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Degradation of phenol and toxicity of phenolic compounds: a comparison of cold-tolerant *Arthrobacter* sp. and mesophilic *Pseudomonas putida*

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Abstract Phenol degradation efficiency of cold-tolerant *Arthrobacter* sp. AG31 and mesophilic *Pseudomonas putida* DSM6414 was compared. The cold-tolerant strain was cultivated at 10°C, while the mesophile was grown at 25°C. Both strains degraded 200 mg and 400 mg phenol/l within 48–72 h of cultivation, but the cold-tolerant strain produced more biomass than the mesophile. Both strains oxidized catechol by the *ortho* type of ring fission. Catechol 1,2 dioxygenase (C1,2D) activity was found intra- and extracellularly in the absence and in the presence of phenol. In the presence of 200 mg phenol/l, C1,2D activity of the mesophile was about 1.5- to 2-fold higher than that of the cold-tolerant strain. However, an initial phenol concentration of 400 mg/l resulted in a comparable enzyme activity of the cold-tolerant and the mesophilic strain. The two strains differed significantly in their toxicity pattern towards 12 aromatic (mostly phenolic) compounds at different growth temperatures, which was determined via growth inhibition in the presence of nutrients and toxicants. For the cold-tolerant strain, toxicity was significantly lower at 10°C than at 25°C. The mesophile showed a significantly lower susceptibility to high hydrocarbon concentrations when grown at 25°C compared to 10°C.

Keywords *Arthrobacter* · Biodegradation · Catechol dioxygenases · Cold tolerant · Phenol · *Pseudomonas*

Introduction

Phenol and phenolic compounds are widely distributed in nature and as environmental pollutants. They are common constituents of many industrial wastewaters such as those produced from crude oil refineries, coal gasification plants, phenolic resin industries, plastics manufacturing, stainless steel production, etc. Due to their toxicity to microorganisms, phenolic compounds may often cause the breakdown of wastewater treatment plants by inhibition of microbial growth (Ren and Frymier 2003), even at relatively low concentrations such as 2 mM (Li and Humphrey 1989) or even 0.25 mM (Gurujeyalakshmi and Oriel 1989).

Many microorganisms utilize phenol, phenolic and other aromatic compounds as the sole carbon and energy source. Most studies have reported phenol degradation by mesophilic aerobic microorganisms at 30°C, predominantly bacteria of the genus *Pseudomonas* (Allsop et al. 1992; Hinteregger et al. 1992). Thermophilic bacteria, mainly bacilli, have been shown to utilize and degrade phenol at 60–65°C (Mutzel et al. 1996; Feitkenhauer et al. 2001).

However, there is little information about cold-tolerant phenol degraders, although successful hydrocarbon biodegradation at low temperatures has been observed in various cold environments (for a review, see Margesin and Schinner 2001). In cold climatic regions, industrial wastewater temperature can often decrease to temperatures around and below 10°C due to seasonal fluctuations. The activity of mesophilic degraders is severely limited at this temperature, whereas cold-adapted microorganisms have evolved a series of adaptation strategies that enable them to compensate for the negative effects of low temperatures on biochemical reactions (Margesin et al. 2002). Kotturi et al. (1991) demonstrated the degradation of concentrations up to 10.6 mM phenol by a cold-tolerant *Pseudomonas putida* at 10°C. Recently, we isolated cold-tolerant bacterial and yeast strains able to utilize up to 12.5 mM and 15 mM phenol at 10°C (Margesin et al. 2003; Margesin and Schinner 2003).

In this study, we compared the phenol degradation efficiency of a mesophilic (*P. putida*, cultivated at 25°C) and a cold-tolerant bacterial strain (*Arthrobacter*

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sp., grown at 10°C). The presence and activity of catechol dioxygenases that are involved in the second step of phenol degradation and catalyze the ring cleavage of catechol was investigated. There are many reports about the intracellular production of catechol dioxygenases by phenol-degrading bacteria (Hinteregger et al. 1992; Hinteregger and Streichsbier 1997; Bastos et al. 2000b) and yeasts (Bastos et al. 2000b; Santos and Linardi 2001). The extracellular presence of these enzymes has been rarely described (Bastos et al. 2000b). Therefore, we determined extra- and intracellular enzyme activity to obtain information on the localization of catechol dioxygenases of the investigated strains. The toxicity pattern of the two strains towards various phenolic compounds and the ability to utilize these compounds for growth was also determined.

