Short Communication

EFFECTS OF Zn^{2+} AND Mn^{2+} ON THE GROWTH OF GENERA *LIPOMYCES* AND *WALTOMYCES*

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Zn^{2+} and Mn^{2+} are essential trace elements for most or all animals, plants and microorganisms, and these ions catalyze enzymes in almost all metabolism (4, 5, 17, 21). Thus, Zn^{2+} and Mn^{2+} are usually added in defined media for the cultivation of microorganisms (3). Control of the metabolism in microorganisms has often been attempted by modifying the concentration of these ions (1, 2, 6–9, 15, 16, 18, 19, 22). We have reported that the growth and lipid accumulation of *Lipomyces starkeyi* IAM 4753, fat yeast, were especially affected by the concentrations of Zn^{2+} and Mn^{2+} in the medium (11–14). One of the two kinds of defined media which was developed in these experiments contained large quantities of Zn^{2+} and Mn^{2+} , and this medium stimulated the total growth of *L. starkeyi* IAM 4753. Another medium with a low concentration of Zn^{2+} and without Mn^{2+} depressed the total growth. These results are of special interest where concentrations of Zn^{2+} and Mn^{2+} affecting the total growth is a special characteristic of *L. starkeyi* IAM 4753 or is a common characteristic of the genus *Lipomyces*.

Here we report the growth of most of the species of *Lipomyces*, *Waltomyces*, and some other yeasts sampled at random for reference, in the defined media with different concentrations of Zn^{2+} and Mn^{2+} , and the degree of sensitivity of yeasts to these ions was investigated.

Yeasts examined in this report are shown in Table 1. A seed culture was prepared as described previously (10), and each yeast was grown aerobically on a reciprocating shaker. The cultivation temperature of yeasts, except *Waltomyces lipofer*, was 29.5°C; *W. lipofer* was grown at 25.0°C.

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The two kinds of media used for this experiment had the same composition except for the concentrations of Zn^{2+} , Mn^{2+} , and monopotassium phosphate. The common composition of the media was: D-glucose, 30 g; $(\text{NH}_4)_2\text{SO}_4$, 5.3 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 g; NaCl, 0.1 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 2.42 mg; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 18.8 μg ; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 3.2 μg ; KI, 13.0 μg ; H_3BO_3 , 42.9 μg ; D-biotin, 0.2 μg ; in 1 l of deionized and distilled water. In one of the media (named LS medium), 4.1 $\mu\text{g/l}$ of Zn^{2+} (with $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and 1.0 g/l of KH_2PO_4 were supplied. Another medium (named CG-P medium) contained 152.3 $\mu\text{g/l}$ of Zn^{2+} (with $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), 21.9 mg/l of Mn^{2+} (with $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$) and 40.0 g/l of KH_2PO_4 . The purpose of supplying a large amount of monopotassium phosphate was to prevent a decrease in the pH of the medium, and it prolonged the physiological effects of Zn^{2+} and Mn^{2+} on the growth of *L. starkeyi* IAM 4753 (13). The pH values of the media were adjusted to 4.5 with 0.2 N KOH before filling up. A 500-ml shaking flask containing 100 ml of the medium was plugged with cotton and was autoclaved at 115°C for 10 min.

Growth was measured by directly counting the cell number using a hemacytometer under a microscope (10).

Figure 1 shows the growth curve of *L. starkeyi* CBS 6132 in which the total growth at the stationary phase was typically affected by the concentrations of Zn^{2+} and Mn^{2+} . There was no difference between growth rates in LS medium (low concentration of Zn^{2+} and without Mn^{2+}) and in CG-P medium (high concentrations of Zn^{2+} and Mn^{2+}) until about 40 h of the cultivation. From then the cell number in the LS medium increased slightly but that in CG-P medium

Fig. 1. Growth of *Lipomyces starkeyi* CBS 6132 grown in media with different concentrations of Zn^{2+} and Mn^{2+} .

○: LS medium with a low concentration of Zn^{2+} (4.1 $\mu\text{g/l}$) and without Mn^{2+} , ●: CG-P medium with high concentrations of Zn^{2+} (152.3 $\mu\text{g/l}$), Mn^{2+} (21.9 mg/l), and monopotassium phosphate (40 g/l) as a buffer chemical.

Table 1. Total growth of yeasts in defined media with different concentrations of Zn²⁺ and Mn²⁺.

