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ORIGINAL ARTICLE

Effect of incubation temperature on growth parameters of *Pseudoalteromonas antarctica* NF_3 and its production of extracellular polymeric substances†

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Keywords
Antarctica, arrhenius model, exopolymer, pseudoalteromonas, square root model, temperature.

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Abstract

Aim: To evaluate the effect of temperature on growth parameters and on extracellular polymeric substance (EPS) production for *Pseudoalteromonas antarctica* NF_3.

Methods and Results: For this purpose, three growth parameters, lag time (λ), maximum growth rate (μ) and maximum population density (A), were calculated with the predictive Gompertz model. To evaluate the variations in μ with respect to temperature, the secondary Arrhenius and the square root models were used. Below the optimal growth temperature (17·5°C), the growth of *P. antarctica* was separated into two domains at the critical temperature of 12°C. Within the suboptimal domain (12–17·5°C), the temperature characteristic was the lowest (5·29 kcal mol^{-1}). Growth population densities were maintained over the entire physiological portion assayed (5–17·5°C). Higher crude EPS production was found at temperatures included in the cold domain (5–12°C).

Conclusions: All calculated parameters revealed an optimal adaptation of this strain to cold temperatures.

Significance and Impact of the Study: The knowledge of the influence of temperature on growth parameters of *P. antarctica* NF_3 and on EPS production could improve the production of this extracellular polymeric substance that is currently being used in the cosmetic and pharmaceutical industries.

Introduction

Pseudoalteromonas antarctica NF_3 is an Antarctic cold-adapted bacterium that produces abundant extracellular polymeric substances (EPS) (Bozal *et al.* 1994; Nevot *et al.* 2006a). It has been suggested that *P. antarctica* EPS could provide a protective barrier around the bacterium based on its ability to coat liposomes and protect them against several surfactants (de la Maza *et al.* 1997; Cócera *et al.* 2001). Other interesting properties found for this bacterial EPS, such as its ability to change ice crystal structure (Rubinsky, personal communication) or contribute to *in vitro* wound healing, have stimulated its use in the cosmetics and pharmaceutical industries (Parente *et al.* 2002).

In Antarctic marine bacteria, several factors affect growth and EPS production, with temperature being one of the most important (Mancuso Nichols *et al.* 2005a). *Pseudoalteromonas antarctica* NF_3 has been defined as a psychrotolerant bacterium, but the influence of temperature on growth parameters and the temperature dependence of EPS production have not yet been studied. One useful way to evaluate bacterial behaviour under different temperatures is to examine the three parameters that characterize the three phases of bacterial growth: the lag time (λ) as a measure of the lag phase, the maximum growth rate (μ) for the exponential growth phase and the maximum population density (A) for the stationary phase. These three parameters can be estimated by the re-parameterized Gompertz equation for bacterial growth. This primary mathematical model fits well the growth of various bacterial species under a variety of culture conditions (Zwietering *et al.* 1990; Shi and Xia 2003; Lu *et al.* 2005). Secondary growth mathematical models, the

modified Arrhenius equation (ln μ *vs* $1/T$) and the square root equations, can be used to study the variation of specific growth rate (μ) as a function of temperature (Arrhenius 1889; Ratkowsky *et al.* 1983; Giannuzzi *et al.* 1998; Cayré *et al.* 2003; Mataragas *et al.* 2006). For several bacteria analysed with the Arrhenius plot, regardless of whether they are psychrophiles, mesophiles or thermophiles, two linear domains appear below the optimal growth temperature (T_{opt}), the slopes of which represent the activation energy for bacterial growth (also called temperature characteristic). The concept of critical temperature ($T_{critical}$) has been introduced to define the point at which the slope changes, thus referring to the border between two temperature domains in which the effect of temperature on growth is different (Harder and Veldkamp 1968; Berger *et al.* 1996; Guillou and Guespin-Michel 1996). Several authors have suggested that, below critical temperature, adaptation to low temperatures could involve the synthesis of stress proteins, use of alternative metabolic pathways and increased energy demand (Choma *et al.* 2000; Tarpgaard *et al.* 2005). The empirical square-root models have also been used successfully for modelling the temperature dependence of bacterial growth of several strains below the T_{opt} and over the full biokinetic temperature range (Ratkowsky *et al.* 1983).

