Effect of Protease Inhibitor (S-PI) on the Growth of Various Yeasts

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An acid protease inhibitor (S-PI*) inhibited various acid proteases. In previous papers,^{1,2)} we reported that S-PI inhibited the growth of *Rhodotorula* at higher temperature, but accelerated it at lower temperature at pH 2.8. This paper describes the effect of S-PI on the growth of various yeasts.

Yeast cells were cultured in S-PI free and S-PI added media containing 7% glucose, 2% polypepton, 0.6% KH_2PO_4 , 0.08% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 4 ppm $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 4 ppm $\text{MnSO}_4 \cdot 6\text{H}_2\text{O}$, 2 ppm thiamine HCl and 0.1% yeast extract at 25°C for 96 hr. The concentration of S-PI in the medium was 60 ppm. The growth of yeast was followed by OD at 660 nm. Fifty nine strains of yeasts were used in this experiment. These strains were stocked cultures in our laboratory.

Figure 1 shows the effect of S-PI on the growth of 4 strains of yeasts. The growth acceleration by S-PI was observed evidently. But the optimum pHs for the growth acceleration were different from each other. The strains whose growth were accelerated by S-PI are listed in Table I. It is evident that S-PI was effective on the acceleration of growth of various yeasts. On the other hand, 7 strains of *Debaryomyces*, 10 strains of *Saccharomyces*, 1 strain of *Lipomyces* etc.

were not effective with S-PI. But taking into consideration that acceleration of growth, caused by S-PI, was related to the cultivation temperature,¹⁾ S-PI might be effective to these strains in some conditions.

The extracellular acid protease production of various yeasts was investigated by Murao *et al.*³⁾ and they found that *Rhodotorula glutinis* K-24 produced a large amount of extracellular acid protease when the yeast grew below pH 4.5, and maximum formation occurred in the cultivation of initial pH 2.8. The physiological changes induced by S-PI addition were observed under the same conditions as the maximum extracellular acid protease production. Thus there seemed to exist some relation between the inhibition of the extracellular acid protease and the physiological changes of the cells. However, in this experiment, S-PI accelerated the cell growth of various yeasts independently of their extracellular protease production. Therefore, it might be reasonable to conclude that physiological changes induced by S-PI was not directly related to the extracellular acid protease production. On the other hand, the intracellular acid proteases play an important role in the yeast growth.⁴⁻⁶⁾ The physiological changes of yeast caused by S-PI might be related to the intracellular acid protease and this problem is now under investigation and will be reported elsewhere.

REFERENCES

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Fig. 1. Effect of S-PI on Growth of Various Yeasts.

Yeast cells were grown in S-PI free and S-PI added medium at 25°C for 96 hr.

A, *Candida japonica* IFO 1119; B, *Candida salmonicola* AJ 4755; C, *Saccharomyces fermentati* IAM 4771; D, *Cryptococcus terreus* IFO 0727. ○, S-PI free medium; ●, S-PI added medium.

* S-PI is the same to Acetyl Pepstatin reported by Aoyagi *et al.*⁶⁾

TABLE I. EFFECT OF S-PI ON THE GROWTH OF VARIOUS YEASTS

The yeast was cultured in S-PI free and S-PI added media at 25°C for 96 hr. And then the growth of yeast in the both media were compared. The productibility of extracellular acid protease by yeast are listed for reference.

Strain	Growth acceleration caused by S-PI					Acid protease production ³⁾
	Initial pH of medium					
	2.8	3.8	4.8	5.8	6.8	
<i>Encomycopsis fibliger</i> IFO 0104	+	++	—	—	—	yes
<i>Saccharomyces fermentati</i> IAM 4771	—	++	+	—	—	no
<i>Hansenula dimennae</i> NRRL YB-3239	—	—	++	+	—	no
<i>Hansenula californica</i> NRRL Y-1680	—	+	—	—	—	no
<i>Cryptococcus neoformans</i> IFO 0699	—	—	+	+	+	no
<i>Cryptococcus terreus</i> IFO 0727	—	—	++	+	—	no
<i>Candida humicola</i> IFO 0753	—	—	—	+	—	no
<i>Candida zeylanoides</i> ATCC 7351	—	+	++	—	—	no
<i>Candida salmonicola</i> AJ 4755	+	++	+	—	—	no
<i>Candida japonica</i> IFO 1119	—	—	++	++	—	no
<i>Tricosporon penicillatum</i> AJ 5106	+	++	+	—	—	no
<i>Tricosporon fermentaus</i> IFO 1199	—	—	+	+	—	no
<i>Rhodotorula peneaus</i> IFO 0930	—	+	+	+	—	no
<i>Rhodotorula rubra</i> IFO 0832	—	++	+	—	—	yes
<i>Rhodotorula glutinis</i> K-24	++	+	—	—	—	yes
<i>Rhodosporidium diobovatum</i> AJ 14179	—	—	++	+	—	
<i>Rhodosporidium sphaerocarpum</i> AJ 14147	—	+	+	—	—	

+: Growth acceleration was detected.

—: Growth acceleration was not detected.

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