Hydantoin cleaving bacterial isolates were recovered from terrestrial soil samples originating from different geographic sources (Antarctica, South Africa and China) using culture-based screening methods (selective agar plates and shake flask cultures supplemented with hydantoins). Thirty-two bacterial isolates possessing the capability to transform the model substrates benzylhydantoin and dihydrouracil to the corresponding N-carbamoyl-amino acids were successfully cultured. Amplification and sequencing of the 16S rDNA revealed that the isolates belonged to the genera *Arthrobacter*, *Burkholderia*, *Bacillus*, *Delftia*, *Enterobacter*, *Flavobacterium*, *Ochrobactrum*, *Pseudomonas* and *Stenotrophomonas*, with one isolate assigned to the family Microbacteriaceae. We have shown that microorganisms with hydantoinase activity are: (i) distributed in various geographically distinct environmental habitats, (ii) distributed worldwide and (iii) found in certain bacterial genera. Furthermore, we have demonstrated the presence of hydantoinase activity in genera in which hydantoinase activity has not previously been reported.

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Keywords: Hydantoinase; Screening; Extreme environment

1. Introduction

Enzymes classed as “hydantoinases” catalyse the hydrolysis of hydantoins in a ring-opening step after which a further enzymatic or chemical hydrolytic step can lead to the formation of amino acids. These enzymes may have different substrate specificities and in general are selective in forming either l- or d-N-carbamoyl amino acids. Hydantoinases are usually grouped, according to their stereospecificity, as d-, l- or non-selective hydantoinases. d-Hydantoinase, together with N-carbamoyl-d-amino acid hydrolase [1] is used in the production of d-amino acids [2].

Hydantoinases are classed as cyclic amidases (EC 3.5.2). In this study, in accordance with Syldatk et al. [3], the name hydantoinase will be employed for all enzymes that hydrolyse hydantoin and/or 5′-monosubstituted hydantoin derivatives and not as a synonym for dihydropyrimidinases, as stated in the EC-nomenclature. It has been shown that dihydropyrimidinases and hydantoinases are not necessarily the same enzyme [3]. The function of dihydropyrimidinases is the hydrolysis of dihydrouracil derivatives, a reaction involved in the reductive pathway of pyrimidine degradation. The in vivo metabolic function of many hydantoinases is still unknown. For example, the hydantoinase from *Agrobacterium* sp. [4] is not able to hydrolyse dihydropyrimidinones, but can hydrolyse hydantoin and 5-monosubstituted hydantoins.

On the basis of amino acid sequence determinations and phylogenetic analyses, it has been shown that the l-hydantoinase from *Arthrobacter aurescens* DSM 3745 belongs to a protein superfamily, which includes dihydropyrimidinases, collagen response mediator proteins, allantoinase, dihydroorotase and ureases. [5]. It is suggested that hydantoinases are members of a very old protein family and have evolved from a common ancestor, and that these first primitive, ancient microorganisms were
able to use abiotically synthesised hydantoins and N-carbamoyl-\(\alpha\)-amino acids as C- and/or N-sources [3]. Gajovic et al. [6] demonstrated that dihydopyrimidinase and, thus the reductive catabolism of pyrimidines, are present in all major eukaryotic kingdoms; a comparison of dihydopyrimidinase-like enzymes showed that the majority of bacterial hydantoinases belong to the same group as the eukaryotic dihydopyrimidinases, and the authors suggest that the ancient progenitor of this group was likely to be a catalytic enzyme.

The question of the natural function, and origins, of hydantoinases remains. This study will not resolve this question, but will give more detailed information on the occurrence of hydantoin cleaving enzymes in nature. Hydantoinase activity has been found in a wide spectrum of microorganisms belonging to, amongst others, the genera *Arthrobacter*, *Pseudomonas*, *Bacillus* and *Flavobacterium* [7,8]. However, no precise data are available of the exact origins and nature of the environmental sample used for the isolation of microorganisms with hydantoinase activity. Thus, we have conducted a screening program for bacteria with hydantoinase activity from terrestrial soil samples of different geographic regions, including extreme habitats. This study has focused on the questions: (i) are microorganisms with hydantoinase activity only found in a small range of similar environmental habitats; (ii) are they distributed worldwide; (iii) are hydantoinases limited to certain bacterial genera?

2. Experimental procedures

2.1. Chemicals

Hydantoins were kindly supplied by Degussa AG, Germany. All other chemicals used were obtained from commercial sources and were of reagent grade.