In addition to growth rates, other physiological characteristics, such as growth yield, change with temperature and affect the adaptation of bacteria to low temperatures (Herbert and Bell 1977; Westermann *et al.* 1989; Knoblauch and Jorgensen 1999). This is the case for *Psychrobacter cryopegella* and other cold-adapted bacteria that maximize or maintain their growth yields at low temperatures to compensate for low growth rates (Harder and Veldkamp 1968; Isaksen and Jørgensen 1996; Bakermans and Nealson 2004).

Recent studies conducted by Nevot *et al.* (2006a,b) revealed that the EPS secreted by *P. antarctica* NF_3 is composed of a capsular polymer and large numbers of outer membrane vesicles (OMV).

This complex structure of EPS has not been demonstrated for other Antarctic marine bacteria, but secretion of EPS seems to be a common trait of many of them (Mancuso Nichols *et al.* 2004). Temperature also seems to influence the production of EPS from marine Antarctic bacteria. One example is the strain CAM025, a psychrotolerant bacterium that also belongs to the genus *Pseudoalteromonas*, which exhibits enhanced production of a high-molecular-weight exopolysaccharide at subzero incubation temperatures (Mancuso Nichols *et al.* 2005a). This temperature-dependent production supports the view that EPS has a cryoprotective role in high-salinity and low-temperature environments and that its extra production could be a strategy for minimizing freeze damage.

The aim of this research was to evaluate the influence of incubation temperature on the growth of the psychrotolerant bacterium *P. antarctica* NF_3. For this purpose, three growth parameters, lag time (λ), maximum growth rate (μ) and maximum population density (A), were calculated with the predictive Gompertz model. The two secondary models most widely used to evaluate the variations in μ with respect to temperature, the secondary Arrhenius-type model and the square root model, were applied between 5 and 30°C. In addition, the influence of incubation temperature on EPS production was analysed.

Materials and methods

Bacterial strain and media

Unless specified, all chemicals and biochemicals were purchased from Fluka (Switzerland). *P. antarctica* NF_3 is a psychrotolerant Gram-negative bacterium isolated from mucous material located only a few centimeters beneath the water surface at the base of a glacier near the inlet of Admiralty Bay (King George Island, South Shetland Islands, Antarctica) and was deposited in the Spanish Type Culture Collection with the reference number CECT 4664. *Pseudoalteromonas antarctica* NF_3 was grown on MM5, a minimal medium described by Bozal *et al.* (1997). A litre of this medium contains 5 g Na_2HPO_4, 2 g KH_2PO_4, 1 g NaCl, 7 g NH_4Cl, 0·5 g $MgSO_4{\cdot}7H_2O$, 0·001 g $FeSO_4{\cdot}7H_2O$, 0·05 g $CaCl_2$ and 20 g glucose, adjusted to a pH of 7·0.

Culture conditions

Two-litre baffled Erlenmeyer flasks containing 250 ml of MM5 were inoculated (1 : 20, v/v) with a growing culture (OD_{530nm} = 0·8) incubated at the test temperature. Duplicate batch cultures, without pH control, were grown at test temperatures with shaking at 150 rev min^{-1} in orbital shaker.

Growth was monitored spectrophotometrically at 530 nm in a Uvikon Spectrophotometer 922 (Kontron Instruments, Milan, Italy), correlating the optical density with cell dry weight measurements. To avoid interference of EPS on OD and cell dry weight measurements, cells were sedimented, washed three times with 1/4 Ringer solution (Oxoid, UK) and finally resuspended to the initial volume. One unit of optical density at 530 nm was shown to be equivalent to 0·45 g (dry weight) of cells per litre.

