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Possible Competition between *S. uvarum* and *Z. mobilis*

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*Saccharomyces uvarum*과 *Zymomonas mobilis*간의 경쟁적 상호작용

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Abstract

Competition between *Saccharomyces uvarum* and *Zymomonas mobilis* in a product-limited continuous culture was investigated at pH 5.0. It was evident that *Z. mobilis* replaced *S. uvarum* completely due to higher enthanol tolerance with *Z. mobilis*.

Introduction

Competition between two microbial populations in a chemostat for a single growth limiting substrate depe-ndes on the relationship between specific growth rate (μ) and substrate concentration (S) of the particular microorganisms. Under certain conditions, one of the competitors may begin to wash out[1~3].

Competition between *Saccharomyces uvarum* and *Zymomonas mobilis* is of a special case in which both microorganisms produce a common autoinhibitor: ethanol that is produced by both microorganisms inhibit the growth of both. The kinetics of both microoganisms have been extensively studied in batch and continuous cultures[4,5] and it has been found that a linear relationship existed between specific growth rate and ethanol concentration for both *S. uvarum* and *Z. mobilis*. However the degree of ethanol inhibition was found to be different.

So far no experimental evidencce has been reported for the case of a mixed culture interaction between *S. uvarum* and *Z. mobilis* in which both are subjected to ethanol inhibition. This was investigated in the present research in a product-limited continuous culture.

Materials and Methods

Organisms and media

The strains used in the evaluation were *S. uvarum* *ATCC* 26602[4] and *Z. mobilis* ZM4[5]. These strains were maintained separately by transferring to fresh agar plates each week and storing at 4°C. The composition of the media per liter was as follows: 150*g* glucose; 5*g* yeast extract (Oxoid); 1*g* $(NH_4)_2SO_4$; 2*g* KH_2PO_4; 1*g* $MgSO_4 \cdot 7H_2O$. Media were sterilized by membrane filtration[6].

Experimental procedure

A strain of *Z. mobilis* was grown in a 1*l* fermentor [7] until it was in the exponential growth phase. Then an inoculum of *S. uvarum* was added to a final concentration of 3.2×10^7 cells/*ml*. Fresh medium was then introduced at a dilution rate of $D=0.15\ h^{-1}$. The time of fresh feed addition is shown as 0hr in Fig. 1.

Analytical methods

S. uvarvm and *Z. mobilis* populations were counted by means of a phase-contrast microscope using a Hawksley haemocytometer of 0.1 *mm* depth for *S. uvarum* and a Thoma haemocytometer of 0.02 *mm* depth for *Z. mobilis*. Correlation factors between total cell numbers and dry weights were made for both strains. There were $3.0\pm0.2\times10^7$ cells/*g* for *S. uvarum* and $1.2\pm0.02\times10^9$ cells/*g* for *Z. mobilis*.

The residual glucose was estimated on the supernatant of a sample after centrifugation (4000 rpm, 10 min) using the dinitrosalicylic acid method[8]. For ethanol estimation, samples were analyzed using a Technicon Autoanalyzer and a procedure developed by Sawyer and Dixon[9].

Results and Discussion

In Fig 1 competition between *S. uvarum* and *Z. mobilis* in a product-limited continuous culture is shown. As can be seen from Fig. 1, *S. uvarum* began to wash out immediately following feed addition. Following an initial decline the *Z. mobilis* population did however increase after 5h eventually completely displacing *S. uvarum*. After 1 day, *S. uvarum* washed out to less than 15% of its initial density while *Z. mobilis* population was maintained at a level of 3×10^9 cells/*ml* which corresponded to a dry weight of 2.5 *g/l*. These results were verified in replicate studies.

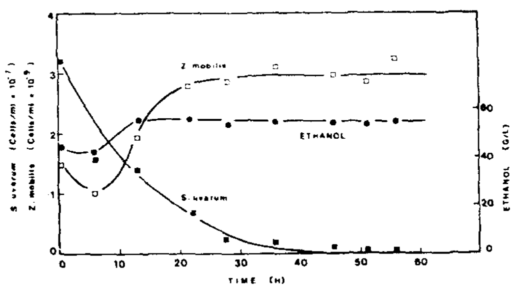

Fig. 1. Competition between *S. uvarum* and *Z. mobilis* in product-limited continuous culture at pH 5.0 and 33°C

