Extremophiles (2003) 7:451–458
DOI 10.1007/s00792-003-0347-2

ORIGINAL PAPER

Rosa Margesin · Silvia Gander · Gabriele Zacke
Anne Monique Gounot · Franz Schinner

Hydrocarbon degradation and enzyme activities of cold-adapted bacteria and yeasts

Received: 1 February 2003 / Accepted: 27 June 2003 / Published online: 26 August 2003
© Springer-Verlag 2003

Abstract The potential of 89 culturable cold-adapted isolates from uncontaminated habitats, including 61 bacterial and 28 yeast strains, to utilize representative fractions of petroleum hydrocarbons (*n*-alkanes, monoaromatic and polycyclic aromatic hydrocarbons) for growth and to produce various enzymes at 10°C was investigated. The efficiency of bacterial and yeast strains was compared. The growth temperature range of the yeast strains was significantly smaller than that of the bacterial strains. Sixty percent of the yeasts but only 8% of the bacteria could be classified as true psychrophiles, showing no growth above 20°C. A high percentage (89%) of the yeast strains showed lipase activity. More than one-third of the 61 bacterial strains produced amylase, β-lactamase, β-galactosidase or lipase; more than two-thirds were protease producers. Only 6% of the bacterial strains but 79% of the yeast strains utilized *n*-hexadecane for growth; 13% of the bacterial strains and 21–32% of the yeast strains utilized phenol, phenanthrene or anthracene for growth. Only four yeast strains but none of the bacterial strains could grow with all hydrocarbons tested. The biodegradation of phenol was investigated in fed-batch cultures at 10°C. Three yeast strains degraded phenol concentrations as high as 10 mM (one strain) or 12.5 mM (two strains). Of eight bacterial strains, two strains degraded up to 10 mM phenol. The optimum temperature for phenol degradation was 20°C for all eight bacterial strains and for two yeast strains. Biodegradation by five yeast strains was optimal at 10°C and faster at 1°C than at 20°C. All phenol-degrading strains produced catechol 1,2 dioxygenase activity.

Keywords Bacteria · Biodegradation · Cold-adapted · Enzymes · Hydrocarbons · Phenol · Psychrophilic · Yeasts

Communicated by K. Horikoshi

R. Margesin (✉) · S. Gander · G. Zacke · F. Schinner
Institute of Microbiology, University of Innsbruck,
Technikerstrasse 25, 6020 Innsbruck, Austria
E-mail: rosa.margesin@uibk.ac.at
Fax: +43-512-5072929

A. M. Gounot
University Claude Bernard Lyon 1, Villeurbanne, France

Introduction

The potential of hydrocarbon-degrading microorganisms has led to the development of bioremediation techniques for contaminated soil and water (Dua et al. 2002). Cold-adapted indigenous microorganisms play a significant role in the in-situ biodegradation of hydrocarbons in cold environments, where temperatures often coincide with their growth temperature range. The aerobic biodegradation of many components of petroleum hydrocarbons, including *n*-alkanes, aromatic and polyclic aromatic hydrocarbons (PAHs), at low temperatures has been reported in arctic, alpine and antarctic environments (for a review, see Margesin and Schinner 2001).

A wide variety of bacteria, fungi and algae have the ability to metabolize aliphatic and aromatic hydrocarbons (Alexander 1999). A large number of degradative cold-tolerant bacteria have been identified, including representatives of heterotrophic aerobic Gram-negative and Gram-positive genera (Whyte et al. 1998; Yakimov et al. 1999; Aislabie et al. 2000; Bej et al. 2000; Baraniecki et al. 2002). Filamentous fungi are known for their potential to degrade PAHs (Cerniglia 1992; Gramss et al. 1999). There is, however, little information about the hydrocarbon-degradative potential of yeasts. Culturable cold-adapted yeasts have been isolated from oil-contaminated antarctic (Aislabie et al. 2001) and alpine soils (Margesin and Schinner 1997a), and from glacier cryoconite (Margesin et al. 2002).

Cold-adapted microorganisms are a potentially exploitable source for cold-active enzymes (Gerday et al.

2000; Margesin 2002). Screenings for cold-active enzyme producers have been mainly focused on bacteria, while yeasts have been rarely considered (Birgisson et al. 2003).

It was the objective of this study to investigate the potential of 89 culturable cold-adapted isolates to utilize aerobically representative fractions of petroleum hydrocarbons (*n*-alkanes, aromatic hydrocarbons, PAHs) for growth, and to produce various enzymes at low temperatures. The efficiency of bacterial and yeast strains and their growth temperature ranges were compared. The biodegradation of phenol was characterized by determining the effect of temperature and the phenol concentration on biodegradation and by determining the presence of catechol dioxygenases.