Calculation of growth parameters

To calculate the growth parameters for each temperature, the experimental data obtained were fitted to the Gompertz

function, using Origin 6·1 software (Microcal Software, Northampton, MA). At time t, the re-parameterized Gompertz model is expressed by the following function:

$$OD_t = OD_0 + A\exp\{-\exp[(\mu e/A)(\lambda - t) + 1]\} \quad (1)$$

where OD_t is optical density at time t; t is time of growth in hours; OD_0, the optical density at $t = 0$; A is the maximum population density (increase of OD between OD_0 and OD_{max}); μ is the maximum or specific growth rate (h^{-1}); λ is the lag time in hours; and e, the base of Napierian logarithm (2·718281).

Lag time–temperature relation

Generation time (GT), defined as the time for the bacterial population to double in cell numbers was calculated from μ by the equation:

$$GT = \log_{10}(2)/\mu \quad (2)$$

The relative lag time (RLT), defined by the amount of work to be done in adjusting to a new environment and the rate at which that work is done (Mellefont *et al.* 2003), was calculated by dividing lag time (λ) by GT.

Growth rate–temperature relation

The effect of incubation temperature on specific growth rate [estimated from the modified Gompertz eqn (1) for each growth temperature] was studied using two common functions to describe temperature relationships: the modified Arrhenius equation and the square root model:

(i) Modified Arrhenius equation

$$\ln\mu = \ln C + (-E_a/R)(1/T) \quad (3)$$

where μ is the specific growth rate, C is the collision or frequency factor, E_a is the activation energy (also called temperature characteristic), R is the universal gas constant and T is the absolute temperature.

(ii) Square root model

The square root model is an empirical model based on the observation that at lower temperatures, the square root of the specific growth rate is linear with temperature. The model was first suggested by Ratkowsky *et al.* (1982) and can be expressed as:

$$\sqrt{\mu} = b(T - T_{min}) \quad (4)$$

where b is a regression coefficient, and T_{min} is the notional minimum temperature at which growth can occur. This relationship fit well the data for temperatures ranging from the minimum temperature at which growth is observed to just below the optimum temperature (T_{opt}) at which maximum growth occurs. At higher temperatures, this equation ceases to model growth adequately owing to the inactivation or denaturation of proteins or to other factors. For this reason, Ratkowsky *et al.* (1983) proposed an extension of eqn (4), which describes bacterial growth throughout the entire temperature range:

$$\sqrt{\mu} = b(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \quad (5)$$

where b and c are constants, T_{min} is the notional minimum temperature at which growth can occur, T_{max} is the notional maximum temperature at which growth can occur, and both are conceptual temperatures and estimated from the extrapolation of the regression line derived from a plot of square root of μ *vs* temperature to the temperature axis.

Extraction of the EPS

For each temperature, cells at the beginning of stationary phase were removed by gentle centrifugation at 4500 ***g*** for 30 min at 5°C, and the sediment was washed with sterile 1/4 Ringer solution (Oxoid) and centrifuged as earlier. Supernatants, containing loose and bound EPS, were filtered through 0·45 μm pore-size filter (Millex-HV_{13}; Millipore). The EPS was precipitated by the addition of three volumes of chilled absolute ethanol. The mixture was held at 5°C overnight and centrifuged (10 000 ***g*** at 5°C for 20 min). Ethanol washes and centrifugation steps were repeated twice. The resulting pellet was dissolved in distilled water and dialysed to remove low-molecular weight sugar, through 10 000 Da cut-off filter (Amicon Ultra-15 centrifugal filter unit; Millipore). Crude EPS solutions were then lyophilized and weighed. The total carbohydrate content was analysed by phenol–sulfuric acid method (Dubois *et al.* 1956) with glucose as the standard. Noninoculated MM5 medium was used as negative control for measurements after exhaustive dialysis. The protein levels in crude EPS samples were determined by the Bradford (1976) method using standard Bio-Rad Reagent (Bio-Rad Laboratories GmbH).