Yeasts		Cells ($\times 10^7$ /ml)		Relative growth	
		LS medium	CG-P medium	LS medium	CG-P medium
<i>Lipomyces starkeyi</i>	CBS 1807	2.2	57.2	1	26.0
	CBS 1809	2.2	47.5	1	21.6
	CBS 2512	2.0	24.7	1	12.4
	CBS 6047	2.1	34.3	1	16.3
	CBS 6132	2.3	59.6	1	25.9
	IFO 0678	2.2	62.9	1	28.6
	KW-4	1.6	30.8	1	19.3
	IAM 4753	1.9	54.4	1	28.6
<i>Lipomyces tetrasporus</i>	CBS 1808	4.9	74.5	1	15.2
	CBS 1810	5.1	51.7	1	10.1
	CBS 2511	2.9	34.5	1	11.9
	CBS 5607	4.2	31.4	1	7.5
	CBS 5910	3.0	42.8	1	14.3
	CBS 6048	3.3	41.5	1	12.6
	JCM 6005	6.7	49.9	1	7.5
	JCM 6006	3.3	59.1	1	17.9
	JCM 6009	6.8	70.1	1	10.3
	JCM 6010	6.5	60.8	1	9.4
<i>Lipomyces kononenkoae</i>	KW-3	6.5	35.5	1	5.5
	JCM 5989	10.0	60.5	1	6.1
	JCM 5990	5.0	40.3	1	8.1
<i>Waltomyces lipofer</i>	CBS 944	3.3	84.4	1	25.6
	CBS 2513	4.2	52.9	1	12.6
	CBS 5841	4.0	90.2	1	22.6
	CBS 5842	4.8	93.5	1	19.5
<i>Hansenula saturnus</i>	R 263	13.8	80.8	1	5.9
<i>capsulata</i>	IFO 0984	2.1	18.4	1	8.8
<i>Candida parapsilosis</i>	AJ 4575	19.5	189.0	1	9.7
<i>valida</i>	K 504	19.2	59.5	1	3.1
<i>Saccharomyces cerevisiae</i>	IAM 4274	8.2	22.2	1	2.7
<i>Torulaspora delbrueckii</i>	IAM 1994	8.4	18.7	1	2.2
<i>Saccharomycodes ludwigii</i>	IFO 1043	1.8	1.8	1	1.0
<i>Cryptococcus albidus</i>	IFO 1320	3.5	152.0	1	43.4

Samples were taken at the stationary phase of growth. LS medium with a low concentration of Zn²⁺ (4.1 μ g/l) and without Mn²⁺. CG-P medium with high concentrations of Zn²⁺ (152.3 μ g/l), Mn²⁺ (21.9 mg/l), and monopotassium phosphate (40 g/l) as a buffer chemical.

increased continuously. The total growth in CG-P medium was about 25.9 times that in LS medium (Table 1). Growth curves of all strains in *Lipomyces* grown in the two media were similar to the curve for *L. starkeyi* CBS 6132 (Fig. 1). However, total growth in LS medium and CG-P medium differed according to the strains (Table 1). The means of the relative growth (CG-P medium/LS medium) of strains in each species were: *L. starkeyi*, 22.3; *L. tetrasporus*, 11.7; *L. kononenkoae*, 6.6.

In all strains of *Waltomyces* (*Lipomyces*) *lipofer* (20), growth curves in the two media were similar to that of *L. starkeyi* CBS 6132 (Fig. 1). The mean of the relative growth of strains in this genus was 20.1 (Table 1).

The total growth of *Hansenula saturnus* R 263, *Hansenula capsulata* IFO 0984, *Candida parapsilosis* AJ 4575, and *Cryptococcus albidus* IFO 1320 were also affected by the concentrations of Zn^{2+} and Mn^{2+} , similar to the genera *Lipomyces* and *Waltomyces* (Table 1). In *Cry. albidus* IFO 1320, particularly, the relative growth was above 43-fold and the strain was the most sensitive to Zn^{2+} and Mn^{2+} of all strains tested in this study (Table 1).

The total growth of *Saccharomyces cerevisiae* IAM 4274, *Torulaspora delbrueckii* IAM 1994, and *Candida valida* K 504 were affected by the concentrations of Zn^{2+} and Mn^{2+} in the medium, but the effect was less than in the genera *Lipomyces* and *Waltomyces* (Table 1). Only *Saccharomycodes ludwigii* IFO 1043 of the 33 strains examined in this experiment was not influenced by the concentrations of Zn^{2+} and Mn^{2+} in the medium at the stationary phase of growth (Table 1).

Apparently Zn^{2+} and Mn^{2+} concentrations in a medium affect the total growth of many yeasts. Further investigations are required to show the classification of the sensitivities of yeast growth to Zn^{2+} and Mn^{2+} . However, the data in this paper indicate that the total growth of most strains in the genera *Lipomyces* and *Waltomyces* are highly sensitive to the concentrations of Zn^{2+} and Mn^{2+} in the medium.

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