2.2. Soil samples

Soil and sediment samples for enrichment experiments were collected in South Africa, Lesotho, Swaziland; China (Yunnan Province, and the Inner Mongolian Autonomous Region), and the McMurdo Valley, McMurdo Dry Valleys, Eastern Antarctica. Soil samples from South African National Parks were collected with the permission of the South African National Parks (SANP). Soil samples from China and Antarctica were provided under the auspices of the EU-MGATech and UWC-Waikato University Antarctica NZ research programs, respectively (DAC).

2.3. Media

The following media were used: Medium I, according to reference [9], used for the screening experiments, contained: 10.0 g/l HMH or IMH, 0.2 g/l fructose, 3.9 g/l \((\text{NH}_4)_2\text{SO}_4\), 0.2 g/l \(\text{MgSO}_4\), 0.02 g/l \(\text{CaCl}_2\cdot 2\text{H}_2\text{O}\), 2.0 g/l \(\text{KHPO}_4\), 1.0 g/l \(\text{KH}_2\text{PO}_4\), 0.2 g/l \(\text{MgSO}_4\cdot 7\text{H}_2\text{O}\), 0.02 g/l \(\text{CaCl}_2\cdot 2\text{H}_2\text{O}\) and 10 ml of a trace element solution at pH 7.0. The trace element solution contained: 50 mg/l \(\text{H}_3\text{BO}_3\), 40 mg/l \(\text{CuSO}_4\cdot 5\text{H}_2\text{O}\), 20 mg/l \(\text{FeCl}_3\), 10 mg/l KI and 4 mg \(\text{CaSO}_4\cdot 2\text{H}_2\text{O}\).

CA-plates, used for the isolation and/or storage of bacterial isolates, contained: 10 g/l casein peptone, 5 g/l yeast extract, 5 g/l glucose, 5 g/l NaCl, 15 g/l agar, pH 7.2 (Media 53, German Collection of Microorganisms and Cell Cultures). CA, used for the storage of bacterial isolates and for the starter cultures, contained: 10 g/l casein peptone, 5 g/l yeast extract, 5 g/l glucose and 5 g/l NaCl.

Medium I, according to [9], used as growth medium for biotransformation assays, contained: 10 g/l glucose, 6.5 g/l \((\text{NH}_4)_2\text{SO}_4\), 0.2 g/l \(\text{MgSO}_4\), 0.02 g/l \(\text{MnCl}_2\cdot 4\text{H}_2\text{O}\), 0.02 g/l \(\text{FeSO}_4\), 0.02 g/l \(\text{CaCl}_2\cdot 2\text{H}_2\text{O}\), 5.54 g/l \(\text{KH}_2\text{PO}_4\), 7.6 g/l \(\text{K}_2\text{HPO}_4\), 0.32 g/l citrate-1-hydrate and 1 g/l IMH at pH 6.8.

TBE (10×), used for agarose gel electrophoresis, was prepared as follows: 108 g Tris and 55 g boric acid were dissolved in 900 ml water. Forty milliliters 0.5 M \(\text{Na}_2\text{EDTA}\) (pH 8.0) was added and the volume adjusted to 1 l.

2.4. Isolation of hydantoin cleaving isolates

Two methods for the isolation of microorganisms possessing hydantoin-cleaving enzymes were used. First, soil samples were enriched aerobically in shake flasks, at 30°C, 160 rpm on medium I supplemented with either IMH or HMH. Chinese soil samples were also incubated at 40 and 50°C. Bacterial isolates were obtained by spreading the enriched medium on agar plates (selective plates) based on medium I, containing the enrichment substrate with addition of 15 g/l agar. Pure isolates were obtained by picking single colonies and replating onto fresh agar plates. Finally, isolates were transferred onto CA-plates. In a second screening method, approximately 1 g of soil sample was added to 10 ml sterile water, containing 0.0005 g nystatin (as fungicide) and 10 μl Triton® X100, mixed well and 100 μl of the resulting suspension was spread on selective plates (see above). Further isolation steps were conducted as described above. Chinese thermal pool sediment samples were also incubated at 40°C and Antarctic soil samples at 4°C. Isolates were stored for short periods at 4°C on CA-plates or long term at −70°C in a sterile solution of 20% glycerol in CA.