Materials and methods

Strains

Eighty-nine isolates, 61 bacterial and 28 yeast strains, were tested. These strains were isolated on complex medium as described from three alpine glacier cryoconite samples (Margesin et al. 2002) and various alpine, Sibirian and antarctic cold environments, such as ice caves, continental ice and mud in the thawing zone at the foot of glaciers. To distinguish between bacterial and yeast cells, the effect of cycloheximide (inhibitor of fungal growth) on growth was determined. Strains were grown on R2A agar plates containing 400 μg cycloheximide ml^{-1} at 10°C for 14 days. Phenol-degrading strains were identified by Margesin et al. (2002) and by DSMZ (German Culture Collection of Microorganisms, Braunschweig, Germany).

Media

The strains were routinely cultivated on R2A agar plates. The ability to utilize various hydrocarbons for growth was tested using a pH-neutral phosphate-buffered mineral medium (Margesin and Schinner 1997b).

Growth temperature range

Suspensions of microbial cells (pre-grown on R2A agar plates at 10°C) in 0.9% NaCl were used to inoculate R2A agar plates which were incubated at 2, 10, 15, 20, 25, 30 and 35°C, using two replicates per strain and temperature. Growth was monitored up to an incubation time of 7–21 days.

Screening for enzyme activities

Amylase, protease, pectolytic enzymes, cellulase, β-galactosidase, lipase and β-lactamase activities were tested on R2A agar plates supplemented with starch, skim milk, polygalacturonic acid (each compound 0.4% w/v), carboxymethylcellulose and trypan blue (0.4% and 0.01% w/v, respectively), lactose and X-Gal (0.2% and 0.004% w/v, respectively), tributyrin (0.5% v/v) or ampicillin (50 μg ml^{-1}). After 3–6 days at 10°C, a positive reaction was noticed when transparent zones around the colonies were directly visible or detected after precipitation or coloration of the undegraded substrate. In the case of β-lactamase activity, a positive reaction was indicated by growth in the presence of ampicillin.

Screening for the microbial utilization of hydrocarbons for growth

All 89 isolates were screened for their ability to utilize aerobically aliphatic and aromatic hydrocarbons in liquid media for growth. Inoculated medium without hydrocarbons, as well as sterile hydrocarbon-containing medium, served as negative controls. Growth was determined spectrophotometrically at 600 nm. Cultures with an OD(600 nm) > 0.2 were scored as positive for growth (Bej et al. 2000). Two to three replicates were used for all experiments and the mean values obtained are reported. The standard deviations obtained were ≤10%.

n-alkanes (n-dodecane, n-hexadecane)

The screening was performed in 100-ml Erlenmeyer flasks containing 10 ml mineral medium. The medium was contaminated with *n*-hexadecane or *n*-dodecane (99% purity, 1 g l^{-1}; corresponding to 4.4 mM hexadecane and 5.9 mM dodecane) and inoculated with 250 μl of a dense suspension of microbial cells (pre-grown on R2A agar plates at 10°C) in 0.9% NaCl. After an incubation period of 14–21 days at 10°C, growth was determined. The residual hydrocarbon concentration was quantified by infra-red-spectroscopy after extraction with trichoro-trifluoro-ethane (McLean et al. 1995).

Aromatic hydrocarbons (phenol)

The screening was done in 100-ml Erlenmeyer flasks containing 10 ml mineral medium. The medium was contaminated with 2.5 mM (235 mg l^{-1}) phenol (99.5% purity) and inoculated with 250 μl of a dense suspension of microbial cells (pre-grown on R2A agar plates at 10°C) in 0.9% NaCl. After an incubation period of 7 days at 10°C, growth was determined. The residual phenol concentration was determined according to Bastos et al. (2000a) by measuring the optical density at 270 nm in the culture supernatants using a calibration curve (0–1.4 mM phenol) prepared in mineral medium.

PAHs (phenanthrene, anthracene)

This screening was performed in microtiter plates (flat bottom, 96 wells), using 200 μl mineral medium per well. Each well received 10 μl of a stock solution of anthracene or phenanthrene (> 96% purity) dissolved in acetone to give a final concentration of 10 mg PAH l^{-1} (5.6 μM). The acetone was allowed to evaporate at room temperature. Afterwards, the PAH-containing medium was inoculated with 10 μl of a dense suspension of microbial cells (pre-grown on R2A agar plates at 10°C) in 0.9% NaCl. Microtiter plates were wrapped in plastic bags. After an incubation period of 14 days at 10°C, growth was determined using a microplate reader.

Phenol biodegradation

Fed-batch cultivation

Phenol-degrading strains were cultivated at 10°C and 180 rpm in 100-ml Erlenmeyer flasks containing 10 ml mineral medium that was contaminated with 1 mM phenol. After 7 days of cultivation and after the disappearance of the contamination, the same culture was re-contaminated with increasing phenol concentrations, ranging from 2.5 to 15 mM phenol. With each phenol amendment, the volume was adjusted to 10 ml by adding fresh mineral medium (2–3 ml). Growth and the residual phenol concentration were monitored at regular time intervals.

Effect of temperature on phenol biodegradation

Phenol-degrading strains were cultivated in phenol-containing mineral medium at 1, 10 and 20°C (15°C for strain AG17, which cannot grow at 20°C) and 180 rpm. Growth and the residual phenol concentration were monitored up to an incubation time of 16 days.