Statistical analysis

The results are expressed as mean and standard error of the mean (±SE). Statistical comparisons were made using one-way ANOVA (Microcal Software), with $P < 0{\cdot}05$ indicating significance. To compare models, mean square error (MSE) and regression coefficient (R^2) were used. The lower MSE defines the better adequacy of the model to describe the data (Sutherland *et al.* 1994).

Results

The Gompertz growth model was used to estimate the three major components of bacterial fitness, the maxi-

Table 1 Growth parameters of *Pseudoalteromonas antarctica* NF_3 grown at different temperatures. Specific growth rate (μ), lag phase duration (λ) and maximum population density (A) were obtained by fitting experimental data to the Gompertz function using Origin 6·1 software (Microcal Software, Inc.)

Temp. (°C)	μ (h^{-1})	±SE	λ(h)	±SE	A	±SE	R^2
5	0·0850	0·0028	27·9658	0·8677	4·5836	0·0471	0·9889
7·5	0·1067	0·0031	24·0236	0·6023	4·5431	0·0434	0·9790
10	0·1559	0·0057	21·2445	0·5116	4·4695	0·0596	0·9987
12·5	0·1806	0·0035	17·0955	0·2320	4·5180	0·0334	0·9895
15	0·1927	0·0059	16·4369	0·3474	4·4489	0·0455	0·9989
17·5	0·2121	0·0069	14·6858	0·3517	4·8552	0·0652	0·9908
20	0·2015	0·0039	6·1091	0·2184	4·2338	0·0165	0·9995
22·5	0·1554	0·0067	5·7640	0·5065	3·5083	0·0328	0·9976
25	0·1337	0·0036	3·4475	0·2689	2·7790	0·0253	0·9987
27·5	0·0574	0·0017	5·5661	0·2739	1·1049	0·0107	0·9883
30	0·0005	0·0002	–	–	0·0636	0·0162	0·9716

SE, standard error; R^2, coefficient of determination.

mum growth rate (μ), the lag time (λ) and maximum population density (A). Table 1 shows results obtained when applying the Gompertz model to the experimental data of *P. antarctica* NF_3 grown over the range of temperatures from 5 to 30°C. In all cases, there was a close correlation between the experimental data and the predicted values, as is shown by the high values of coefficients of determination (R^2).

The maximum growth rate (μ)

The secondary models used to describe the temperature effect on maximum growth rate were the Arrhenius and the square root equations and both described well the experimental data of μ. When logarithms of maximum growth rate values were plotted against the reciprocal of the absolute temperature (Arrhenius relationship), linear dependence below the optimal temperature (17–18°C) was found (Fig. 1a). This portion is called the Arrhenius portion or the physiological or normal portion. At temperatures above the normal portion, the specific growth rate decreased until temperatures near 30°C were reached, at which growth ceased. The Arrhenius portion (5–17·5°C) had a low coefficient of determination ($R^2 = 0{\cdot}9241$). This portion was best fitted by two linear segments whose convergence determined the critical temperature at approximately 12°C (Fig. 1b). This temperature separates two linear domains below the optimal temperature: the cold domain (from 5°C to *c.* 12°C) and the suboptimal domain (from 12 to 17·5°C). Segment slopes determined two temperature characteristics. Over the