Materials and methods

Strains

Mesophilic *Pseudomonas putida* DSM6414 was purchased from the German Culture Collection (DSMZ, Braunschweig). The strain utilizes aromatic compounds (benzene, toluene, ethylbenzene, phenol) at 25–30°C (Gibson et al. 1973) and degrades up to 5 mM phenol. Cold-tolerant *Arthrobacter* sp. AG31 was isolated from an Alpine ice cave in Salzburg, Austria and was found to utilize up to 10 mM phenol at 10°C (Margesin et al. 2003). The strains were routinely cultivated on standard, environmental medium R2A agar plates, containing 0.05% yeast extract, 0.05% peptone, 0.05% tryptone, 0.05% glucose, 0.05% starch, 0.03% sodium pyruvate, 0.03% K_2HPO_4, 0.005% $MgSO_4 \times 7H_2O$, and 1.5% agar (pH 7). The utilization of hydrocarbons was determined in a phosphate-buffered minimal medium composed of 0.35% $Na_2HPO_4 \times 2H_2O$, 0.2% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.005% $Ca(NO_3)_2 \times 4H_2O$, 0.001% ammonium iron(III) citrate, and 0.02% $MgSO_4 \times 7H_2O$ (pH 7).

Effect of temperature on growth

The strains were cultivated aerobically at various temperatures (1–37°C) and 180 rpm in 100-ml Erlenmeyer flasks containing 20 ml of either complex (R2A) or minimal medium amended with phenol, using precultures of cells grown in complex or minimal medium. Growth was monitored at regular time intervals by measuring the optical density at 600 nm (OD_{600}).

Activity of catechol dioxygenases during phenol biodegradation

Cultivation

Cultivation was done aerobically in 100-ml Erlenmeyer flasks containing 20 ml medium, using three replicates. *P. putida* DSM6414 was cultivated at 25°C, *Arthrobacter* sp. AG31 at 10°C. To determine enzyme activities in the absence and in the presence of phenol, the strains were grown either in R2A medium without phenol or in minimal medium containing phenol (200 mg/l and 400 mg/l), using precultures of cells grown in complex or minimal medium, respectively. Sterile controls were used to determine abiotic phenol loss. Growth (OD_{600}) was determined after 24, 48, and 72 h of cultivation. The residual phenol concentration was quantified as described (Margesin et al. 2003) by measuring the optical density at 270 nm in the culture supernatants.

Preparation of enzyme extracts

To determine extracellular activity, culture supernatants (15 min at 12,000 rpm and 4°C) were tested for activity. Intracellular enzyme activity was measured in cell-free extracts. After centrifugation of 2 ml of culture broth, cells were suspended in 2 ml of 50 mM potassium-phosphate buffer (pH 7.5) and disrupted by 3 min of supersonic treatment (30-s intervals of sonication, followed by 30-s intervals of interruption, were applied over 6 min) in an ice-cooled bath. Solids were removed by centrifugation (15 min at 12,000 rpm and 4°C), and the supernatant was used for enzyme assays. All enzyme extracts were kept on ice and used immediately for enzyme assays.

Enzyme assays

Enzyme activities were determined after 24, 48, and 72 h of cultivation in the absence of phenol (cells grown in the complex medium) and in the presence of phenol (cells grown in minimal medium with phenol as the carbon source). Measurements of activities were performed with a recording spectrophotometer at 25°C. Catechol-1,2-dioxygenase activity (C1,2D) was determined according to Nakazawa and Nakazawa (1970) by measuring the product formation (*cis,cis*-muconic acid) from catechol at 260 nm using 50 mM potassium phosphate buffer (pH 7.5). Activity of catechol-2,3-dioxygenase (C2,3D) was assayed by the method of Nozaki (1970) by measuring the product formation (2-hydroxymuconic semialdehyde) from catechol at 375 nm using 50 mM potassium phosphate buffer (pH 7.5). One unit was defined as the amount of enzyme that produced 1 μmol of product. Specific activity was expressed in micromoles of product liberated per minute per unit of optical density at 600 nm.

