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Effect of Temperature on Growth of Psychrophilic and Psychrotrophic Members of *Rhodotorula aurantiaca*

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Abstract

The thermodependence of growth kinetic parameters was investigated for the Antarctic psychrophilic strain *Rhodotorula aurantiaca* and a psychrotrophic strain of the same species isolated in Belgium (Ardennes area). Cell production, maximum growth rate (μ_{max}), and half-saturation constant for glucose uptake (*Ks*) of both yeasts were temperature dependent. For the two yeasts, a maximum cell production was observed at about 0°C, and cell production decreased when temperature increased. The μ_{max} values for both strains increased with temperature up to a maximum of 10°C for the psychrophilic strain and 17°C for the psychrotrophic strain. For both yeasts, *Ks* for glucose was relatively constant at low temperatures. It increased at temperatures above 10°C for the psychrophilic strain and 17°C for the psychrotrophic strain. Although its glucose affinity was lower, the psychrotrophic strain grew more rapidly than the psychrophilic one. The difference in growth rate and substrate affinity was related to the origin of the strain and the adaptation strategy of *R. aurantiaca* to environmental conditions.

Index Entries: *Rhodotorula aurantiaca*; growth kinetics; psychrophilic; psychrotrophic; cold adaptation.

Introduction

Microorganisms can grow at temperatures ranging from subzero to boiling point and represent a thermal continuum. Psychrophiles, mesophiles, and thermophiles have traditionally been defined in relation to their cardinal temperature for growth. Psychrophilic microorganisms are of special interest in the cold environment, because their average growth range

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is –10 to +20°C *(1,2)*. Together with psychrotrophic microorganisms (0–40°C) *(3)*, they are cold-adapted organisms and are found in both aquatic and terrestrial cold environments. Psychrophilic microorganisms play an essential role in the ecology of permanently cold regions, which cover a significant portion of the planet. In view of the importance of cold habitats to global ecology, it is surprising that psychrophiles and psychrotrophs have not been studied more intensively. It is hoped that the current wave of interest in the environment and the desire to save energy will contribute to a much needed resurgence of interest in the research of psychrophiles and psychrotrophs. Besides their ecological significance, they could be used in low-energy biotechnological processes such as bioremediation of polluted cold soil *(4)*.

Compared with psychrotrophs, psychrophiles grow over a narrower temperature range. Although not all cold-adapted microorganisms fit neatly into this classification, it is a useful scheme because it relates to the environmental origins of the two groups: psychrophiles tend to be isolated more frequently from thermally stable cold habitats (<5°C), whereas psychrotrophs are more characteristic of thermally unstable cold habitats *(2,3)*. It is well known that growth in cold environments is usually determined by membrane fluidity affecting the transport of nutrients *(2,5–7)*, forces affecting tertiary and quaternary structures of enzymes *(8–10)*, and temperature-dependent interactions of regulatory compounds at the enzyme, ribosome, RNA, or DNA level *(11)*.

In the present study, we studied growth behavior in response to temperature change of two cold-adapted yeasts. The two strains are psychrophilic and psychrotrophic members of the *Rhodotorula aurantiaca* species. Consequently, they are particularly suitable organisms to test the relationship between cardinal growth temperature and physiological characteristics.

Materials and Methods

Origin of Collection Strains

Yeasts were collected near the Antarctic Station Dumont d'Urville (66° 40'S, 140° 01'E) by Prof. C. Gerday (University of Liege, Belgium). The psychrotrophic strain, *R. aurantiaca* 31345, was purchased from the Mycotheque of the University of Louvain-la-neuve (MUCL). The psychrophilic strain of *R. aurantiaca* coming from Antarctic was deposited at the MUCL under registration no. 40267.

Identification of Strains

Identification of strains were made at 10°C and were based on a metabolic profile determined by the analytical profile index (API) 20C AUX (BioMérieux, Marcy e' étoile France).

Growth Conditions

The cardinal temperature of growth for Antarctic strains was determined with the following rich medium: 1% glucose, 1% yeast extract, 1% peptone, and 2% agar. For kinetic study, the psychrophilic and psychrotrophic strains of *R. aurantiaca* were cultivated in YNB medium (Difco, Detroit, MI) supplemented with 1% glucose and 0.5% ammonium sulfate. Cultures were carried out at 0, 4, 10, and 17°C for the psychrophilic strain and 4, 10, 17, 25, and 32°C for the psychrotrophic strain.

Sample Analysis

Periodically, samples were taken and analyzed for cell production and glucose consumption. Cell density was obtained by counting with a Bürker chamber using a phase-contrast microscope. The residual concentration of glucose in medium was measured spectrophotometrically using the Glucose Enzymatique PAP500 kit (BioMérieux) as described in the Biomérieux technical notice.

