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Removal of nitrogen by Antarctic yeast cells at low temperature

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Abstract: Nitrate removal in a medium (at 5°C) and the effect of culture temperature on the fatty acid composition were investigated using *Candida* sp. which was isolated from the upper layer of Lake Vanda in the McMurdo Dry Valleys, Antarctica. The strain was cultured at 5°C aerobically, on a synthetic medium containing potassium nitrate (NO_3-N, 100 mg l^{-1}) as a nitrogen source, and examined the effects of pH and chlorine on growth and NO_3-N removal in the medium. Within the pH of 3 to 7 the yeast cells exhibited a similar removal of nitrate level. The strain grew well and also removed nitrate at chlorine concentrations of 5 and 10 mg l^{-1} but did not grow at chlorine concentration of 20 mg l^{-1}. Decreasing the growth temperature induced an increase in the content of linolenic acid (18 : 3) in the yeast cells.

key words: *Candida,* fatty acid, nitrate, psychrophilic, wastewater

Introduction

Nitrate increase in ground water is becoming an important problem in water supply. Increase of nitrate concentration in ground water may be a result of fertilization of soil in agriculture. Anion exchange resins are widely used to remove nitrate in contaminated water, but regeneration of the resins produces nitrate-rich wastewater. The use of yeast in biological wastewater treatment is advantageous because yeast grows well at low pH, reduces organic matter, and may be able to recover biomass. Several yeast strains have been researched in regard to the biological treatments of industrial and domestic wastewater (Thanh and Simard, 1973; Senjyu *et al.*, 1989; Ohno *et al.*, 1991). Most biological treatments of wastewater were performed at room temperatures (20–28°C). However, little is known about the biological treatment of wastewater at low temperatures.

In previous papers, we reported the treatment of dissolved organic matter at low temperatures (0 and 5°C) by the psychrophylic yeast *Candida* sp. which was isolated from water samples from Lake Vanda in Antarctica (Katayama-Hirayama *et al.*, 1997), and also reported the results of a basal experiment on nitrate removal in the presence of NaCl at low temperature (5°C) using *Candida* sp. (Katayama-Hirayama *et al.*, 1998).

In the conventional wastewater treatment system using yeasts, addition of chlorine to the raw wastewater before the yeast treatment tank and pH control in the treatment

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were carried out, in order to maintain the domination of the strain and to avoid bacterial contamination (Senjyu *et al.*, 1989; Ohno *et al.*, 1991). Here we describe the results of a basal experiment using an Antarctic yeast *Candida* sp. on the removal of various nitrogen sources, the effect of pH and chlorine on the growth and nitrate removal at 5°C, and the effect of temperature on the fatty acid composition of *Candida* sp. to investigate the psychrophilic property.

Materials and methods

Candida sp. and the culture

Candida sp. isolated from the upper layer of Lake Vanda in the McMurdo Dry Valleys in Antarctica was used in this study. The strain was kindly offered by Mr. J. Nishikawa, Science University of Tokyo. The physiological characteristics of *Candida* sp. are as follows: DNase activity (+), nitrate utilization (+), sensitivity for cycloheximide (+), splitting of arbutin (−), starch test (−), vitamin requirement (−), acid formation (+), NaCl tolerance 0–5% (w/v) (+), 50% of glucose (+), splitting of fat (+), ester production (−), DBB reaction (−), gelatin liquefaction (−) and urease activity (−). It had fermentable activity and produced acid substances from various types of sugar, but produced none from lactose (Nagashima *et al.*, 1990). The stock cultures of the strain were maintained on YM agar slants that contained 5 g of peptone, 3 g of yeast extract, 3 g of malt extract, 10 g of glucose and 20 g of agar in 1000 m*l* of distilled water. The pH of the medium was adjusted to 6.5 before sterilization. The culture was prepared by transferring a loop of the stock culture and putting it into 50 m*l* of liquid YM medium in a 200-m*l* flask, and incubating it at 5°C at 130 rpm on a rotary shaker.

Nitrogen removal in *Candida* sp.

The yeast cells collected (0.1 g, dry weight) were suspended in 100 m*l* of the synthetic medium that contained nitrate (KNO_3), nitrite (KNO_2) or ammonium ($(NH_4)_2SO_4$) as a nitrogen source (Table 1). The C/N ratio (weight) of each medium was 40.

Table 1. Composition of a synthetic medium for *Candida* sp.

Constituent	
Glucose	10 g
Nitrogen source*	100 mg
KH_2PO_4	1 g
$MgSO_4 \cdot 7H_2O$	500 mg
$CaCl_2 \cdot 2H_2O$	100 mg
NaCl	100 mg
Vitamin solution**	1 m*l*
Distilled water	1000 m*l*

*KNO_3, KNO_2 or $(NH_4)_2SO_4$

**Vitamin solution (μg ml^{-1}) contains biotin 2, Ca-pantothenate 400, inositol 2000, thiamine-HCl 400, piridoxine-HCl 400, nico-tinic acid 400 and *p*-aminobensoic acid 200.