Utilization of phenolic compounds for growth

Growth in minimal medium can be taken as an indicator of hydrocarbon biodegradation since pre-investigations revealed a good correlation between hydrocarbon loss and growth. The utilization for growth of ten phenolic and two other aromatic hydrocarbons was tested using microtiter plates (flat bottom, 96 wells). An incubation temperature of 10°C (cold-tolerant strain) or 25°C (mesophilic strain) was used. Each well received 50 μl of a suspension of microbial cells pre-grown in minimal medium with 200 mg phenol/l and washed in minimal medium (OD_{600} adjusted to 0.2). Afterwards, 150 μl of hydrocarbon-amended minimal medium was added to each well to give a final concentration of 200 mg/l of one of the following compounds: phenol, *o*-cresol (*o*-methylphenol), *m*-cresol (*m*-methylphenol), *m*-cresol (*p*-methylphenol), catechol (*o*-hydroxyphenol), resorcinol (*m*-hydroxyphenol), hydroquinone (*p*-hydroxyphenol), guaiacol (*o*-methoxyphenol), *p*-nitrophenol, *p*-nitrotoluene, salicylate (carboxyphenol), or benzoate (carboxybenzene). Inoculated medium without hydrocarbons and sterile hydrocarbon-containing medium served as negative controls. Three replicates were used per compound and strain. Microtiter plates were incubated in a moist chamber to avoid evaporation. Growth (OD_{600}) was determined at regular time intervals up to an incubation time of 17 days using a microplate reader.

Toxicity of phenolic compounds

An approach to toxicity testing is to measure the impact of toxicants on growth inhibition in the presence of nutrients. The toxic effect of 12 hydrocarbons was determined for both strains at 10°C

and 25°C using microtiter plates. Each well received 50 µl of a suspension of microbial cells (OD_{600} adjusted to 0.2) pre-grown in half-concentrated nutrient broth. Then, 150 µl of hydrocarbon-amended, half-concentrated nutrient broth was added to each well to give a final concentration of 50, 100, and 200 mg/l of one of the following compounds: phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, resorcinol, hydroquinone, guaiacol, *p*-nitrophenol, *p*-nitrotoluene, salicylate, or benzoate. Inoculated medium without hydrocarbons served as positive control, and sterile hydrocarbon-containing medium was used as negative control. Three replicates were used per compound, strain and test temperature. Growth (OD_{600}) was determined at regular time intervals up to an incubation time of 8–12 days. The inhibitory effect of hydrocarbons on growth was calculated from optical densities obtained in the presence and absence of hydrocarbons at the beginning of the stationary growth phase. Significant differences ($P < 0.05$) were determined by analysis of variance and multiple range analysis (least significant difference). *t*-Test analysis was used to determine whether the cultivation temperature had a significant effect on toxicity.

Results

Strain *Arthrobacter* sp. AG31

The cold-tolerant strain investigated in this study was tentatively assigned to *Arthrobacter* sp. (Keddie et al. 1986). The cells were Gram-positive, non-motile, and non-spore-forming pleomorph rods. On R2A agar, colonies had a yellow, non-fluorescent pigment. This pigment was lost when the strain was grown on minimal medium plates containing phenol as the carbon source. Activities of catalase, urease, and nitrate reduction were present, while arginine dihydrolase activity, ornithin decarboxylase, and acetoin production were not detected. The strain did not produce acid from glucose, ribose, cellobiose, fructose, raffinose, mannitol, trehalose, lactose, maltose, mannose, and saccharose. Partial 16SrDNA sequencing revealed a similarity of 96.4% with *Arthrobacter sulfureus*.

Effect of temperature on growth

The mesophilic and cold-tolerant character of the strains investigated became apparent in the different growth temperature ranges (Fig. 1). In complex medium such as R2A, mesophilic *Pseudomonas putida* showed optimum growth (in terms of maximum cell density) at 20–30°C and growth was still good at 35°C; high cell density was also observed at 10–15°C after 72 h. Growth in minimal medium containing phenol as the carbon source was optimal at 25–30°C and was considerably reduced at 20°C and 35°C. Phenol was not utilized at 1–15°C.

The cold-tolerant *Arthrobacter* sp. exhibited the properties of a facultative psychrophile (Morita 1975), showing growth at 1°C and above 20°C. There was no growth at 35°C. After 24 h of cultivation in complex medium, growth was optimal at 25°C and was weak at 30°C (Fig. 1). However, after 48 h and 54 h of cultivation, the optimum temperature range for growth was extended to 15–25°C and 10–25°C, respectively (data not shown). The ability to utilize phenol for growth was optimal at 15°C and was lost at 30°C (Fig. 1). Interestingly, both strains lost their pigmentation when cultivated on agar plates with phenol-containing minimal medium.