2.5. Assay of enzyme activity

For the detection of hydantoinase activity in the bacterial isolates, biotransformation experiments were conducted as follows: a loopfull of bacterial biomass was inoculated into 5 ml CA at 30°C and incubated over night as a starter culture. Two milliliter starter culture was added to 50 ml of GM (supplemented with IMH as inducer for enzyme expression) and incubated at 30°C and 160 rpm. Cells were harvested after 48 h by centrifugation (10 min, 8000 rpm and 4°C; Beckman, AvantiTM J-25). Resting cells were obtained by washing twice with 100 mM K-phosphate buffer (pH 8.0), followed by centrifugation and resuspension in the same buffer. The substrate \(\text{BnH}\) was dissolved in 100 mM K-phosphate buffer (pH 8.0), assisted by a 30-min sonication. The assay substrates (30 mM DU or 10 mM BnH) dissolved in 100 mM K-phosphate buffer (pH 8.0) were pre-incubated
cycles were used: Initial denaturation at 94 °C, 0.5 mM forward primer, 0.5 mM reverse primer, 1.5 mM MgCl2 under the following conditions: 0.2 mM dNTPs (Promega, and performed using a ThermoHybaid PCRsprint Thermocycler (PCR) was used for the amplification of the 16S rRNA gene standard gene fragments of 10 and 25 ng. Polymerase chain reaction (10 gel electrophoresis in TBE buffer stained with ethidium bromide. DNA was checked for quality and quantity using 1% agarose gel. DNA, and PCR water combined to a total volume of 50 μl. The reaction solution was added to 800 μl Ehrlich Reagent (1 g 4-dimethylaminobenzaldehyde, 5 ml H2O, 5 ml 6 M HCl) and 900 μl water. The quantification of the yellow product was performed photometrically at 430 nm ([10], using a Nucleodex detector). The flow rate was 0.2 ml/min.

2.7. 16S rDNA preparation and sequencing

Genomic DNA from the bacterial isolates was extracted and purified using the Quiagen DNeasy Tissue Kit (Quiagen), following the manufacturers instructions for Gram-positive bacterium. DNA was checked for quality and quantity using 1% agarose gel electrophoresis in TBE buffer stained with ethidium bromide (10 μl/100 ml) and quantified under UV-light in comparison to standard gene fragments of 10 and 25 ng. Polymerase chain reaction (PCR) was used for the amplification of the 16S rRNA gene and performed using a Thermohybird PCRscript Thermocycler under the following conditions: 0.2 mM dNTPs (Promega, USA), 5 μl Taq-Polymerase 10× reaction buffer (Promega), 0.5 mM forward primer, 0.5 mM reverse primer, 1.5 mM MgCl2 (Promega), 0.5 μl Taq-Polymerase (Promega), 1 μl genomic DNA, and PCR water combined to a total volume of 50 μl. The following Universal Bacillus oligonucleotide primer sequences were used: forward primer 9F (5′-GGAGTATCCTGCAGTAG11) and reverse primer U1510R (5′-GGTTACCTTGGTACGACTT12). For the amplification the following cycles were used: Initial denaturation at 94 °C for 1 min followed by 30 amplification cycles with template DNA denaturation at 94 °C for 1 min, primer annealing at 51 °C for 1 min and primer extension at 72 °C for 1 min and final extension at 72 °C for 10 min.

Amplified DNA was purified from the reaction mixture by agarose gel purification using the GFXTM PCR-DNA and gel band purification kit (Amersham Bioscience, New York). PCR products were sequenced by the Department of Molecular and Cell Biology, University of Cape Town, South Africa, using the oligonucleotide primer E9F. The resulting nucleotide sequences were analysed using the BioEdit Sequence Alignment Editor software (Copyright® 1997–2001 Tom Hall, Department of Microbiology, North Carolina State University). Nucleotide sequence homology searches were carried out using the BlastN electronic mail server from the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/).

3. Results and discussion

Bacterial isolates with the ability to grow on IMH as a nutritional source were recovered from enrichment cultures, but interestingly, no hydantoinase-positive bacteria were found in enrichment cultures using HMH. Since all bacterial isolates were isolated using IMH as an enrichment substrate, this hydantoin was routinely used as an inducer for hydantoinase expression in bioconversion experiments. Isolates with activity towards BnH and DU were characterized using 16S rRNA gene amplification, sequencing and database comparisons. The isolates were assigned according to the closest 16S rDNA match (Table 1). Further characterization via classical bacterial identification is in progress.

In total, 32 bacterial strains were isolated from terrestrial soil samples of different environmental sources (see Table 1). Pseudomonas putida strains and an Agrobacterium tumefaciens strain have previously been isolated from South Africa and characterized [13]. Pseudomonads have been isolated from a wide variety of sources, including soils, fresh or sea water, sewage, foodstuffs and food industry wastes [14].

