Factors Affecting Oxygen Uptake by Yeast *Issatchenkia orientalis* as Microbial Feed Additive for Ruminants

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ABSTRACT : The objective of this work was to evaluate a thermotolerant yeast *Issatchenkia orientalis* DY252 as a microbial feed additive for ruminants. In the present study, the influence of volatile fatty acids (VFA) and temperature on oxygen uptake rate by *I. orientalis* DY 252 was investigated. It was evident that the oxygen uptake rate was decreased gradually as the VFA concentrations increased in a range of 30 to 120 mM. Although the oxygen uptake rate was not greatly affected by temperature in the range 37 to 43°C, a maximum value of 0.45 mg O_2/g cell/ min was obtained at 39°C. With regard to the oxygen uptake rate by yeast, viability was found to be less important than the metabolic activity of yeast. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 7 : 1011-1014)***

Key Words : *Issatchenkia orientalis*, Oxygen Uptake Rate, Viability, Vitality, Volatile Fatty Acids, Yeast Culture

INTRODUCTION

Direct-fed microbial feed additives (previously called probiotics) are currently used as feed additives for the improvement of health and production of livestock. The term direct-fed microbials (DFM) has been employed to describe microbial-based products (Martin and Nisbet, 1992). Many microorganisms have been used in DFM formulations. The most common organisms found in DFM products include *Saccharomyces*, *Bacillus*, and *Aspergillus* (Reynolds, 1998). Yeasts, in particular *S. cerevisiae*, have been widely used in brewing, wine-making, and baking processes for several thousand years. Yeast culture using *S. cerevisiae* is one of the DFM available on the market. The yeast culture is defined as a dry product composed of yeast and the media on which it was grown, dried in such a manner as to preserve the fermenting capacity of the yeast (Rose, 1987).

Increasing number of ruminants has been receiving yeast culture as a dietary supplement. Most studies indicated that the yeast culture increases feed intake rather than altering feed conversion efficiency. The yeast culture often increases the rate of fiber breakdown in the rumen, which in turn will enhance feed intake (Wallace, 1996). The most reproducible effect of the yeast culture on rumen fermentation is that it stimulates the bacterial viable counts of cellulolytic and lactic acid utilizing species (Wiedmeier et al., 1987; Harrison et al., 1988; Newbold et al., 1995). The rumen environment is essentially anaerobic. More than 99% of rumen bacteria are strict anaerobes (i.e. they cannot tolerate even small amounts of oxygen), so traces of oxygen entering the rumen could be detrimental to the fermentation (Wallace, 1996). However, low levels of dissolved oxygen (no more than 3 μmol per L) can be detected around the time of feeding (Scott et al., 1983; Hillman et al., 1985). Rose (1987) previously proposed that one of the beneficial functions that yeast could perform in the rumen was the removal of molecular oxygen. It becomes clear that the main effect of yeast is to protect rumen bacteria from the effects of oxygen, and the stimulation of rumen bacteria is partly dependent on its respiratory activity (Newbold et al., 1996; Wallace, 1996).

In our previous work, a thermotolerant yeast *I. orientalis* DY 252 was isolated and demonstrated as a potential candidate for use as a microbial feed additive for ruminants (Lee et al., 2002). Growth characteristics of *I. orientalis* DY 252 in aerobic batch and fed-batch cultures were previously studied in detail (Shin et al., 2002). The present study was conducted to investigate factors affecting the oxygen uptake rate by *I. orientalis* DY 252. For comparative purposes, the oxygen uptake rate by *Saccharomyces cerevisiae* NCYC 240 was also studied since this strain was reported as one of the best strains in terms of oxygen uptake ability (Newbold et al., 1996).