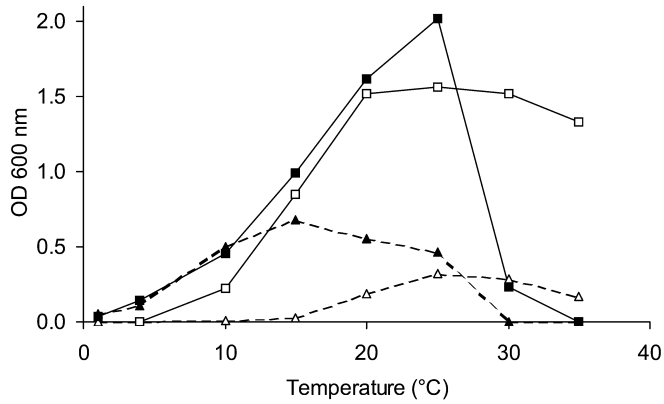

Fig. 1 Effect of temperature on growth of cold-tolerant *Arthrobacter* sp. AG31 (■ ▲) and mesophilic *Pseudomonas putida* DSM6414 (□ △) after 24 h of cultivation in complex medium (*solid lines*, *squares*) and after 48 h of cultivation in minimal medium with phenol as the sole carbon source (*dashed lines*, *triangles*)

Activity of catechol dioxygenases during phenol biodegradation

Phenol biodegradation

A cultivation temperature of 10°C or 25°C was selected for the cold-tolerant and mesophilic strain, respectively, because high cell densities were obtained in the presence and absence of phenol at these temperatures (Fig. 1). Comparable cell densities (OD_{600} of 1.4–1.5) were obtained after 72 h of cultivation of the two strains in complex medium. Biomass production was significantly lower in minimal medium with phenol as the sole carbon source, whereby the higher phenol concentration resulted in a higher cell density (Fig. 2). This can be explained by the growth-limiting factor of the substrate (C source) concentration. After 48 h of growth with phenol, the biomass production of the cold-tolerant strain was higher by 30% (200 mg phenol/l) and 70% (400 mg/l) compared to the mesophile. After 72 h, biomass production by the cold-tolerant strain was still higher by 20–30% (Fig. 2). Since no abiotic loss of phenol was detected in sterile controls, the measured phenol disappearance could be attributed to biodegradation. Phenol degradation was well correlated with growth. Biodegradation by the cold-tolerant strain at 10°C was comparable to that by the mesophile at 25°C; 200 mg and 400 mg phenol/l were fully degraded within 72 h of cultivation. However, the mesophilic strain had a 20–50% higher specific degradation activity (phenol loss per unit of optical density at 600 nm) than the

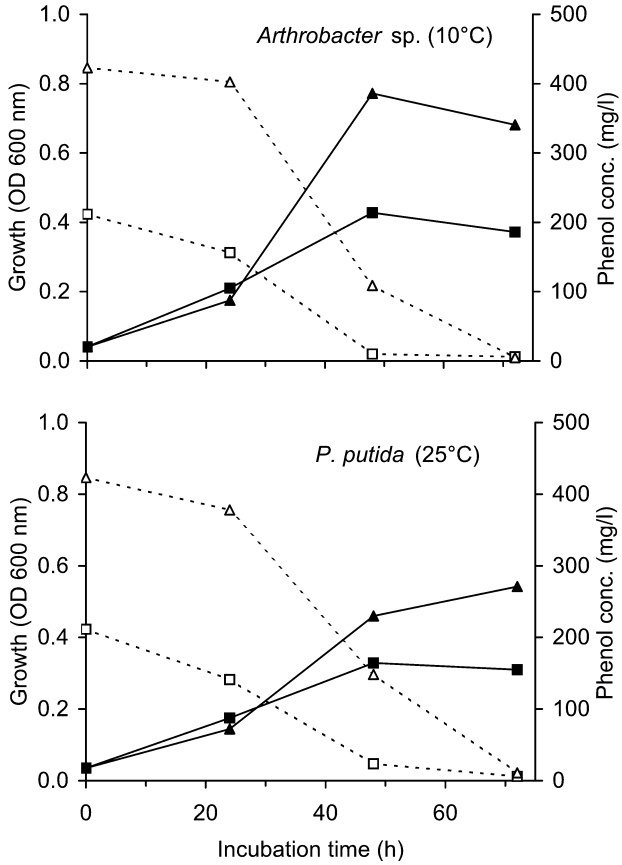

Fig. 2 Growth (*solid lines*, *solid symbols*) and phenol degradation (*dashed lines*, *open symbols*) by cold-tolerant *Arthrobacter* sp. AG31 cultivated at 10°C and mesophilic *P. putida* DSM6414 cultivated at 25°C. Initial phenol concentration: ■, □ 200 mg/l; ▲, △ 400 mg/l

cold-tolerant strain due to the lower mesophilic biomass production.