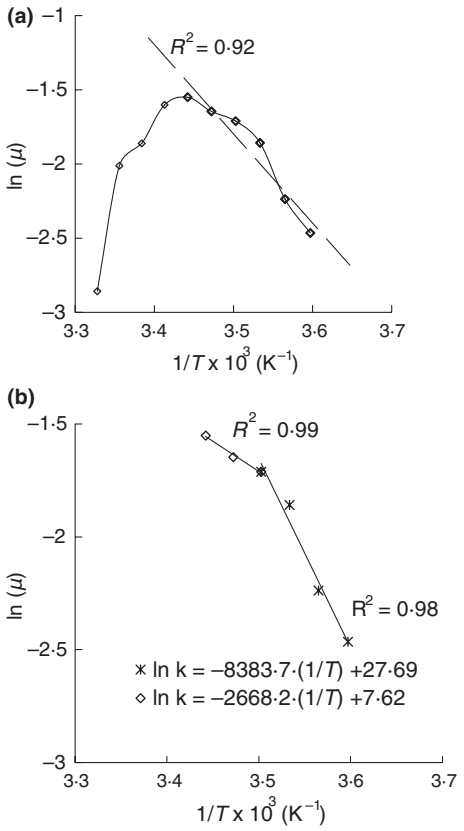

Figure 1 Arrhenius plot (ln μ *vs* the reciprocal of the absolute temperature) for *Pseudoalteromonas antarctica* NF_3 grown at different temperatures. (a) Interpolation of data points (solid line) and fitted curve for the Arrhenius law between 5 and 30°C. (b) Fitted curves for the cold domain (*) and for the suboptimal domain (◇). The maximum specific growth rate (μ) is expressed in h^{-1}, and the temperature is expressed in degrees Kelvin.

cold domain, the temperature characteristic was 16·7 kcal mol^{-1} (1 cal = 4·184 J); and over the suboptimal temperature domain, it was 5·3 kcal mol^{-1}.

The application of the square root eqn (4) in the physiological temperature range showed a linear dependence below the optimal temperature with a low coefficient of determination ($R^2 = 0{\cdot}94$) (Fig. 2a). This portion, as in the Arrhenius representation, was best fitted by two segment lines with convergence at approximately 12°C (Fig. 2b).

To evaluate the influence of temperature on the growth rate throughout the full temperature assayed range, the expanded square root eqn (5) was applied. The equation fitted consistently with data ($R^2 = 0{\cdot}99$), and in addition, it allowed us to estimate the 'cardinal temperatures' ($T_{min} = -9{\cdot}24 \pm 2{\cdot}55$, $T_{max} = 30{\cdot}27 \pm 0{\cdot}15$ and $T_{opt} = 17{\cdot}29 \pm 0{\cdot}004$), which characterized the *P. antarctica* NF_3 growth (Fig. 3).

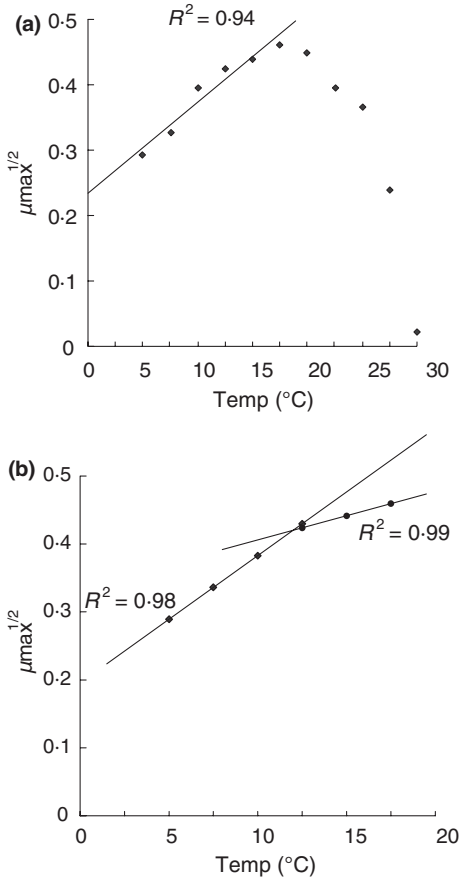

Figure 2 Effect of temperature on specific growth rate of *Pseudoalteromonas antarctica* NF3 according to square root model for suboptimal growth temperatures. (a) Data adjusted to a linear segment; (b) data adjusted to two linear segments.