Presence of catechol dioxygenases

The presence of catechol dioxygenases, which catalyze the ring cleavage of catechol, was tested using qualitative assays in microtiter plates. Catechol 1,2 dioxygenase activity (C1,2D) was tested as described by Neidle and Ornston (1986) and Birger et al. (1997). A solution (150 μl) containing 0.004% phenol red, 1 mM EDTA and 10 mM catechol (pH 7.5 adjusted with ammonium hydroxide) was added to 50 μl of the liquid culture of phenol-degrading strains [OD(600 nm) adjusted to 1]. The presence of C1,2D resulted in a color change from red to yellow-orange within 10 min in the dark at room temperature. Catechol 2,3 dioxygenase activity (C2,3D) was determined as described by Morgan et al. (1989) and Birger et al. (1997). A solution (150 μl) containing 90 mM catechol in 50 mM Tris-acetate buffer (pH 7.5) was added to 50 μl of the liquid culture of phenol-degrading strains [OD(600 nm) adjusted to 1]. The presence of C2,3D resulted in the formation of a green-brownish color within 2 h in the dark at room temperature.

Results

Growth temperature range of bacterial and yeast strains

All strains could grow at temperatures ranging from 2 to 15°C; only few (3% of the bacteria and yeasts, respectively) showed no growth at 20°C. Interestingly, the growth temperature range of the yeast strains was significantly smaller than that of the bacterial strains. Compared to bacteria, only a small proportion of the yeasts could grow at 25 and 30°C (Fig. 1). Sixty percent of the yeasts but only 8% of the bacteria could be classified as true psychrophiles, showing no growth above 20°C.

Screening for enzyme activities

A considerable number of isolates produced enzymes. All 28 yeast strains had β-lactamase activity, and a high percentage (89%) of the yeast strains showed lipase activity, but none of them produced β-galactosidase or pectolytic enzymes. More than one-third of the 61 bacterial strains produced amylase, β-lactamase, β-galactosidase or lipase; more than two-thirds were protease producers (Table 1).

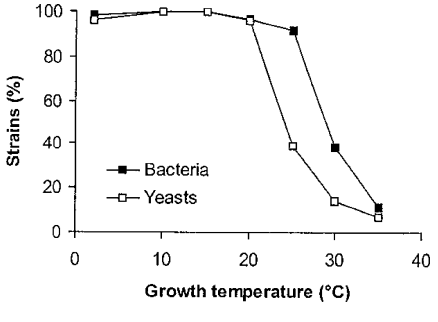

Fig. 1 Growth temperature range of cold-adapted bacteria (61 = 100%) and yeasts (28 = 100%)

Screening for the microbial utilization of hydrocarbons for growth

n-alkanes (n-dodecane, n-hexadecane)

Due to the different melting points of the two tested *n*-alkanes, *n*-dodecane with a melting point of −12°C remained liquid at 10°C, whereas *n*-hexadecane with a melting point of 18.2°C became solid at 10°C. This may have influenced the bioavailability of the compounds to the microorganisms. There was also a significantly higher abiotic loss of *n*-dodecane (14, 28, 48 and 67% after 1, 3, 6 and 10 days, respectively, at 10°C) than of *n*-hexadecane (8 and 10% after 8 and 14 days, respectively, at 10°C) in sterile controls.

In spite of the solidification of hexadecane at 10°C, 26 of the 89 tested strains could utilize this *n*-alkane for growth. Among these strains were 4 bacterial and 22 yeast strains (6.6% of the tested bacterial strains and 78.6% of the 28 yeast strains). However, only 7 of the 89 strains showed an OD(600 nm) > 0.2 when *n*-dodecane was present (Table 2). The *n*-dodecane utilizers were yeast strains and were also able to utilize *n*-hexadecane for growth.

The degradation of *n*-dodecane and *n*-hexadecane by a selected yeast strain that showed significantly better growth than any other strain [OD(600 nm) > 1], was investigated. This strain degraded 39.9% and 35.4% of hexadecane and dodecane (1 g l^{-1}), respectively, after 8 days at 10°C. After 5 days at 15°C, 50% and 73% of hexadecane and dodecane were degraded. The strain has been identified as *Yarrowia lipolytica* RM7/11 and has been found to be an efficient diesel-oil degrader at low temperatures (Margesin and Schinner 1997a), showing maximum oil-degradation activity at 0–20°C and no growth above 30°C.