Catechol 1,2 dioxygenase (C1,2D)

C1,2D activity of mesophilic *P. putida* (cultivated at 25°C) and cold-tolerant *Arthrobacter* sp. (cultivated at 10°C) in the absence and presence of phenol is shown in Fig. 3. Extra- and intracellular enzyme activity were determined in crude extracts. Both strains produced a remarkable amount of C1,2D in the absence of phenol. Total (intra- and extracellular) enzyme activity in complex medium was generally higher for the mesophile than for the cold-tolerant strain. The higher total specific activity of the cold-tolerant strain after 24 h of cultivation is a consequence of the lower biomass production (logarithmic growth phase) compared to the mesophile that had already reached the stationary growth phase. After 72 h, both strains had produced a comparable amount of biomass, but the mesophile showed still an about twofold higher total C1,2D activity than the cold-tolerant strain (Fig. 3).

Both strains showed a lower relative enzyme activity in the presence of 200 mg phenol/l than in complex medium. The specific total C1,2D activity was generally higher in minimal medium with phenol as the sole carbon source than in complex medium, which can be attributed to the high biomass obtained in complex medium. In the presence of the lower phenol concentration tested (200 mg/l), total (specific and relative) C1,2D activity of the mesophile was about 1.5- to 2-fold higher than that of the cold-tolerant strain, despite comparable phenol biodegradation in terms of phenol loss by the two strains. However, the presence of 400 mg phenol/l resulted in an identical relative enzyme activity of the two strains after 24 h of cultivation at 10°C (*Arthrobacter* sp.: 5% phenol loss) and 25°C (*P. putida*: 11% phenol loss). After 48 h and 72 h, the relative enzyme activity of the cold-tolerant strain was lower by only about 10% compared to that of the mesophile.

Total (intra- and extracellular) specific and relative C1,2D activity was highest after 24 h of cultivation when phenol biodegradation had just started (400 mg/l) or was still low (200 mg/l) and decreased with incubation time. The same time course was observed in the absence of phenol.

Catechol 2,3 dioxygenase (C2,3D)

No C2,3D activity was found in the supernatant or in cell extracts of the two strains. However, the presence of this enzyme was noticed when using a quantitative test system (Margesin et al. 2003). This discrepancy might be explained by the significantly longer reaction time (2–3 h) used for the quantitative test and could also be attributed to inefficient cell disruption. This became apparent when whole cells (washed in buffer) were assayed. With this method, a low C2,3D activity was detected in the absence of phenol (50 U/OD_{600} and 39 U/OD_{600}, produced by *Arthrobacter* sp. and *P. putida*, respectively). The maximum C2,3D activity of the strains in the presence of phenol was about 18-fold (*Arthrobacter* sp.) or 34-fold (*P. putida*) lower than maximum C1,2D activity, which indicates that the presence of phenol induced the *ortho*-cleavage of catechol in both cases.

Utilization for growth and toxicity of phenolic compounds

The two strains utilized various aromatic compounds for growth (Table 1). The cold-tolerant strain showed the same pattern of hydrocarbon utilization at 10°C and 25°C (data not shown). However, there was no relation between the utilization of hydrocarbons for growth (Table 1) and the toxicity of these compounds (Fig. 4). Toxicity was tested at 10°C and 25°C since both strains showed good growth in complex medium at these temperatures (Fig. 1).