MATERIALS AND METHODS

Strains

Issatchenkia orientalis DY 252 used in this study was isolated at this laboratory as described previously (Lee et al., 2002). Characteristics of *I. orientalis* DY 252 are

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Table 1. Characteristics of *Issatchenkia orientalis* DY252

Characteristic	Result	Reference
Origin	Molasses	Lee et al. (2002)
Bio Safety	Level 1	
Carbon source utilization[a]	D-Glucose (+)	Barnett et al. (2000)
	D-Fructose (+)	
	Sucrose (-)	
	Maltose (-)	
	Ethanol (+)	
Optimum		
pH for growth	4.0	Shin et al. (2002)
temperature for growth	32°C	
Resistance to		
pH	2.0	Lee et al. (2002)
bile	0.9% (w/v)	
volatile fatty acids[b]	80 mM	

[a] (+) positive, (-) negative.
[b] Acetate/propionate/butyrate (70:20:10, by mol %).

summarized in Table 1. *S. cerevisiae* NCYC 240 was obtained from the National Collection of Yeast Cultures (Norwich, UK). These strains were maintained by transferring to fresh YM broth (Difco) agar plates biweekly and were stored at 4°C.

Culture conditions

Single yeast colonies were taken from a stock culture plate and transferred to a sterilized Erlenmeyer flask containing 50 mL of a YM broth medium. The shaking incubation of the flask was carried out at 30°C for 16 h. Five mL of the inoculum was transferred to a flask containing 50 mL of the fresh YM broth medium and then incubation was carried out further at 30°C for 6 h prior to use.

Measurement of oxygen uptake rate

A 2.5-L jar fermentor (Kobiotech, Korea) containing 960 mL of a 50 mM potassium phosphate buffer (pH 6.5) mixed with volatile fatty acids plus 0.5% (w/v) glucose was used to measure the oxygen uptake rate. Acetate/propionate/butyrate (70:20:10, by mol percent) were mixed and the mixture was added to the buffer solution at a concentration of 0, 39, 78, and 117 mM. The fermentor was flushed with N_2 gas or air in order to calibrate the dissolved oxygen probe (Mettler-Toledo GmbH, Germany) in the fermentor. The stirring speed was controlled at 500 rpm. After allowing the dissolved oxygen tension reading to stabilize at 100% (i.e. 6.7 mg O_2/L at 39°C), 40 mL of the active yeast broth was added to the fermentor very rapidly and then the decrease in the dissolved oxygen concentrations was monitored for 10 min. The rates of the oxygen uptake under various conditions were calculated from the phase of the decline.

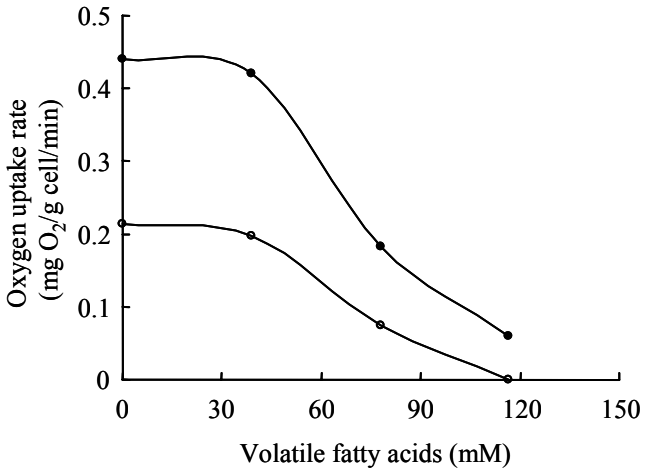

Figure 1. The effect of volatile fatty acids concentration on oxygen uptake rate by *I. orientalis* DY 252 along with *S. cerevisiae* NCYC 240 at 39°C. *I. orientalis* DY 252 (●), *S. cerevisiae* NCYC 240 (○).