The effect of an initial pH of 2–8 on the removal of nitrate was examined using the synthetic medium containing nitrate as a sole nitrogen source. The pH of the medium was adjusted by 1M HCl or 1M NaOH.

Chlorine was added as sodium hypochlorite solution before the incubation. The supernatants of the media which were filtered through membranes (pore size of 0.45 μm) were used for the analysis.

Effect of temperature on the fatty acid composition

Fatty acid compositions of the cells that were cultured on YM medium in the range of 0 to 20℃ were analyzed. Total lipids were extracted by the partially modified method of Bligh and Dyer (Morris *et al.*, 1981). Fatty acid methyl esters were prepared according to PSJ (2000).

Analytical methods

Nitrate, nitrite and ammonium nitrogens and residual chlorine were analyzed according to Standard Methods (APHA *et al.*, 1992). Fatty acid methyl esters were analyzed according to PSJ (2000).

Results and discussion

Figure 1 shows the growth of *Candida* sp. and the removal of various nitrogen sources in the medium at 5℃. Nitrite was not observed in the cultured broth throughout the experiments. *Candida* sp. can remove more than 90% of nitrate, nitrite and ammonium nitrogen in the medium within 4 days. The strain showed similar growth in each nitrogen source.

Figure 2 shows the effect of initial pH of 3 to 8 on the removal of nitrate in the medium and the growth of *Candida* sp. at 5℃. Within the pH of 3 to 8, the yeast cells

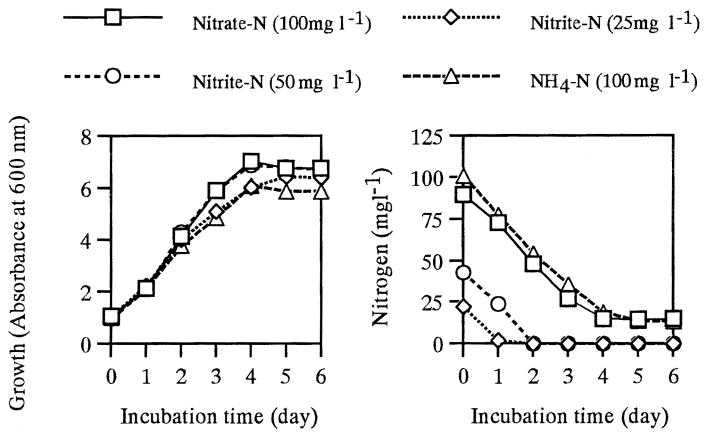

Fig. 1. The growth of *Candida* sp. and time variation of nitrogen concentration in the medium. The strain was cultured on a synthetic medium containing various nitrogen sources under the culture of pH 6, at 5℃.

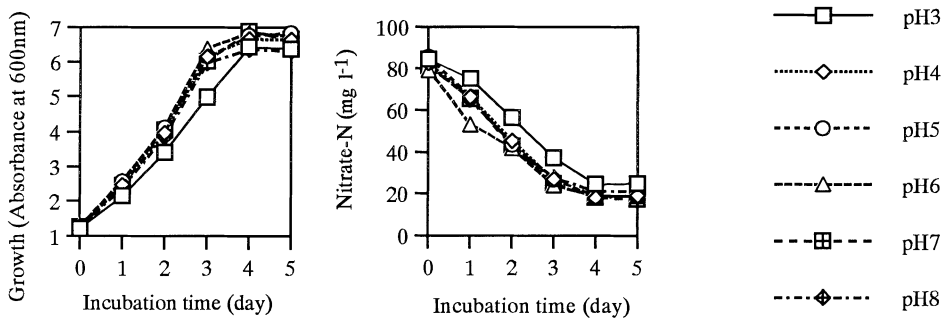

Fig. 2. Effect of initial pH of 3 to 8 on the removal of nitrate-N in the medium and the growth of *Candida* sp. at 5°C.