Growth of the cold-tolerant strain in the presence of nutrients and hydrocarbons at 10°C was significantly increased (up to 38%) in the presence of most of the 12

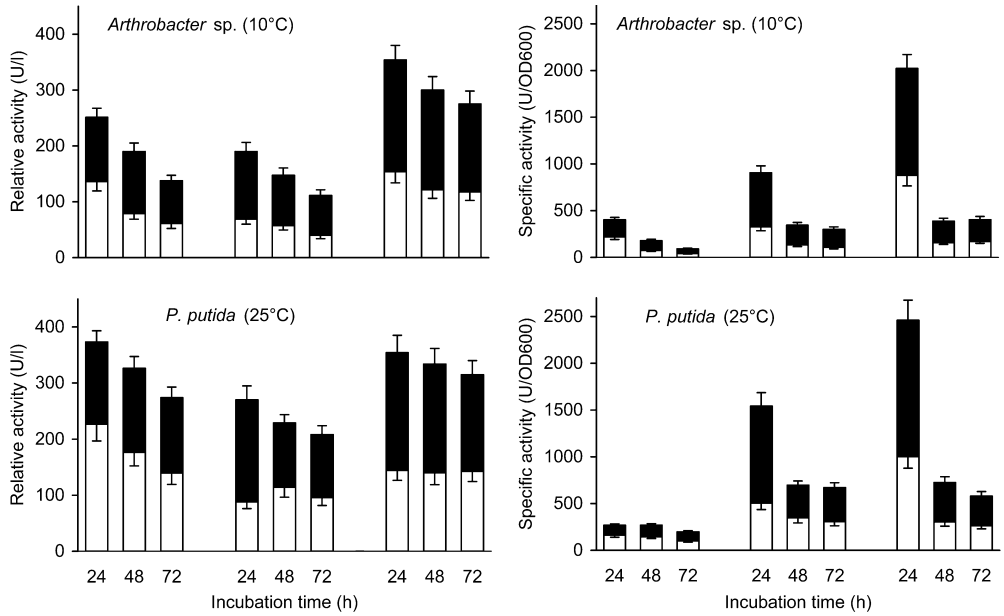

Fig. 3 Effect of phenol on extracellular (*white columns*) and intracellular (*black columns*) catechol 1,2 dioxygenase (C1,2D) activity of cold-tolerant *Arthrobacter* sp. AG31 cultivated at 10°C and mesophilic *P. putida* DSM6414 cultivated at 25°C. The first, second, and third group of the three columns represent growth in complex medium, and growth in minimal medium with 200 mg and 400 mg phenol/l, respectively

aromatic hydrocarbons tested. *p*-Nitrophenol was growth stimulating (40%) at 50 mg/l, but growth inhibiting (16–82%) at higher concentrations. Hydroquinone inhibited growth significantly (49–56%), while *p*-nitrotoluene had no significant effect on growth at all three concentrations tested. A completely different toxicity pattern was obtained at a growth temperature of 25°C. None of the compounds tested had a growth-stimulating effect, while most of the 12 compounds inhibited growth if applied at a concentration higher than 50 mg/l. The highest inhibition resulted from *p*-nitrophenol (82%) and hydroquinone (57%). Resorcinol, benzoate, and salicylate had no effect (Fig. 4).

Growth of the mesophilic strain at 10°C was significantly inhibited in the presence of 9 of the 12 compounds, 200 mg *p*-nitrophenol/l resulted in total growth inhibition. Low stimulation of growth occurred only in the presence of 50 mg *p*-cresol/l (6%) and catechol (10–14% at 50–200 mg/l). Benzoate and *p*-nitrotoluene had no significant effect. At a cultivation temperature of 25°C, toxicity of high hydrocarbon concentrations (200 mg/l) was significantly lower than at 10°C. Only hydroquinone had a growth-stimulating (up to 20%) effect (Fig. 4).

Statistical evaluation showed that hydrocarbon toxicity (in terms of growth inhibition) for the cold-tolerant strain was significantly ($P < 0.001$) lower at 10°C than at 25°C, independent of the hydrocarbon concentration tested. The mesophile showed a significantly ($P < 0.05$) lower susceptibility to high hydrocarbon concentrations when grown at 25°C compared to 10°C.