Estimation of viability

A 1 mL sample was diluted serially and plated in duplicate to obtain the viable count. The culture medium used for plates was a YM broth agar medium. The plates were incubated at 30°C for 48 h, and the final colony count was taken as the average of the two plates for the dilution containing 30 to 300 colonies per plate. The total number of cells per mL was estimated from the count using a haemocytometer of 0.1 mm depth. The percentage of viability was determined by dividing the viable count of the total count. Viability was also estimated by the methylene blue staining (Gilliland, 1959). The dead cells were judged by their darker appearance. The percentage of such cells was estimated and subtracted from 100 to give the percentage viability.

RESULTS AND DISCUSSION

Effect of volatile fatty acids (VFA) on oxygen uptake rate

Rumen fermentation provides small molecular weight products such as acetate, propionate, and butyrate. The acetate: propionate: butyrate ratio in the rumen is approximately 70:20:10 by mol percent, and the concentrations of the total VFA are within a range of 70 to 130 mM. Figure 1 shows the effects of the total VFA concentrations on the oxygen uptake rate by *I. orientalis* DY 252 along with *S. cerevisiae* NCYC 240. For this particular study, a mixture of VFA, a composition similar to that reported by Agarwal et al. (2000), was used. These experiments were carried out at 39°C, because this is the body temperature of ruminants. As can be seen from Figure 1, the oxygen uptake rate was not greatly affected by the total VFA concentrations in a range of 0 to 30 mM.

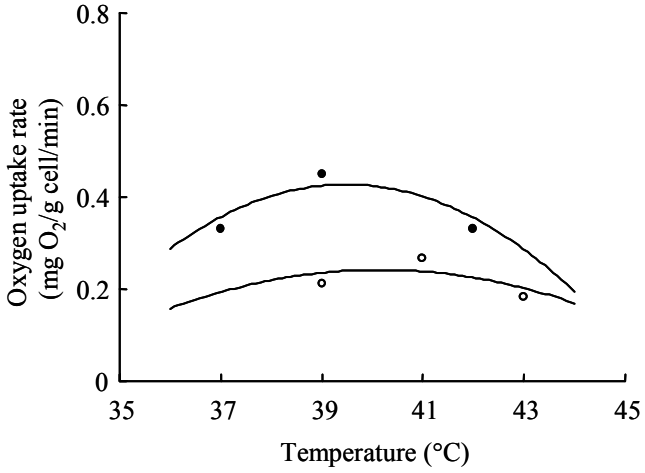

Figure 2. The effect of temperature on oxygen uptake rate by *I. orientalis* DY 252 together with *S. cerevisiae* NCYC 240. *I. orientalis* DY 252 (●), *S. cerevisiae* NCYC 240 (○).

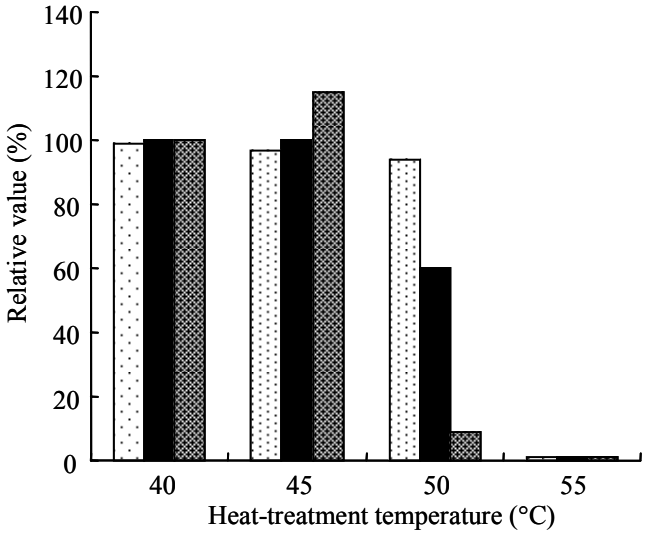

Figure 3. The effect of heat-treatment temperature for *I. orientalis* DY 252 cells on viability and oxygen uptake rate at 39°C. Vitality ( ), Oxygen uptake rate ( ), Viability ( ).