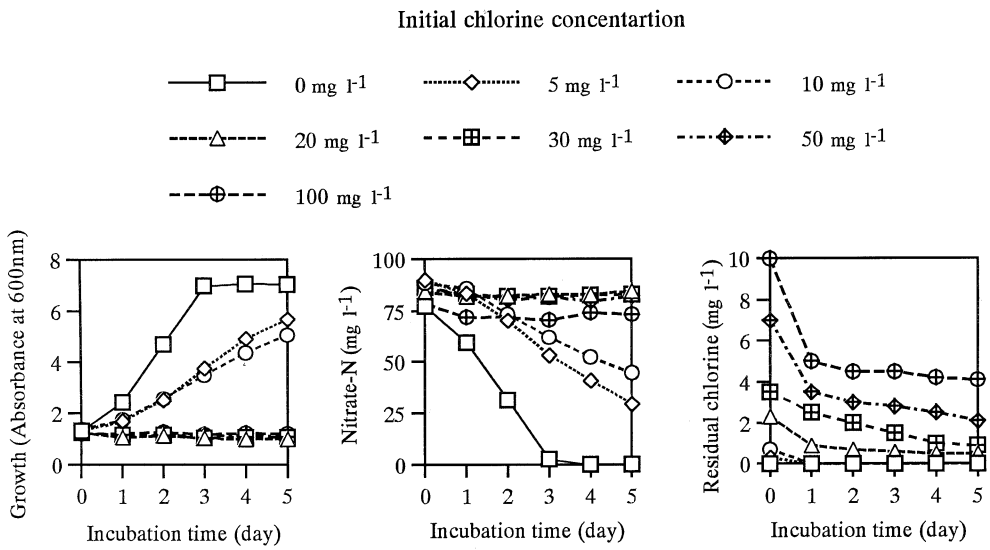

Fig. 3. Effect of chlorine on the growth of *Candida* sp., nitrate removal and the residual chlorine concentration in the medium under the culture of pH 6, at 5°C.

grew well and showed a similar nitrate removal level. The growth and nitrate removal at pH 3 and at low temperature of 5°C may be advantageous for application to an actual wastewater treatment plant. The pH of the medium increased to the range of 7.6–8.2 after incubation.

Figure 3 shows the effect of chlorine on the growth of *Candida* sp., nitrate removal and the residual chlorine concentration in the medium. Removal of nitrate decreased with increase of the chlorine concentration. The strain removed nitrate in the medium and grew well at chlorine concentrations of 5 and 10 mgl^{-1} but did not grow at chlorine concentration of 20 mgl^{-1} or more. The residual chlorine in the medium at concentration of more than 2 mgl^{-1} affected the growth and nitrate removal of the strain.

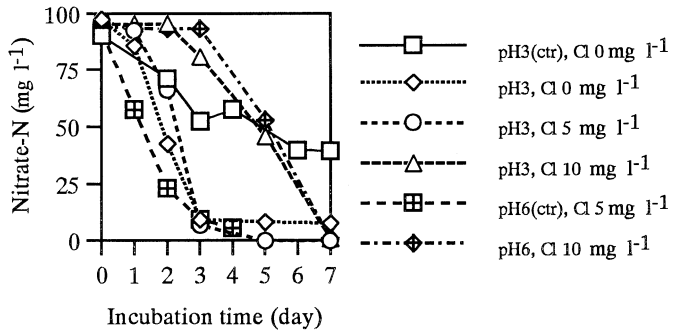

Fig. 4. Effects of pH and chlorine concentration on the removal of nitrate in the medium by the *Candida* sp. culture. ctr: pH of the medium was controlled constantly by a pH controller.

Figure 4 shows the effect of pH and chlorine on the removal of nitrate by cells of *Candida* sp. In this figure, 'ctr' means that the pH of the medium was controlled constantly by a pH controller. In this experiment, the medium was not sterilized for application to actual wastewater treatment. Each medium contained about 5000 CFU of heterotrophic bacteria ml^{-1} of medium. The added chlorine (5 to 100 mgl^{-1}) completely killed the bacteria.

At controlled pH of 3 during the experimental period, the nitrate removal was decreased but the removal rate was still maintained at about 60% of the initial rate. Addition of chlorine to the medium at the concentration of 5 mgl^{-1} did not affect the nitrate removal rate. At chlorine concentration of 10 mgl^{-1}, nitrate removal was inhibited in the early period but it recovered to almost 100% at the end of the incubation time.

These results suggest that the addition of chlorine (10 mgl^{-1}), pH control (pH 3) and low temperature (5℃) are also effective to maintain the domination of yeast cells, and may be applicable to the practical use of the strain.

Table 2 summarizes the effect of culture temperature on the fatty acid composition of the cells of *Candida* sp. The total unsaturated fatty acids (%) were almost equal at 90% at each cultivating temperature. Decreasing the growth temperature induced an increase in the linolenic acid content (18 : 3, m.p. −11℃). The strain may adapt to temperature changes by changing its fatty acid composition to maintain cellular fluidity (Russell, 1978, 1984; McGibbon and Russell, 1983).

Table 2. Effect of temperatures on the fatty acid composition of *Candida* sp.

Temperature (℃)	Fatty acid (%)						Total unsaturated fatty acids (%)
	16:0	16:1	18:0	18:1	18:2	18:3	
0	8.2	1.2	2.4	15.8	32.5	40.0	89.5
5	9.2	5.2	0.9	20.7	37.8	26.3	90.0
10	10.1	5.3	0.9	19.0	44.7	20.1	89.1
20	14.1	1.4	2.4	34.1	38.0	10.1	83.6

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