Theoretical analyses have been reported for the case of competition for a single growth limiting substrate between two populations which produced a common inhibitor, and various possibillities have been shown.[10,11] In the present investigation, however, the specific growth rates of both strains were controlled not by substrate limitation but by product inhibition because the residual glucose in the fermentor was in excess(viz. 40*g/l*). As shown in Fig. 2 which shows the effect of dilution rate on ethanol concentration at steady-state with pure cultures of *S. uvarum*, the specific growth rate of both strains was linearly affected by ethanol concentration within the range studied. Thus mathematical equations for this situation are given by:

$$\frac{dX_i}{dt}=\mu_i\cdot X_i-D\cdot X_i,\quad i=1,2 \qquad (1)$$

$$\mu_i=\mu_{mi}\cdot\left(1-\frac{P}{P_{mi}}\right),\quad i=1,2 \qquad (2)$$

The solution of equation (1) with an initial condition of $X_i(0)=X_{0i}$ is given by:

$$X_i(t)=X_{0i}\cdot \mathrm{Exp}(\mu_i-D)\cdot t \qquad (3)$$

Consequently X_i decreases if the sepecific growth rate is smaller than the fixed dilution rate in a product-limited continuous culture.

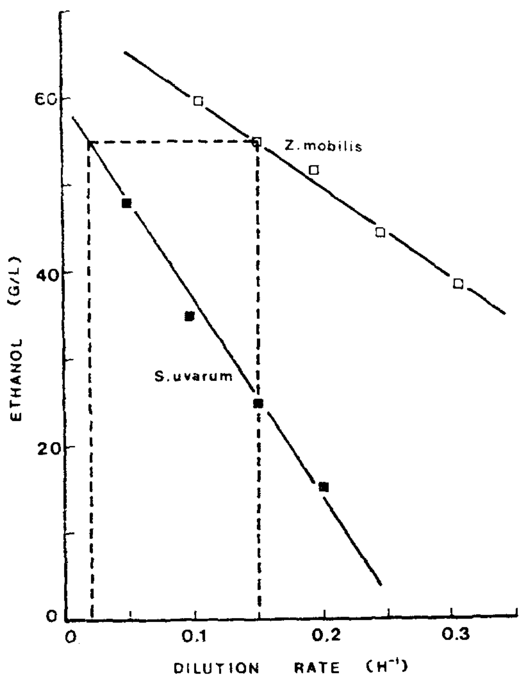

Fig. 2. Effect of dilution rate on ethanol concentration with pure cultures of *S. uvarum* and *Z. mobilis* at pH 5.0 and 33°C

As shown in Fig. 2, ethanol concentration of 55g/l was obtained with *Z. mobilis* at $D=0.15h^{-1}$. However, the specific growth rate of *S. uvarum* at the same ethanol concentration was determind to be 0.02 h^{-1} which was much smaller than the fixed dilution rate of $D=0.15\ h^{-1}$. Therefore, *S. uvarum* washed out according to equation (3). Since steady-state data of ethanol concentration for *Z. mobilis* were always higher than those for *S. uvarum* at all dilution rates at pH5.0 as shown in Fig. 2, it is clear that there is no fear of contamination by *S. uvarum* in a product-limited continuous culture.

It should be mentioned that although *Z. mobilis* replaced *S. uvarum* at pH 5.0 the reverse was evident at pH 4.0. As the strain of *Z. mobilis* was found to be quite sensitive to pH, there was no growth below pH 3.6. For most strains of yeast, however, the absolute limits of pH for growth have been reported to be 2.4 and 8.6[12]. It appears that the wide pH range for growth results in the survival of yeast in the chemostat at the lower pH.

Nomenclature

D: dilution rate, 1/h

P: ethanol concentration, g/l

P_m: maximum ethanol concentration above which cells do not grow, g/l

S: substrate concentration, g/l

t: time, h

X: biomass concentration, g/l

μ: specific growth rate, 1/h

μ_m: maximum specific growth rate, 1/h

Acknowledgments

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요 약

발효로부터 생성되는 에타놀에 의해서 균체성장속도가 제한되는 연속식 배양 시스템에서, 에타놀 생성균주인 *Saccharomyces uvarum*과 *Zymomonas mobilis* 간의 경쟁적 상호 작용을 연구한 결과 *Zymomonas mobilis*가 *Saccharomyces uvarum*에 비하여 에타놀에 대한 耐性이 크기 때문에 *Zymomonas mobilis* 만이 생존할 수 있다.

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