Determination of Kinetic Factors

After inoculation, growth at each temperature was monitored by periodic measurement of the culture turbidity at 540 nm. The maximum growth rate (μ_{max}) at each temperature was established from the linear part of the plot of optical density vs time. Values of specific growth rate (μ) and residual glucose concentration (S) were fitted by the Monod equation:

$$\mu = \mu_{max} \times S/Ks + S$$

The Ks value was deduced from a Lineweaver-Burk plot.

Results

Twenty yeasts collected in the Antarctic were analyzed for their growth temperature range. They mainly consisted of psychrophilic strains. All strains were able to grow at 0°C. Eighteen strains were unable to grow near 20°C, and only two strains showed weak growth at 25°C and were unable to grow at 30°C (Table 1).

To determine the biochemical and physiological characteristics that differentiate psychrophiles and psychrotrophs, the growth kinetic of the psychrophilic strain *R. aurantiaca* A19 was analyzed and compared to that of the psychrotrophic one of the same species (the MUCL *R. aurantiaca* 31345). Cultures were achieved at different temperatures as described in Materials and Methods.

Effect of Temperature on Cell Density

The cell density was maximal at low temperature for both strains and decreased when temperature increased (Fig. 1). Table 2 gives the maximum cell concentrations reached with both strains at different temperatures. For

Table 1
Identification and Maximum Growth Temperature for Antarctic Yeasts[a]

Strain	Identification	Maximum temperature for growth (°C)
A2	*Candida ciferrii*	20
A4	*Candida ciferrii*	20
A5	*Tricosporon cutaneum*	20
A9m	*Tricosporon* sp.	15
A9l	*Cryptococcus albidus*	15
A11	*Candida* sp.	20
A16	*Rhodotorula aurantiaca*	15
A17	*Cryptococcus* sp.	20
A18	*Candida colliculosa*	15
A19	*Rhodotorula aurantiaca*	20
A19B	*Mrakia gelida*	15
N1	*Cryptococcus albidus*	15
N8	*Candida tropicalis*	20
N9	*Candida lusitaniae*	20
N10B	*Tricosporon* sp.	25
N13	*Tricosporon cutaneum*	20
N14	*Candida* sp.	15
N41	*Bullera* sp.	20
N42	*Bullera* sp.	20
N85	*Cryptococcus* sp.	25

[a]Identification was made using the API. Growth temperature range was determined on agar-rich medium.

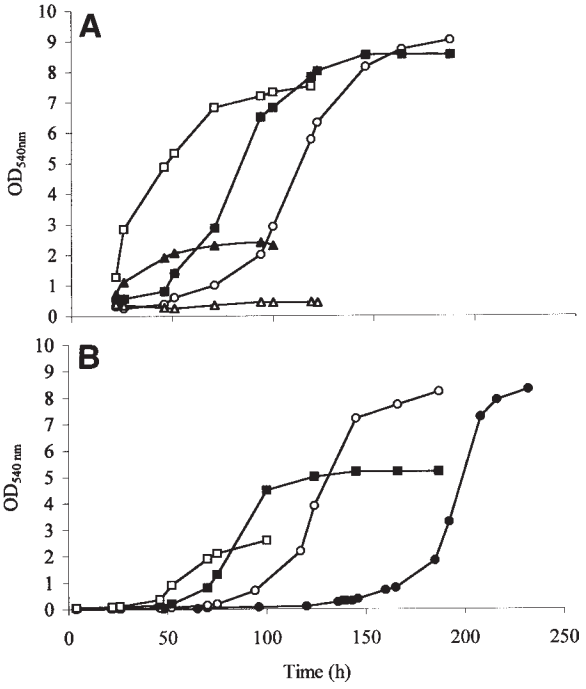

Fig. 1. Growth of the two strains of *R. aurantiaca*: **(A)** psychrotrophic strain cultivated at 4 (○), 10 (■), 17 (□), 25 (▲), and 30°C (△); **(B)** psychrophilic strain cultivated at 0 (●), 4 (○), 10 (■), and 17°C (□).

Table 2
Cell Production in Batch Culture Experiments
by Psychrophilic and Psychrotrophic Strains of *R. aurantiaca* at Different Temperatures

	Culture temperature (°C)				
	0	4	10	17	25
Psychrophilic strain	$8.2 \times 10^8 \pm 0.4 \times 10^8$	$8.2 \times 10^8 \pm 0.4 \times 10^8$	$5.3 \times 10^8 \pm 0.2 \times 10^8$	$2.5 \times 10^8 \pm 0.5 \times 10^8$	ng[b]
Psychrotrophic strain	nd[a]	$7.1 \times 10^8 \pm 0.3 \times 10^8$	$6.9 \times 10^8 \pm 0.4 \times 10^8$	$5 \times 10^8 \pm 0.2 \times 10^8$	$1.7 \times 10^8 \pm 0.4 \times 10^8$

[a]Not determined.
[b]No growth.