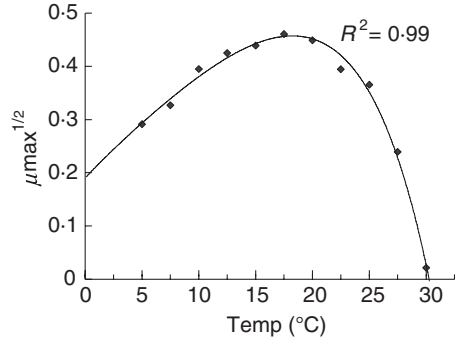

Figure 3 Effect of temperature on specific growth rate of *Pseudoalteromonas antarctica* NF3 according to square root model for the full biokinetic temperature range.

The lag time (λ)

The temperature had a significant effect on *P. antarctica* NF_3 lag time (λ). As shown in Table 1, the lag phase duration was notably reduced from approximately 28 h at 5°C to 3·4 h at 25°C. Above 25°C, the lag time began to increase until growth ceased at temperatures near 30°C. The lag time value at 30°C was omitted in Table 1 because the Gompertz model yielded a negative value, which is mathematically but not microbiologically possible. RLT were used to evaluate the temperature effect on this kinetic parameter (Table 2). In the suboptimal temperature range (12·5–17·5°C), RLT values were very similar. Below this range (cold domain), the RLT differed significantly ($P < 0·05$) from each other.

Table 2 Relative lag time (RLT) obtained for *Pseudoalteromonas antarctica* NF_3 grown at different incubation temperatures

Temperature (°C)	RLT	±SE
5	7·90	0·50
7·5	8·52	0·46
10	11·01	0·67
12·5	10·26	0·34
15	10·53	0·55
17·5	10·35	0·59

The maximum population density (A)

The results obtained for the kinetic parameter of growth yield (A) for *P. antarctica* NF_3 cultures at the different temperatures are listed in Table 1. The maximum value was reached near the optimal temperature of growth (17–18°C), although this parameter showed no significant changes owing to temperatures in the normal or physiological range of growth (5–18°C). At higher temperatures, this parameter diminished drastically.

EPS production by *Pseudoalteromonas antarctica* NF_3

The EPS yield was estimated at the beginning of the stationary phase when EPS accumulation reached the maximum. The EPS yield of *P. antarctica* NF_3 was maximal at lower incubation temperatures (≤10°C), with more than 200 mg of crude EPS per gram of dry weight (Fig. 4a). EPS yield decreased at temperatures above the critical temperature, and at temperatures around the optimal (18°C), it was four times less than the yields obtained in the cold domain. EPS production was negligible at temperatures above 25°C.

The protein content of the total crude EPS was high at all temperatures, containing an average of 55% protein. The highest protein percentages were detected at 12·5 and 10°C, being 65·7% and 63·2%, respectively, but differences were not significant with respect to other temperatures (Fig. 4b). Finally, EPS composition showed that

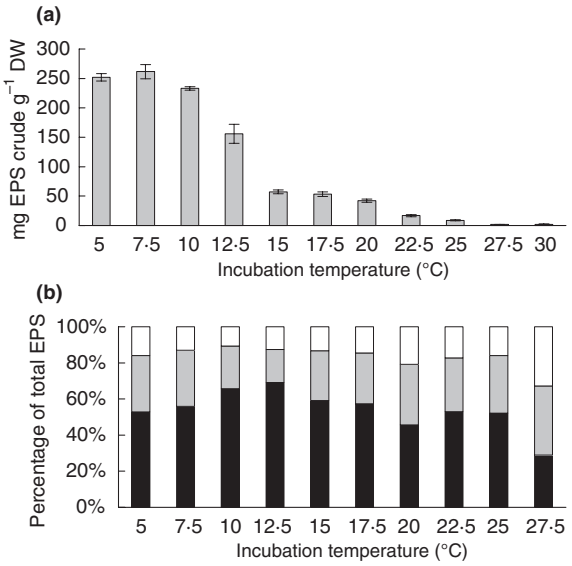

Figure 4 (a) Yield of extracellular polymeric substances (EPS) (milligram EPS per gram dry weight of cells) from batch cultures of *Pseudoalteromonas antarctica* NF_3 incubated at different temperatures. (b) Composition of crude EPS produced by *P. antarctica* NF_3 at different incubation temperatures. (■), % protein; (▨), total carbohydrate; (□), % other components.

carbohydrate percentages were very similar at different temperatures.