Table 1 Utilization of aromatic hydrocarbons (200 mg/l) for growth by cold-tolerant *Arthrobacter* sp. AG31 cultivated at 10°C and mesophilic *Pseudomonas putida* DSM6414 cultivated at 25°C

Compound	*Arthrobacter* sp.	*P. putida*
Phenol	+	+
o-Cresol (*o*-methylphenol)	+	+
m-Cresol (*m*-methylphenol)	+	+
p-Cresol (*p*-methylphenol)	–	–
Catechol (*o*-hydroxyphenol)	+	+
Resorcinol (*m*-hydroxyphenol)	–	–
Hydroquinone (*p*-hydroxyphenol)	(+)	+
Guaiacol (*o*-methoxyphenol)	–	–
p-Nitrophenol	–	–
p-Nitrotoluene	–	–
Salicylate (carboxyphenol)	+	+
Benzoate (carboxybenzene)	(+)	–

Discussion

The aerobic phenol degradation pathways in microorganisms involve the hydroxylation of phenol to catechols, which are the substrate of ring-cleaving enzymes that catalyze the second step of phenol degradation by either *ortho*- (C1,2D) or *meta*-cleavage (C2,3D) of catechol (Fritsche 1998). The genetic potential for both the *ortho*- and the *meta*-cleavage of catechol has been reported for some phenol-degrading bacteria (Nakazawa and Yokota 1973; Hinteregger et al. 1992). Both strains investigated in this study oxidized catechol by the *ortho* type of ring fission. Most bacteria degrade phenol by *ortho*-cleavage of catechol (Birger et al. 1997; Hinteregger and Streichsbier 1997; Bastos et al. 2000b), but there are also reports about phenol degraders with high C2,3D activity (Hinteregger et al. 1992; Natarjan et al. 1994; Mutzel et al. 1996). According to Müller and Babel (1994) *ortho*-cleavage is 9–23% more efficient than *meta*-cleavage on a biomass production basis. Hamzah and Al-Baharna (1994) demonstrated that induction of the ring-cleavage pathways for catechol depends on the compound supplied as the sole carbon source.