Various members of the genus Pseudomonas with the ability to produce carbamoyl-amino acids and/or amino acids have been reported, including a Pseudomonas desmolyticus isolate with the capability to produce 3-phenylglycine [15] and various P. putida strains [16–18]. In this study, bacterial isolates with hydantoinase activity, belonging to the genus Pseudomonas, were found in a variety of soil samples including dry alpine soils, compost and oligotrophic gravels from Antarctica. All isolates showed degradation of the test substrates DU and BnH, but at different levels of conversion (Table 1).

Of particular interest are two Pseudomonas strains isolated from Antarctic soil samples, one (isolate G7) from Bratina Island and the other (isolate N7) from the Miers Valley, Antarctica. These dry valley deserts of Eastern Antarctica are generally accepted to be some of the harshest environments on earth and have formerly been considered to be highly unfavourable to life [19]. No Antarctic bacterial isolates have been reported previously as having hydantoinase activity. The discovery of Pseudomonas strains from this environment is not unexpected; previous literature reports describe 32 psychrophilic bacteria belonging to the genus Pseudomonas having been isolated from an Antarctic cyanobacterial mat. Three novel species were named (Pseudomonas antarctica sp. nov., Pseudomonas meridiana sp. nov. and Pseudomonas proteolytica sp. nov. [20]). Psychrophiles are defined as microorganisms possessing cardinal growth temperatures of 15 °C (optimal), 20 °C (maximal) and 0 °C (minimal) [21]. Recent growth experiments showed that the Pseudomonas strains G7 and N7 were able to grow well under low temperature and had higher growth rate at 20 than at 30 °C (data not shown). The ability of all isolates to grow at approximately 30 °C is fully consistent with the growth characteristics of psychrotrophic organisms.
Table 1
Hydantoinase positive bacterial isolates

<table>
<thead>
<tr>
<th>Description of soil sample</th>
<th>Isolate no.</th>
<th>Genus</th>
<th>%Homology bp</th>
<th>DU-biotransform</th>
<th>BullH-biotransform</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA, Beach mountain, 700 m a.s.l., soil</td>
<td>N1</td>
<td>Stenotrophomonas sp.</td>
<td>99%/472</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>SA, Stellenbosch, pine forest, soil</td>
<td>K3</td>
<td>Unclassified Microbacteriae</td>
<td>100%/445</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Veldrift, &quot;guano&quot; from a salt lake with bird dung</td>
<td>F16</td>
<td>Bacillus</td>
<td>99%/585</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SA, Stellenbosch, Reliance Compost Company, compost of wine yards</td>
<td>F17</td>
<td>Bacillus</td>
<td>100%/585</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Stellenbosch, Reliance Compost Company, shredder material</td>
<td>K5</td>
<td>Ochrobactrum sp.</td>
<td>98%/356</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Stellenbosch, Reliance Compost Company, shredder material (5 weeks old)</td>
<td>F7</td>
<td>Enterobacter sp.</td>
<td>97%/384</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SA, Table mountain, 700 m a.s.l, soil</td>
<td>L9</td>
<td>Pseudomonas sp.</td>
<td>99%/584</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Worchester, Distell Brandy Distillery, biofilm of a RBC</td>
<td>K18</td>
<td>Bacillus</td>
<td>99%/585</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SA, Stellenbosch, pinewood forest, soil</td>
<td>G8</td>
<td>Pseudomonas sp.</td>
<td>99%/592</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Stellenbosch, Reliance Compost Company, compost of fiber material</td>
<td>F8</td>
<td>Flavobacterium sp.</td>
<td>98%/563</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Stellenbosch, Reliance Compost Company, shredder material (12 weeks old)</td>
<td>F9</td>
<td>Pseudomonas sp.</td>
<td>99%/592</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, West Coast N.P., &quot;guano&quot; from a small pond at Langebaan lagoon</td>
<td>M18</td>
<td>Pseudomonas sp.</td>
<td>99%/445</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, West Coast N.P., &quot;guano&quot; from a small pond at Langebaan lagoon</td>
<td>H20</td>
<td>Bacillus sp.</td>
<td>100%/565</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SA, Blue Mountain Pass, soil, 2500 m a.s.l</td>
<td>K20</td>
<td>Ochrobactrum sp.</td>
<td>100%/587</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SA, Cederberg mountains, 1100 m a.s.l, dry soil</td>
<td>E21</td>
<td>Ochrobactrum sp.</td>
<td>99%/585</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Smitskamma N.P., soil of an Afriomontane</td>
<td>M18</td>
<td>Pseudomonas sp.</td>
<td>99%/445</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Veldrift, &quot;guano&quot; from a salt lake with bird dung</td>
<td>F16</td>
<td>Bacillus sp.</td>
<td>100%/520</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SA, Stellenbosch, Reliance Compost Company, compost of fiber material</td>
<td>F17</td>
<td>Bacillus sp.</td>
<td>100%/588</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Worchester, Distell Brandy Distillery, biofilm of a RBC</td>
<td>F21</td>
<td>Ochrobactrum sp.</td>
<td>100%/535</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Stellenbosch, pinewood forest, soil</td>
<td>K18</td>
<td>Bacillus sp.</td>
<td>99%/585</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Blue Mountain Pass, soil, 2500 m a.s.l</td>
<td>K20</td>
<td>Ochrobactrum sp.</td>
<td>100%/587</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SA, Cederberg mountains, 1100 m a.s.l, dry soil</td>
<td>F21</td>
<td>Ochrobactrum sp.</td>
<td>100%/535</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, Stellenbosch, Reliance Compost Company, shredder material (12 weeks old)</td>
<td>E21</td>
<td>Ochrobactrum sp.</td>
<td>99%/585</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>SA, West Coast N.P., &quot;guano&quot; from a small pond at Langebaan lagoon</td>
<td>M18</td>
<td>Pseudomonas sp.</td>
<td>99%/445</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