Discussion

Several predictive models, such as the Gompertz, the logistic and others, are commonly used to determine important biokinetic parameters relating to bacterial growth (Zwietering *et al.* 1990; Skinner *et al.* 1994). In our study, these parameters were calculated by fitting the experimental data of *P. antarctica* NF_3, grown over the range of temperatures from 5 to 30°C, to the Gompertz model. This model estimated soundly biokinetic parameters of bacterial growth, as demonstrated by the high coefficients of determination (R^2) obtained. The use of this model allowed us to calculate the three parameters simultaneously and compare easily the effect of temperature on the biokinetic parameters studied. Separate calculations without a coherent model would be confusing and produce biased estimates (Shi and Xia 2003).

Pseudoalteromonas antarctica NF_3 is considered a psychrotolerant bacterium particularly abundant in Antarctic coastal water (Bozal *et al.* 1997; Bowman 1998). Adaptation of *P. antarctica* NF_3 to the different temperatures studied was determined by means of the Arrhenius law and the square root model. Under laboratory conditions, *P. antarctica* has a T_{opt} around 17–18°C, at which it displays its maximum growth rate, and a $T_{critical}$ of 12°C. The value of this critical temperature was the same when obtained from the Ratkowsky profile in suboptimal conditions that was characterized by two slopes joined at a temperature called the change temperature in agreement with Bajard and Brouillaud (1995). These calculated temperatures demonstrate the capacity of *P. antarctica* NF_3 to grow at temperatures considerably above those *in situ* temperature of the Antarctic environment from which it was isolated, which could be relevant from an ecological point of view, if the bacterium is confronted with thermal fluctuations of its environment. Recently, we verified the capacity of *P. antarctica* NF_3 to grow over a wider range of temperatures from −4 to 30°C (data not shown), in agreement with the predicted cardinal temperatures obtained when the extended equation of Ratkowsky was applied. The mean annual temperature in King George Island (South Shetland, Antarctica) is approximately −2°C, but temperatures of 8·2°C have also been attained (March 1987). These temperatures are still under the $T_{critical}$, but we have to recall that $T_{critical}$ was determined from growth in flasks where the bacteria encountered a sole source of substrate (glucose) at a concentration higher than would be expected in the Antarctic environment. This analysis also excludes the influence of other parameters, such as substrate affinity or growth yield that may affect the bacterial growth and competitiveness of micro-organisms in their habitats (Nedwell and Rutter 1994; Isaksen and Jørgensen 1996; Arnosti *et al.* 1998; Mancuso Nichols *et al.* 2005b; Tarpgaard *et al.* 2005). This situation has been commonly observed among other cold-adapted micro-organisms cultivated under laboratory conditions and does not mean they are poorly adapted to their environmental temperature (Russell and Hamamoto 1998; Cavicchioli *et al.* 2003).

In the cold domain from 5°C to *c.* 12°C, the temperature characteristic (16·7 kcal mol^{-1}) was about three times higher than in the suboptimal temperature domain (5·3 kcal mol^{-1}) from *c.* 12°C to 17–18°C. Similar Arrhenius profiles have been reported for other psychrotolerant bacteria, such as *Pseudomonas fluorescens* MF0 (Guillou and Guespin-Michel 1996), *Pseudomonas putida* 01G3 (Chablain *et al.* 1997), *Bacillus cereus* TZ415 (Choma *et al.* 2000) and *Erwinia carotovora* ssp. *atroseptica* CFBP 6276 (Smadja *et al.* 2004). It has been hypothesized that critical temperature is a pivotal temperature, which exists as a result of increased energy demands, the synthesis of stress proteins and/or the use of alternative metabolic pathways at low temperatures (Guillou and Guespin-Michel 1996; Choma *et al.* 2000; Bakermans and Nealson 2004). This low temperature characteristic for *P. antarctica* NF_3 could mean that it uses less energy to grow at low temperatures, and thus, would be well adapted to cold environments.