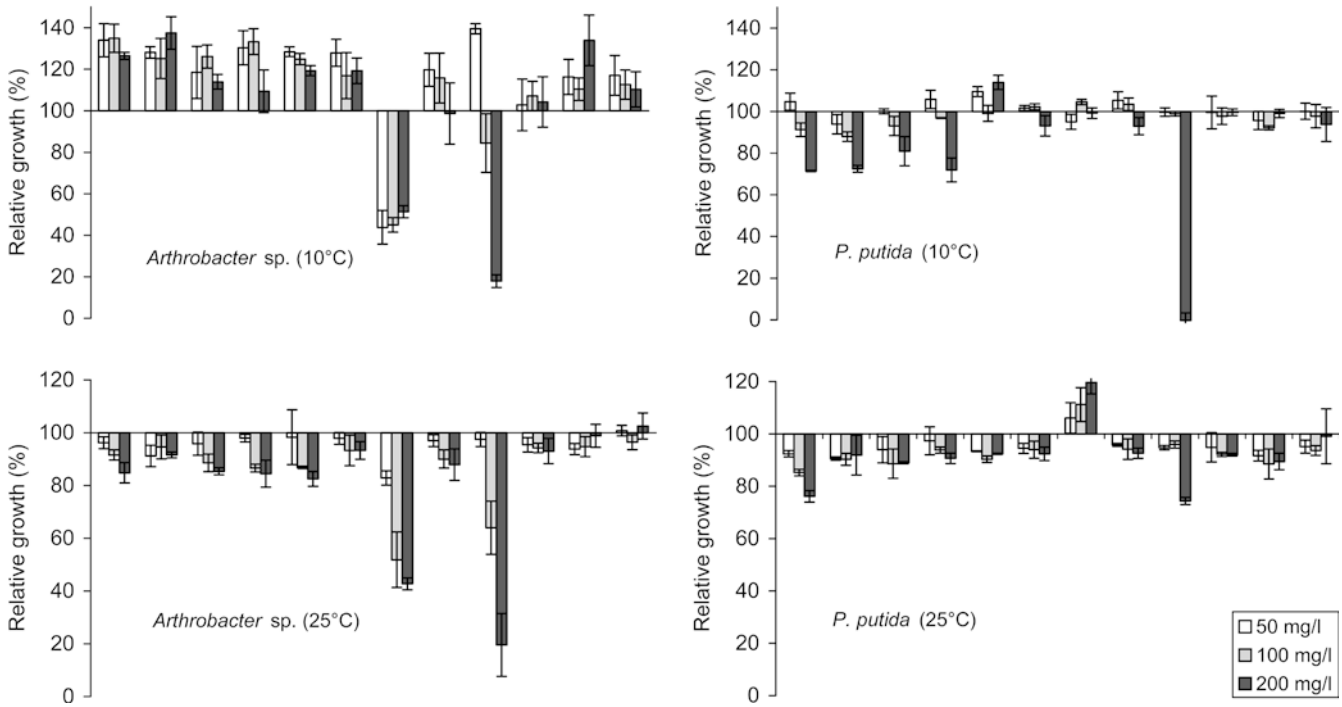

Fig. 4 Growth of cold-tolerant *Arthrobacter* sp. AG31 and mesophilic *P. putida* DSM6414 in the presence of nutrients and 12 aromatic hydrocarbons (100% = control = growth without hydrocarbons; <100% = growth inhibition; >100% = growth stimulation). Strains were cultivated at 10°C and 25°C. The 12 groups of columns represent the compounds tested (from *left to right*): phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, resorcinol, hydroquinone, guaiacol, *p*-nitrophenol, *p*-nitrotoluene, salicylate, and benzoate

In this study, C1,2D activity of the two strains was detected both intra- and extracellularly. The extracellular presence of these enzymes has been rarely described (Bastos et al. 2000b). Extracellular phenol oxidases are produced by some fungi for the degradation of lignin, a complex phenolic compound (Lengeler et al. 1999). The capability of microorganisms to produce extracellular enzymes during the degradation of aromatic compounds might be important as a protection mechanism against high (toxic) concentrations. The activity and stability of enzymes secreted in the surrounding medium is influenced by chemical-physical conditions; therefore, the knowledge of the extracellular presence of degrading enzymes could be useful for improving bioremediation processes.

It was remarkable that both strains produced a considerable amount of C1,2D in the absence of phenol (the relative activity in complex medium was even higher than in medium containing 200 mg phenol/l), which indicates that a certain level of enzyme production might be constitutively expressed. The constitutive expression of enzymes involved in the degradation of phenol is not common; so far it has been described for C2,3D of a thermophilic *Bacillus* sp. (Mutzel et al. 1996). This feature might be especially important for the immediate microbial response to a contamination event.

Both the mesophilic and the cold-tolerant strain investigated in this study were able to degrade 200 mg and 400 mg phenol/l within 48–72 h of cultivation at 10°C (cold tolerant) or 25°C (mesophile). In comparison, a cold-tolerant *Pseudomonas putida* grew and degraded phenol at rates that were about 65–80% lower than those obtained with mesophiles (Kotturi et al. 1991). Mesophilic bacteria were reported to degrade 200 mg and 600 mg phenol/l within 20 h and 50 h at 30°C (*P. putida*, Hinteregger et al. 1992), and to utilize 700 mg/l (7.5 mM) within 96 h at 29°C (*Alcaligenes faecalis*, Bastos et al. 2000a). In our study, a relatively high phenol concentration (400 mg/l, corresponding to about 4.3 mM) resulted in a comparable enzyme activity of the cold-tolerant *Arthrobacter* sp. and the mesophilic *P. putida*. The cold-tolerant strain produced much more biomass than the mesophile. These results demonstrate the efficiency of cold-tolerant microorganisms for biodegradation processes at low temperatures. Bioremediation in cold climates is based on the ability of cold-adapted microorganisms to degrade organic contaminants under cold conditions.

Phenol biodegradation is often a prerequisite for the treatment of mixed pollutants. Phenolic compounds may affect adversely the efficiency of wastewater treatment and lead to decreased effluent quality (Ren and Frymier 2003). Such compounds usually exhibit toxicity by the polar narcosis mechanism; this mode of action is unique to phenols and some other compounds with related structures (Verharr et al. 1992). The two strains tested in this study differed significantly in their toxicity pattern towards 12 aromatic (mostly phenolic) compounds at different growth temperatures. Growth of the cold-tolerant strain at 10°C was mainly stimulated in the

presence of the tested hydrocarbons, while at 25°C, this stimulation turned often into inhibition. For the cold-tolerant strain, toxicity was significantly lower at 10°C than at 25°C, independent of the hydrocarbon concentration. On the other hand, the mesophile showed a significantly higher sensitivity to high hydrocarbon concentration (200 mg/l) when grown at 10°C compared to 25°C. This reflects the adaptation of the cold-tolerant and the mesophilic strain to their respective optimal growth temperature ranges; strain-specific differences may also play a role. Microorganisms able to cope with a wide range of phenolic compounds at low temperatures, such as the cold-tolerant strain described in this study, could be useful for the low-energy treatment of industrial effluents.

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