Aromatic hydrocarbons (phenol)

Fifteen of the tested 89 strains showed an OD (600 nm) > 0.2 when grown with 2.5 mM phenol. Eight of these strains were bacteria (13.1% of 61 bacterial strains) and 7 were yeasts (25% of 28 yeast strains) (Table 2). All strains degraded 2.5 mM phenol within 3–7 days at 10°C. Bacterial strains were identified as *Arthrobacter* spp. (two strains) or *Pseudomonas* spp. (six strains) (Margesin et al. 2002). Yeast strains were classified as *Rhodotorula* spp. (three strains), *Candida* sp. (one strain) or *Cryptococcus* spp. (two strains) (Table 3). Both *Cryptococcus* spp. strains were tentatively

Table 1 Potential of 61 bacterial (=100%) and 28 yeast (=100%) strains to produce enzymes at 10°C

Enzyme activity	Bacteria (100% = 61)		Yeasts (100% = 28)		Total (100% = 89)	
	No.	%	No.	%	No.	%
Amylase	23	37.7	7	25.0	30	33.7
Protease	43	70.5	6	21.4	49	55.1
Cellulase	7	11.5	7	25.0	14	15.7
β-galactosidase	22	36.1	0	0	22	24.7
β-lactamase	31	50.8	28	100	59	66.3
Lipase	23	37.7	25	89.3	48	53.9
Pectolytic enzymes	4	6.6	6	21.4	10	11.2

Table 2 Potential of 61 bacterial (=100%) and 28 yeast (=100%) strains to utilize hydrocarbons for growth at 10°C [OD(600 nm) > 0.2]

Hydrocarbons	Bacteria (100% = 61)		Yeasts (100% = 28)		Total (100% = 89)	
	No.	%	No.	%	No.	%
n-dodecane	0	0	7	25.0	7	7.9
n-hexadecane	4	6.6	22	78.6	26	29.2
Phenol	8	13.1	7	25.0	15	16.9
Phenanthrene	8	13.1	6	21.4	14	15.7
Anthracene	8	13.1	9	32.1	17	19.1

identified as *Cryptococcus aerius* Nannizzi within the *Cryptococcus albidus* group. One yeast strain could not be classified as belonging to any of the described yeast species; however, the strain could be identified as a heterobasidiomycete.

PAHs (phenanthrene, anthracene)

The two tested three-ring aromatics were utilized to a similar extent. After 14 days at 10°C, 14 and 17 of the tested 89 strains utilized phenanthrene or anthracene, respectively, for growth [OD(600 nm) > 0.2]. Among these strains were 13% of the 61 bacterial strains, and 21 or 32% (phenanthrene or anthracene) of the 28 yeast strains. All these strains originated from alpine glacier cryoconite. The best growth was observed with two bacterial strains showing an OD(600 nm) > 0.8, which was higher than the OD measured in controls containing glucose as carbon source (data not shown). Both strains were isolated from different samples of alpine-glacier cryoconite and were tentatively identified as belonging to the genus *Chryseomonas* sp. (Margesin et al. 2002).

Considering the data given here on the microbial utilization of aliphatic and aromatic hydrocarbons for growth, the following pattern was obtained. The ability to utilize at least one of the tested hydrocarbons (hexadecane, phenol, phenanthrene or anthracene) for growth [OD(600 nm) > 0.2], was restricted to 41 of the 89 strains investigated. Among these strains were 16 bacterial strains (26.2% of 61 strains) and 25 yeast strains (89.3% of 28 strains). Only 4 yeast strains (three *Rhodotorula* spp. and one *Candida* sp.) but none of the bacterial strains could grow with all hydrocarbons; all of them were isolated from alpine glacier cryoconite and did not grow at temperatures above 20°C.

Biodegradation of phenol

Fed-batch cultivation

The 15 strains that utilized phenol for growth were selected to study the effect of the phenol concentration on biodegradation using fed-batch cultivation, whereby the same culture was re-contaminated with increasing phenol concentrations as soon as the phenol from the previous amendment had disappeared. The same pattern was observed with all strains: growth increased as long as phenol was degraded but decreased rapidly when the phenol concentration became toxic. With increasing phenol concentration, the lag-phases increased due to substrate inhibition by the toxic substrate phenol. Figure 2 shows the changes in OD and the remaining phenol concentration during the cultivation of two yeast strains that tolerated high phenol concentrations. The drop in OD at the time of phenol amendments was caused by the addition of fresh medium (volume adjustment). Since there was no abiotic loss of phenol in sterile controls, phenol disappearance had to be attributed to biodegradation.

Three yeast strains (two *Cryptococcus* spp. and one heterobasidiomycete; Table 3) degraded phenol concentrations as high as 10 mM (one strain; Fig. 2, upper part) or 12.5 mM (two strains; Fig. 2, lower part). Up to 5 mM phenol was degraded within 3 days, while 7 days were needed for the degradation of 7.5 and 10 mM phenol. The degradation of 12.5 mM phenol was complete after 18 days (Fig. 2). The rapid decrease in microbial growth after the last phenol addition might be caused by the toxic effects of high phenol concentrations or by the accumulation of toxic intermediates.

Four yeast strains from alpine glacier cryoconite (Table 3) degraded up to 5 mM phenol over a total cultivation period of 24 days (7 days each with 1 and 2.5 mM, and 10 days with 5 mM). However, no complete degradation was observed; 1.3–1.5 mM phenol remained after 10 days of incubation with 5 mM phenol. A brownish color was noticed in the cultures of all four strains, indicating the presence of phenol oxidases.