However, increases in the total VFA concentrations from 40 mM to 120 mM resulted in gradually decreased the oxygen uptake rate. The oxygen uptake rate was reduced by half at a total VFA concentration of 70 mM. As shown in Figure 1, it is evident that *I. orientalis* DY 252 showed 2-fold higher ability to take up oxygen than *S. cerevisiae* NCYC 240. The oxygen uptake rate by the other yeast strain isolated by us from a commercially available yeast culture in Korea was also measured. The oxygen uptake rate by the isolated strain was found to be far lower than that by *S. cerevisiae* NCYC 240 (data not shown).

Effect of temperature on oxygen uptake rate

In Figure 2, the temperature effect within a range of 37 to 43°C on the oxygen uptake rate by *I. orientalis* DY 252 together with *S. cerevisiae* NCYC 240 is shown. It appears from Figure 2 that the oxygen uptake rate was not greatly affected by the temperature, with a maximum value at about 40°C for both strains. The differences in the oxygen uptake rates between the two strains reduced as the temperature was increased from 39°C to 41°C to 43°C, indicating that *I. orientalis* DY 252 was more sensitive to higher temperatures than *S. cerevisiae* NCYC 240. A maximum value of 0.45 mg O_2/g cell/ min was obtained at 39°C with *I. orientalis* DY 252. The value of oxygen uptake rate in the absence of VFA with *S. cerevisiae* NCYC 240 was about 10-fold lower than that reported previously in the paper by Newbold et al. (1996) as shown in Table 2. A possible reason for this was probably due to the difference in the level of oxygen concentrations during the measurements of oxygen uptake rate.

Measurement of oxygen uptake rate with heat-treated yeast cells

In order to study the effect of yeast viability on its respiratory activity, the effect of heat-treatment temperature for *I. orientalis* DY 252 cells on both viability and oxygen uptake rate at 39°C is shown in Figure 3. In these experiments, flasks containing 40 mL of a yeast broth were heat-treated at 40, 45, 50 and 55°C for 1 h before inoculation. As the heat-treatment temperature was increased from 40°C to 45°C to 50°C to 55°C, the relative viability which was determined using a standard plate count was found to be 100% to 115% to 9% to 0%, respectively. The relative increase in viability at 45°C was probably due to the growth occurred during the heat treatment of 1 h.

Table 2. Comparison of differences in specific oxygen uptake rates by various yeast strains

Strain	O_2 concentration[a]	Specific O_2 uptake rate	Reference
S. cerevisiae			
NCYC 1026	13 μM	1.1 mg O_2/g cell/min	Newbold et al. (1996)
NCYC 240	13 μM	2.1 mg O_2/g cell/min	Newbold et al. (1996)
I. orientalis			
DY 252	215 μM	0.45 mg O_2/g cell/min	This work
S. cerevisiae			
NCYC 240	215 μM	0.2 mg O_2/g cell/min	This work

[a] Experiments were carried out at indicated levels of O_2 concentration.

When viability based on the methylene blue staining technique was examined microscopically, it is evident from Figure 3 that viability was not greatly affected up to 50°C of the heat-treatment temperature. Also shown in Figure 3 is the relative value of the oxygen uptake rate. When the cells were heat-treated at 50°C, 94% of the cells were unstained. Although 94% of the cells were still living and 9% of the cells were able to reproduce only, 61% of the relative oxygen uptake rate was achieved using the cells. It is generally agreed that viability refers to whether a yeast cell population is "alive" or "dead", and vitality is a measure of how alive or active the "live" yeast cell population (Heggart et al., 1999). The percent viability determined using a standard plate count method is the true estimate of the percentage of cells which are able to reproduce. Accordingly, as far as the oxygen uptake rate by yeast is concerned, it is concluded that viability was found to be less important than vitality (i.e. the metabolic activity of yeast).

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