Both secondary models used in our study to describe the effect of temperature on growth rate were statistically valid to adjust the values obtained from the primary model. Some authors suggest that the root square model is better to interpret the effect of temperature than the Arrhenius-type model (Stannard *et al.* 1985; Cayré *et al.* 2003) and some of them attribute this to the fact that the Arrhenius equation was originally used to describe the thermodependence of specific growth in simple chemical reactions (Ratkowsky *et al.* 2005). In our case, the adjustment with both models was similar although the root square equation was better (lower MSE) than the Arrhenius model to predict *P. antarctica* growth. Nevertheless, the Arrhenius-type model was useful to interpret thermodynamically the effect of temperature on the growth rate below the T_{opt}.

Concerning the effect of temperature on the maximum population densities (A), we found for *P. antarctica* NF_3 that the growth yield peaked near the optimal temperature and then stayed nearly constant between T_{opt} and the minimum assayed temperature (5°C). Similar results were also reported for other psychrotolerant bacteria that maximize or maintain their growth yields below the optimal temperature and throughout the normal or physiological temperature range (Isaksen and Jørgensen 1996; Knoblauch and Jorgensen 1999). Bakermans and Nealson (2004) postulated that the maximization or maintenance of biomass and not of growth rates is the most beneficial survival strategy for growth at low temperatures.

Temperature variation in *P. antarctica* NF_3 cultures also influenced lag phase duration. For *P. antarctica*, in the normal physiological range (from 18 to 5°C), it was apparent that, as incubation temperature decreased, lag time increased. The lag phase duration is affected by many variables, and bacterial responses to changes in the environment are complex and difficult to characterize. To evaluate the effects of temperature on the lag times, the RLT concept was employed following Mellefont *et al.* (2003). These authors sought to quantify the amount of work that a population has to perform in order to adjust to a new environment, regardless of the rate at which that work is done. For *P. antarctica* NF_3, if RLT are plotted against a scale reflecting the magnitude of the change in the environment, a horizontal straight-line relationship is observed in the suboptimal domain. This constancy means no extra work was required to respond to changes in this portion of temperatures. For the cold domain, RLT decreased with temperature, meaning *P. antarctica* needs less work to adapt to the environment and thus is well adapted to this range of temperatures.

Another response to low temperatures frequently observed in Antarctic strains from the genus *Pseudoalteromonas* is the production of high concentrations of EPS (Bozal *et al.* 1997; Mancuso Nichols *et al.* 2004, 2005a). Exopolymer production requires a significant carbon and energy investment for the bacterial cell. However, benefits derived from exopolymer production are evident, such as the enhancement of bacterial growth and survival under harsh environmental conditions (Wolfaardt *et al.* 1999; Mancuso *et al.* 2005b). For *P. antarctica* NF_3, the highest crude EPS production was found at temperatures included in the cold domain (5–12°C). At the suboptimal domain, crude EPS production decreased, being almost four times lower at the optimal temperature than in the cold domain. The crude EPS obtained from the culture broths of *P. antarctica* NF_3 grown at various temperatures consisted mainly of proteins and carbohydrates being part of a capsular polymer and large numbers of OMV (Nevot *et al.* 2006a,b).

The use of the predictive Gompertz model together with the secondary Arrhenius-type and the square root models allowed us to calculate the biokinetic growth parameters easily and to evaluate the influence of incubation temperature on them for the psychrotolerant bacterium *P. antarctica* NF_3. All calculated parameters reveal an optimal adaptation of this strain to cold temperatures. The highest yields of EPS by *P. antarctica* NF_3 obtained in the cold domain suggests that EPS may have a role in facilitating fitness to low temperatures, although more studies need to be conducted to define the specific actions of EPS in the cold.

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