Table 3 Characterization of phenol-degrading cold-adapted yeasts and bacteria (T_{max} maximum temperature at which growth in R2A medium occurred; *C1,2D* presence of catechol 1,2 dioxygenase; *C2,3D* presence of catechol 2,3 dioxygenase; + positive reaction; (+) weak reaction, − negative reaction; [*Phenol*$_{max}$] highest phenol concentration degraded at 10°C)

Strain	Isolated from	Identification	T_{max} for growth (°C)	Effect of temperature (°C) on phenol degradation	Effect of temperature on biomass formation (OD)	C1,2D	C2,3D	[$Phenol_{max}$] (mM)
Yeasts								
AG 15	Alpine ice cave (A)	*Cryptococcus* sp.	20	20 > 10 > 1	10 > 1 > 20	+	−	12.5
AG 21	Alpine glacier foot (mud in the thawing zone; F)	*Cryptococcus* sp.	20	10 > 1 > 20	10 > 20 = 1	+	−	12.5
AG 17	Alpine ice cave (A)	Heterobasidiomycete	15	10 > 1 > > 15	10 > 1 > 15	+	−	10
A 10	Alpine glacier cryoconite (A)	*Rhodotorula* sp.	20	10 > 1 > 20	10 > 20 = 1	+	−	5
A 11	Alpine glacier cryoconite (A)	*Rhodotorula* sp.	20	10 > 1 > > 20	10 > 1 > > 20	+	−	5
A 19	Alpine glacier cryoconite (A)	*Candida* sp.	20	1 = 10 > > 20	1 > 10 > 20	+	−	5
A 43	Alpine glacier cryoconite (A)	*Rhodotorula* sp.	20	20 > 10 > 1	10 > 20 > 1	+	+	5
Bacteria								
AG 30	Alpine ice cave (A)	*Arthrobacter* sp.	25	20 > 10 > 1	1 > 10 > 20	+	+	10
AG 31	Alpine ice cave (A)	*Arthrobacter* sp.	25	20 > 10 > 1	1 > 10 > 20	+	+	10
B41	Alpine glacier cryoconite (A)	*Pseudomonas* sp.	25	20 > 10 > 1	10 > 1 = 20	+	(+)	2.5
B 48	Alpine glacier cryoconite (A)	*Pseudomonas* sp.	30	20 > 10 > 1	10 > 1 = 20	(+)	−	2.5
C 16	Alpine glacier cryoconite (A)	*Pseudomonas* sp.	25	20 > 10 > 1	1 > 10 > 20	+	(+)	2.5
C 31	Alpine glacier cryoconite (A)	*Pseudomonas* sp.	25	20 > 10 > > 1	10 > 20 > 1	+	(+)	2.5
C 32	Alpine glacier cryoconite (A)	*Pseudomonas* sp.	25	20 > 10 > 1	10 > 1 > 20	+	(+)	2.5
C 34	Alpine glacier cryoconite (A)	*Pseudomonas* sp.	25	20 > 10 > 1	10 > 1 > 20	+	(+)	2.5

Of the eight bacterial strains, six strains (*Pseudomonas* spp.) could not degrade phenol concentrations higher than 2.5 mM, while two strains (*Arthrobacter* spp.) degraded 10 mM phenol within 7 days (Table 3).

Effect of temperature on phenol biodegradation

The optimum temperature for phenol degradation was 20°C for all eight bacterial strains and for two yeast strains. Five yeast strains, however, had an optimum temperature of 10°C, and biodegradation was faster at 1°C than at 20°C or 15°C. In one case, biodegradation performance at 1°C and 10°C was comparable (Table 3). Biomass formation (OD) was always greater at 10°C than at 20°C. Three bacteria and one yeast strain showed even higher biomass production at 1°C (Table 3).

Presence of catechol dioxygenases

C1,2D was detected with all strains tested, indicating the oxidation of catechol by the *ortho* type of ring cleavage. Some strains also produced C2,3D; these strains are able to oxidize catechol by the *meta* type of ring cleavage (Table 3).

Discussion

A wide variety of bacteria, fungi and algae have the ability to metabolize aliphatic and/or aromatic hydrocarbons. Cold-adapted hydrocarbon-degrading microorganisms are predominantly bacteria (Chablain et al. 1997; Aislabie et al. 1998, 2000; Whyte et al. 1998; Yakimov et al. 1999; Bej et al. 2000). We observed a significantly lower percentage of culturable *n*-dodecane utilizers compared to *n*-hexadecane utilizers, which we attribute to the higher abiotic loss and probably also to the higher toxicity of *n*-dodecane. Olivera et al. (1997) attributed the low dodecane biodegradation to the rapid evaporation of this compound. In our study, we found a higher percentage of culturable yeast than of bacterial strains able to utilize *n*-hexadecane, phenol or two three-ring aromatics (phenanthrene and anthracene) for growth at 10°C (Table 2). The ability to utilize *n*-hexadecane was especially widespread among the investigated yeast strains. Interestingly, only four yeast strains but none of the bacterial strains could grow with both aliphatic and aromatic hydrocarbons. Thus, cold-adapted yeasts appear to have a versatility to utilize hydrocarbons. In comparison, many of the 200 investigated bacterial strains mineralized aliphatic or aromatic hydrocarbons but not both (Foght et al. 1990). About one-third of hydrocarbon-degrading bacteria had both catabolic pathways for aliphatic and aromatic hydrocarbons degradation (Sotsky and Atlas 1994).