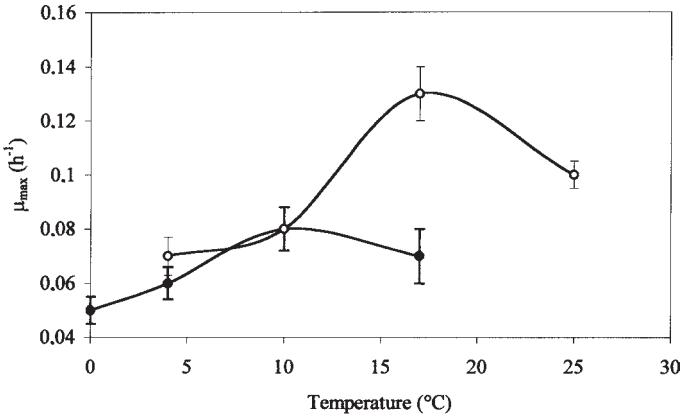

Fig. 2. Maximum growth rates (μ_{max}) of the psychrophilic (●) and the psychrotrophic (○) strains of the *R. aurantiaca* as a function of temperature. Strains were grown in batch culture, and maximum growth rates during the exponential phase were calculated.

the psychrophilic strain, the cell densities below 4°C were 1.5- and 3.5-fold higher, respectively, than those produced at 10 and 17°C. The upper limit temperature for this strain was 20°C. A similar behavior was observed with the psychrotrophic strain. The higher cell densities were produced at 4 and 10°C. These values were 1.4- and 4.2-fold higher than those at 17 and 25°C, respectively. The growth-limiting temperature for the psychrotrophic strain was 32°C.

Effect of Temperature on μ_{max} *and* Ks

The μ_{max} values were measured for each organism (Fig. 2). The optimum temperature for the psychrophilic strain was about 10°C (μ_{max}, 0.08 h^{-1}). For the psychrotrophic strain, it was 17°C (μ_{max}, 0.13 h^{-1}). Figure 3 gives the glucose half-saturation constant for both strains at each temperature. Below optimal temperature (10 and 17°C, respectively, for the psychrophilic and psychrotrophic strains), the *Ks* did not vary in function of the temperature for both strains. When the temperature increased beyond the optimum, the *Ks* greatly increased, showing that there was a great decrease in affinity for glucose. By comparing *Ks* values for the two organisms, the psychrotrophic strain showed lower *Ks* values, especially below 15°C. This result suggests that the glucose uptake system of the psychrophilic strain was better adapted to function at low substrate concentration.

Specific Affinity

Figure 4 shows the specific affinity (μ_{max}/Ks) for glucose uptake for the two strains. It evolves similarly for both strains and it increases with temperature up to an optimum, and then decreases. A greater μ_{max}/Ks was observed at temperatures giving the fastest growth (10 and 17°C, respectively, for the psychrophilic and psychrotrophic strains). The μ_{max}/Ks

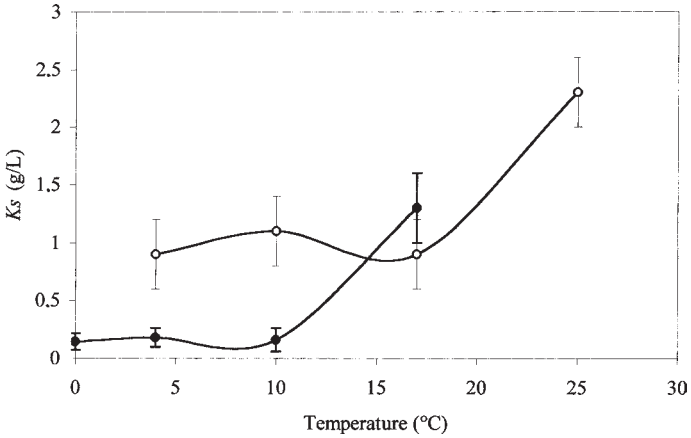

Fig. 3. Temperature dependence of glucose half-saturation constant (*Ks*) of the *R. aurantiaca*: the psychrophilic strain (●) cultivated at 0, 4, 10, and 17°C, and the psychrotrophic strain (○) cultivated at 4, 10, 17, and 25°C. The *Ks* values were deduced from Lineweaver-Burk plots.

