some information about immunological distances.

It was demonstrated by AXELSEN (5) that cytoplasmic substances of *Candida albicans* were a good immunogen and antigen. Moreover, GUINET et al. (6) showed that antisera prepared after AXELSEN were effective for learning the relatedness of *C. albicans* to *C. tropicalis*. We tried to prepare the antisera against the cytoplasmic extract of *T. beigelii* whose surface compounds had been proved to be a poor immunogen.

One ml of the concentrated extract (protein 10 mg/ml) of *T. beigelii* SH 204

Organism			Strain	Source	Precipitation lines with disrupted antigen													
					Antiserum A						Antiserum B							
					a	b	b'	c	d	e	f	a	b	b'	c	d	e	f
<i>T. beigelii</i>			SH 204	IFO	1200													
"			SH 211	IAM	12250													
"			SH 216	IAM	12246													
"			SH 284	IFO	0173													
"			SH 287	IFO	1198													
"			SH 288	IFO	1200													
"			SH 291	IFO	1500													
Group I																		
"			SH 283	IFO	0116													
"			SH 285	IFO	0174													
"			SH 286	IFO	0598													
"			SH 289	IFO	1202													
"			SH 290	IFO	1489													
"			SH 215	IAM	12206													
"			SH 207	MP	79-4a													
"			SH 203	MP	79-6a													
Group II																		
<i>T. pullulans</i>			SH 293	IFO	0114													
"			SH 294	IFO	1232													
<i>C. curvata</i>			SH 201	IAM	12183													
<i>Cr. albidus</i> var. <i>aerius</i>			SH 228	IAM	4317													
<i>Cr. laurentii</i>			SH 212	IAM	12264													
<i>Cr. luteolus</i>			SH 202	IAM	12207													
<i>Cr. neoformans</i>			SH 213	IAM	12253													
<i>Sp. odoratus</i>			SH 209	IAM	12258													
<i>Sp. salmonicolor</i>			SH 208	IAM	12249													
<i>Cr. himalayensis</i>			SH 222	IAM	4963													
<i>R. rubra</i>			SH 227	IAM	4938													
<i>T. brassicae</i>			SH 280	IFO	1584													
<i>G. candidum</i>			SH 234	RIFY	No. 16													
<i>G. fici</i>			SH 237	RIFY	F-2-9													
<i>G. rectangulatum</i>			SH 236	RIFY	B91-1													
<i>G. sp</i>			SH 276	AJ	4941													
<i>Deb. hansenii</i>			SH 254	IAM	4978													
<i>H. fabianii</i>			SH 239	IAM	12212													
<i>H. petersonii</i>			SH 241	IAM	12216													
<i>H. silvicola</i>			SH 243	IAM	12218													
<i>N. elongata</i>			SH 232	IAM	4973													
<i>P. pinus</i>			SH 248	IAM	12224													
<i>S. bisporus</i>			SH 268	IAM	12235													

Fig. 1. The results of gel diffusion precipitation of *T. beigeli* and related species.

Antiserum A=against the disrupted extract of *T. beigeli* SH 204, Antiserum B=against the autolysate of *T. beigeli* SH 204, ↓=short and weak precipitation lines.

(IFO 1200—CBS 2530) prepared from the cells grown in Sabouraud dextrose broth on a reciprocal shaker by mechanical disruption with Yeda press (Yeda Research and Development Co., Ltd., Israel) was emulsified with 1 ml of Freund's complete adjuvant. Sera of rabbits which were injected with the emulsion three times at intervals of three weeks gave six precipitation lines in gel-diffusion test with the homologous antigen. The results obtained when the sera were reacted with the disrupted cell extracts of various yeasts are shown in Fig. 1. Only the strains which were designated as *T. beigeli* gave two or more precipitation lines. They may be divided tentatively into two groups according to the number of precipitation lines. Two strains of *T. pullulans* gave only one sharp precipitation line which linked with one of the lines given by *T. beigeli*.

Seven strains, from *Candida curvata* SH 201 to *Sporobolomyces salmonicolor* SH 208, gave a short and vague precipitation line respectively between the serum

well and the antigen well. However, they were too short and vague to know the relationship between them and those of *T. beigelii*.

Other yeasts of the genus *Trichosporon* not shown in Fig. 1 including *T. capitatum*, and other yeasts of 65 species belonging to 19 genera, gave no precipitation line. These yeasts will be listed in another paper.

The antisera scarcely agglutinated living and killed cells of all tested strains of *T. beigelii*. Number of precipitation lines did not vary after the sera were absorbed by living or killed cells of *T. beigelii*. The extracts were shown to give no precipitation line after heating or treating with pronase.

Though the antisera can effectively pick up the strains of *T. beigelii* when their extracts are used as antigen, it is usually difficult to get an extract of yeast without using some appropriate apparatus. Yeda press cannot give a satisfactory yield of soluble proteins. Thus a laboratory devoid of facilities for disruption cannot try the effectiveness of the sera. As some commercial yeast enzymes are solubilized with the process of autolysis, isolated, and purified, if the antigenicity of the cytoplasmic proteins is not damaged by the autolysis, the antisera are expected to be utilized widely. The extracts of 10 strains of *T. beigelii* obtained by autolysis for 48 hr at room temperature under toluene layer in respective glass centrifuge tubes gave lesser precipitation lines than Yeda press extracts but their patterns were similar. It is clear that the autolysis does not at least damage the antigenicity of some cytoplasmic proteins of *T. beigelii*.

Moreover, if the autolysate is proved to be effective as an immunogen, serological method utilizing cytoplasmic substances may be adopted widely. The good antisera were obtained from rabbits given the autolysate of *T. beigelii* SH 204, and the results of reactions between the sera and the extracts of various yeasts are shown also in Fig. 1. The range of yeasts which gave short and vague precipitation line extended and some ascomycetous yeasts were included. But it is clear that the sera can discriminate the strains of *T. beigelii* well.

Hereafter, it is expected that serological studies of yeasts not based on antigenicity of surface substances might make a contribution to identification and classification of yeasts. Moreover, if it might be ascertained that the antigenicity and immunogenicity of the cytoplasmic proteins of many yeasts might not be damaged during the process of autolysis, the method would be more easily practicable.

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