Special attention was payed in this study to the degradation of phenol. Phenolic compounds are common

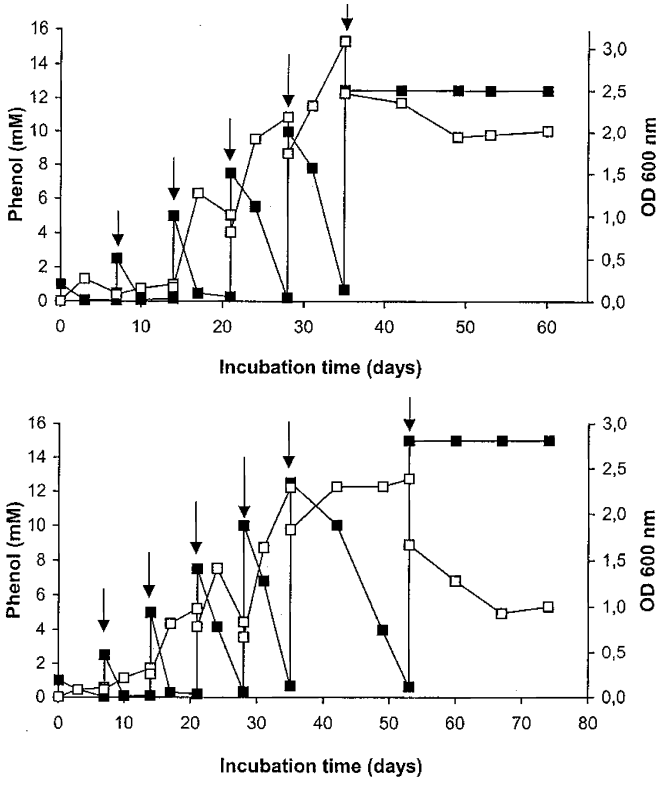

Fig. 2 Residual phenol concentration (■) and growth (optical density, □) as a function of incubation time at 10°C in the fed-batch cultures of two psychrophilic yeast strains (*upper part*: heterobasidiomycete strain AG 17; *lower part*: *Cryptococcus* sp. strain AG 21). Phenol amendments are represented by *arrows*

constituents of wastewaters from the oil industry. Due to their toxicity to microorganisms, phenolic compounds can cause the breakdown of wastewater-treatment plants by inhibition of microbial growth, even at relatively low concentrations such as >2 mM (Li and Humphrey 1989). In our study, phenol concentrations as high as 12.5 mM could be degraded at 10°C by two yeast strains using fed-batch cultivation. This cultivation method has been proven to be efficient for the selection and acclimation of a phenol-degrading bacterial consortium (Guieysse et al. 2001). There is little information about cold-adapted phenol degraders. Kotturi et al. (1991) demonstrated low temperature degradation of 10.6 mM phenol (1 g l^{-1}) by a cold-tolerant *Pseudomonas putida*. In our study, bacterial strains (*Arthrobacter* spp.) degraded up to 10 mM, while yeast strains (*Cryptococcus* spp.) tolerated and degraded up to 12.5 mM phenol at 10°C. Mesophilic degraders of high phenol concentrations include bacterial and yeast strains. Among mesophilic yeasts, strains of *Candida tropicalis* degraded 16 mM phenol within 6 days at 29°C (Bastos et al. 2000a) and even 27 mM phenol at 30°C (Krug et al. 1985), *Trichosporon* sp. degraded 18 mM phenol during 5 days at 30°C (Santos and Linardi 2001). An inhibitory effect on yeasts was observed at concentrations higher than 11 mM phenol (Santos and Linardi 2001). Mesophilic bacteria of the genus *Pseudomonas* were reported to degrade 16 mM phenol (Babu et al. 1995) and 10.6 mM within 5 days (Hinteregger et al. 1992). In our study, 10 mM phenol was utilized both by bacteria and yeasts within 7 days at 10°C, while 14–18 days were needed for the degradation of 12.5 mM phenol.

Interestingly, the strains investigated in this study were isolated from cold environments that were not hydrocarbon-contaminated, which indicates the ubiquity of hydrocarbon-degrading bacteria and yeasts. Microorganisms able to efficiently degrade oil hydrocarbons (Margesin and Schinner 1998) and phenol (Bastos et al. 2000b) have been isolated from uncontaminated environments. However, Aislabie et al. (2001) detected culturable yeasts only in oil-contaminated antarctic soils but occasionally in pristine control soils. These authors attributed the significant enhancement in numbers of culturable yeasts and filamentous fungi in oil-contaminated cold soils to the important role of fungi in the degradation of hydrocarbons or their metabolites.