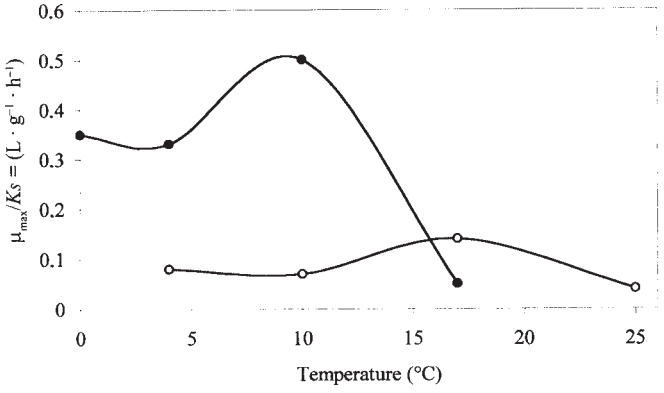

Fig. 4. Temperature dependence of specific affinity (μ_{max}/Ks) for the psychrophilic (●) and the psychrotrophic (○) strains of the *R. aurantiaca*.

shown by the psychrophilic strain was about fivefold greater than for the psychrotrophic strain below 10°C.

Discussion

The cardinal temperature determined for the Antarctic yeast collection clearly showed a higher proportion of psychrophiles than psychrotrophs. This predominance can be related to the permanently cold temperature in the Antarctic. Indeed, psychrophiles are generally less frequently isolated but are more likely to be found in habitats having a stable thermal regime *(2)*. In addition to temperature, other factors, including the availability of nutrients, influence the proportion of psychrotrophic and psychrophilic

microorganisms in an environmental niche *(2)*. The relation between nutrient level and temperature is difficult to analyze *in situ*. Nevertheless, it is reflected in the general observation that higher proportions of psychrophiles occur in Antarctic, compared to Arctic, marine ecosystems because the latter are more influenced by terrestrial nutrient input *(12)*. Indigenous psychrophilic yeasts in Antarctic deserts have simple growth requirements that enable them to colonize a severely nutrient-limited environment *(13)*.

In this work, we studied the strategy of adaptation of *R. aurantiaca* to cold environments. Two strains of this species were isolated from environments where thermal regimes were different. One strain was from Belgium (Ardennes area), where temperature fluctuates daily and seasonally. The other came from the Antarctic, where temperature is stable. Consequently, temperature ranges of growth of the two members of this species were different. The Antarctic strain can be considered psychrophilic because it was unable to grow beyond 20°C, whereas the strain from Belgium was psychrotrophic because its higher growth-limiting temperature was 32°C. For both strains, the optimal temperature for production of the highest final cell density was about 0°C. For the specific growth rate, the temperatures that gave the best μ_{max} were 17 and 10°C, respectively, for the psychrotrophic and the psychrophilic strains. Our results suggest that the optimum temperature for both strains was not easy to define. Indeed, the definition of the optimum must integrate several parameters: μ_{max}, *Ks* for substrate uptake, cell production, and physiological state. These parameters do not have the same evolution in response to thermal fluctuations. Similar observations were reported for several bacteria *(8–10,14,15)* and suggest that the optimal temperature for growth rate is not necessarily the one that gives the best physiological state.

The ability of one microorganism to outcompete with other microorganisms is the result of its higher level of adaptation to the environmental conditions. This will be determined by efficiency of nutrient uptake and utilization (cell yield). Thus, substrate affinity was crucial for any cell development. For both yeasts, glucose affinity had similar evolution in response to temperature; it was maximal below temperatures that gave the fastest growth rate and increased when the temperature of culture was close to the higher limit for growth. Similar observations were made by Herbert *(16)* and Nedwell and Rutter *(15)*, respectively, with the psychrophilic *Vibrio* sp. AF1 and the psychrotrophic *Brevibacterium* sp. 1/15. Russell *(17)* suggests that the loss of membrane selective permeability at high temperatures could be the cause of the upper limit of growth.

It has been previously proposed that the ability of an organism to sequester substrate is best defined not by the *Ks* value alone, but by specific affinity μ_{max}/Ks *(15,18–22)*. The specific affinity emphasizes the importance of taking into account not only the half-saturation constant (*Ks*) but also the maximum growth rate μ_{max}, when comparing substrate-sequestering abilities of microorganisms. In both cases, the specific affinity for glucose increased with temperature, reached an optimum, and then decreased.

Below 15°C, the μ_{max}/Ks of the psychrophilic strain for substrate was greater than that of the psychrotrophic, suggesting that the psychrophilic strain was able to outgrow the psychrotrophic strain at low substrate concentration.

Our results underline the adaptive changes in cellular physiology of *R. aurantiaca* in relation to its environmental conditions. The Antarctic strain in response to the weak level of nutrients developed a greater affinity for the substrate. By contrast, the psychrotrophic strain, which comes from an area where nutrients are less limiting, had a relatively weak substrate affinity. However, its growth rate was greater than that of the psychrophilic strain.

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