Screenings for cold-active enzyme producers have been mainly focused on bacteria, while yeasts have been rarely considered (Birgisson et al. 2003). Our data show that cold-adapted yeast strains are potentially useful as lipase producers. Lipases from yeasts are gaining industrial interest with applications in laundry detergent and dairy industries (Burden and Eveleight 1990). The use of cold-active enzymes in detergents allows colder washing cycles and thus energy-saving by lowering the temperature without a loss of enzyme activity.

Many of the yeast strains (60%) investigated in this study were true psychrophiles, showing a maximum growth temperature of 20°C, while the majority of the bacteria were cold-tolerant (92%) and could grow at temperatures above 20°C (Fig. 1). Accordingly, the optimum temperature for phenol degradation was generally higher for bacteria (20°C) than for yeast strains (10°C). Five yeast strains degraded phenol even faster at 1°C than at 20°C. Psychrophiles are found in permanently cold habitats such as ice caves, cryoconite on melting glacier ice (Gounot 1999) and melting sea ice (Bowman et al. 1997). However, even in permanently cold environments, at least 50% of the bacteria (Delille and Perret 1989) are not psychrophilic. Significantly lower numbers of bacteria that could grow at 2°C than at 20°C were found in alpine glacier cryoconite, while the opposite pattern was observed for yeasts (Margesin et al. 2002). Due to this apparently restricted growth temperature range and the low optimum temperature for efficient biodegradation, psychrophilic yeasts may offer a great potential for bioremediation processes in permanently cold climatic regions or environments where temperatures of 20°C are not exceeded, such as groundwater and subsoils. They could be useful for the construction of biosensors for the selective, sensitive and rapid monitoring, or in-situ analysis of pollution, since the broad measuring ranges and long-term viabilities of many yeast strains make them good candidates for use

as biocatalysts (Alkasrawi et al. 1999; Trosok et al. 2002).

In conclusion, cold-adapted microorganisms show a great potential for the low-temperature biodegradation of hydrocarbons. The contribution of yeasts to the biodegradation of hydrocarbons in the environment and as potentially exploitable enzyme producers may be much more important than currently expected, considering their great metabolic versatility.

References

Aislabie J, McLeod M, Fraser R (1998) Potential for biodegradation of hydrocarbons in soil from the Ross Dependency, Antarctica. Appl Microbiol Biotechnol 49:210–214

Aislabie J, Foght J, Saul D (2000) Aromatic hydrocarbon-degrading bacteria from soil near Scott base, Antarctica. Polar Biol 23:183–188

Aislabie J, Fraser R, Duncan S, Farrell RL (2001) Effects of oil spills on microbial heterotrophs in Antarctic soils. Polar Biol 24:308–313

Alexander (1999) Biodegradation and bioremediation, 2nd edn. Academic, London

Alkasrawi M, Nandakumar R, Margesin R, Schinner F, Mattiasson B (1999) A microbial biosensor based on *Yarrowia lipolytica* for the off-line determination of middle-chain alkanes. Biosensors Bioelectron 14:723–727

Babu KS, Ajithkumar, Kunhi AAM (1995) Mineralization of phenol and its derivatives by *Pseudomonas* sp.strain cp4. World J Microbiol Biotechnol 11:661–664

Baraniecki CA, Aislabie J, Foght JM (2002) Characterization of *Sphingomonas* sp. Ant 17, an aromatic hydrocarbon-degrading bacterium isolated from Antarctic soil. Microb Ecol 43:44–54

Bastos AER, Tornisielo VL, Nozawa SR, Trevors JT, Rossi A (2000a) Phenol metabolism by two microorganisms isolated from Amazonian forest soil samples. J Ind Microbiol Biotechnol 24:403–409

Bastos AER, Moon DH, Rossi A, Trevors J, Tsai SM (2000b) Salt-tolerant phenol-degrading microorganisms isolated from Amazonian soil samples. Arch Microbiol 174:346–352

Bej AK, Saul D, Aislabie J (2000) Cold-tolerant alkane-degrading *Rhodococcus* species from Antarctica. Polar Biol 23:100–105

Birger A, Krauss G, Kiesel B, Dermietzel J, Gläßer W (1997) Abbaupotential für aliphatische und aromatische Kohlenwasserstoffe in bakteriellen und heterotrophen Communities differenter Grundwasser-Biozönosen. In: Kreysa G, Wiesner J (eds) Möglichkeiten und Grenzen der Reinigung kontaminierter Gewässer. Dechema, Frankfurt, pp 571–581

Birgisson H, Delgado O, Garcia Arroyo L, Hatti-Kaul R, Mattiasson B (2003) Cold-adapted yeasts as producers of cold-active polygalacturonases. Extremophiles 7:185–193

Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA (1997) Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 63:3068–3078

Burden DW, Eveleight DE (1990) Yeasts—diverse substrates and products. In: Spencer JFT, Spencer DM (eds) Yeast technology. Springer, Berlin Heidelberg New York, pp 199–227

Cerniglia CE (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3:351–368

Chablain PA, Philippe G, Groboillot A, Truffaut N, Guespin-Michel JF (1997) Isolation of a soil psychrotrophic toluene-degrading *Pseudomonas* strain: influence of temperature on the growth characteristics on different substrates. Res Microbiol 148:153–161

Delille D, Perret E (1989) Influence of temperature on the growth potential of southern polar marine bacteria. Microb Ecol 18:117–123

Dua M, Singh A, Sethunathan N, Johri AK (2002) Biotechnology and bioremediation: successes and limitations. Appl Microbiol Biotechnol 59:143–152

Foght JM, Fedorak PM, Westlake DWS (1990) Mineralization of (^{14}C)hexadecane and (^{14}C)phenanthrene in crude oil: specificity among bacterial isolates. Can J Microbiol 36:169–175

Gerday C, Aittaleb M, Bentahir M, Chessa J-P, Claverie P, Collins T, D'Amico S, Dumont J, Garsoux G, Georlette D, Hoyoux A, Lonhienne T, Meuwis M-A, Feller G (2000) Cold-adapted enzymes: from fundamentals to biotechnology. Trends Biotechnol 18:103–107

Gounot AM (1999) Microbial life in permanently cold soils. In: Margesin R, Schinner F (eds) Cold-adapted organisms. Springer, Berlin Heidelberg New York, pp 3–15

Gramss G, Voight KD, Kirsche B (1999) Degradation of polycyclic aromatic hydrocarbons with three to seven aromatic rings by higher fungi in sterile and unsterile soils. Biodegradation 10:51–62

Guieysse B, Wikström P, Forsman M, Mattiasson B (2001) Biomonitoring of continuous microbial community adaptation towards more efficient phenol degradation in a fed-batch bioreactor. Appl Microbiol Biotechnol 56:780–787

Hinteregger C, Leitner R, Loidl M, Ferschl A, Streichsbier F (1992) Degradation of phenol and phenolic compounds by *Pseudomonas putida* EFII. Appl Microbiol Biotechnol 37:252–259

Kotturi G, Robinson CW, Inniss WE (1991) Phenol degradation by a psychrotrophic strain of *Pseudomonas putida*. Appl Microbiol Biotechnol 34:539–543

Krug M, Ziegler H, Straube G (1985) Degradation of phenolic compounds by the yeast *Candida tropicalis* HP15. J Basic Microbiol 2:103–110

Li JK, Humphrey AE (1989) Kinetic and fluorimetric behaviour of a phenol fermentation. Biotechnol Lett 11:177–182

Margesin R (2002) Cold-active enzymes as new tools in biotechnology. In: Gerday C (ed) Extremophiles. Encyclopedia of life support systems. EOLSS, Oxford (in press)

Margesin R, Schinner F (1997a) Effect of temperature on oil degradation by a psychrotrophic yeast in liquid culture and in soil. FEMS Microbiol Ecol 24:243–249

Margesin R, Schinner F (1997b) Bioremediation of diesel-oil contaminated alpine soils at low temperatures. Appl Microbiol Biotechnol 47:462–468

Margesin R, Schinner F (1998) Oil biodegradation potential in alpine habitats. Arct Alp Res 30:262–265

Margesin R, Schinner F (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl Microbiol Biotechnol 56:650–663

Margesin R, Zacke G, Schinner F (2002) Characterization of heterotrophic microorganisms in alpine glacier cryoconite. Arct Antarct Alp Res 34:88–93

McLean G, Röhler J, Buchser W (1995) Biodegradability of two-stroke cycle outboard engine oils in water. Approved test method CEC L-33-A-93, Brussels

Morgan JAW, Winstanley C, Pickup RW, Jones JG, Saunders JR (1989) Direct phenotypic and genotypic detection of a recombinant pseudomonad population released into lake water. Appl Environ Microbiol 55:2537–2544

Neidle EL, Ornston LN (1986) Cloning and expression of *Acinetobacter calcoaceticus* catechol 1,2-dioxygenase structural gene cat A in *Escherichia coli*. J Bacteriol 168:815–820

Olivera NL, Esteves JL, Commendatore MG (1997) Alkane biodegradation by a microbial community from contaminated sediments in Patagonia, Argentina. Int Biodet Biodegrad 40:75–79

Santos VL, Linardi VR (2001) Phenol degradation by yeasts isolated from industrial effluents. J Gen Appl Microbiol 47:213–221

Sotsky JB, Atlas RM (1994) Frequency of genes in aromatic and aliphatic hydrocarbon biodegradation pathways within bacterial populations from Alaskan sediments. Can J Microbiol 40:981–985

Trosok SP, Luong JHT, Juck DF, Driscoll BT (2002) Characterization of two novel yeast strains used in mediated biosensors for wastewater. Can J Microbiol 48:418–426

Whyte LG, Hawari J, Zhou E, Bourbonnière L, Inniss WE, Greer CW (1998) Biodegradation of variable-chain-length alkanes at low temperatures by a psychrotrophic *Rhodococcus* sp. Appl Environ Microbiol 64:2578–2584

Yakimov MM, Giuliano L, Bruni V, Scarfi S, Golyshin PN (1999) Characterization of Antarctic hydrocarbon-degrading bacteria capable of producing bioemulsifiers. Microbiologica 22:249–256