*Bacterial isolates from the genus Pseudomonas and Bacillus represent the major groups in this screening approach. Both genera were found in South African compost samples and dry soil samples (Fig. 1), whereas two Bacillus strains (isolates F18 and K18) were isolated from a saline environment. Both strains, as well as Ochrobactrum G21 and strain G20, were able to grow under saline conditions (GM supplemented with 10% NaCl), but not at a higher salt content (data not shown). All four strains were therefore designated as moderate halophilic. Different Bacillus strains with the ability to cleave hydantoins have been reported in literature, and most possess thermostable hydantoinases [22–24]. Only one Ochrobactrum anthrophi strain has been reported previously. This strain, isolated from a soil sample from Spain, showed the ability to release methionine from N(2)-(2-methylthioethyl) hydantoin [25]. The hydantoinase of this strain was inducible with N(2)-(2-methylthioethyl) hydantoin and was alkali-labile, with a pH optimum of 9.0. In the present study, a number of Ochrobactrum strains were isolated, mostly from compost samples (see Table 1; Fig. 1) and from a biofilm of a...*
rotating biological contactor (RBC) used for the treatment of a wine distillery wastewater. In addition, one Ochrobactrum sp. strain from a hypersaline salt lake from Inner Mongolia has been isolated from sea water was shown to have the ability to produce d-N-carbamoylphenylglycine [26].

The genus Arthrobacter has previously been shown to be an important source of hydantoinase activities. These Gram-positive organisms are found in a wide variety of ecological habitats: in soils and sewage, and associated with fish and plants [27]. The hydantoinsases of the A. aurescens strains DSM3745 and 3747 have been well-described [28] and both produce α-amino acids from 5-monosubstituted hydantoins [28]. The hydantoinase from A. aurescens DSM 3745 belongs to the amidohydrolase superfamily [5] and has been shown to be a Zn\(^{2+}\)-metalloenzyme [29,30] with L-selectivity in the conversion of L-5-\((\text{3'}\text{-indolylmethyl})\)-hydantoin. The hydantoinase and carbamoylase genes from A. aurescens DSM 3747 and DSM 3745 have each been cloned and their nucleotide sequences determined. These two enzymes show a high degree of nucleotide and amino acid sequence identity (96-98%) [31,32].

In this study, Arthrobacter strains were found both in hot desert (African) and cold desert (Antarctic) soils.

Two other Arthrobacter strains (F7 and G7) were found in sediment of a saline pond from Bratina Island, Antarctica. These bacterial isolates showed the same growth characteristics as the Pseudomonas strains N7 and G7 (data not shown), and have been designated as psychrophilic. The isolation of Arthrobacter strains from this environment is not unexpected, since psychrophilic and psychrotrophic Arthrobacter strains have been reported from samples of subterranean cave silts [33], glacier silts [34] and the soils of Antarctica [35-39]. Psychrophilic bacteria isolated from cyanobacterial mats in the McMurdo Dry Valleys, Antarctica have been recently characterized as Arthrobacter flavus sp. nov. [40] and Arthrobacter roseus sp. nov. [41]. Two isolates from penguin rookery soil samples in Antarctica have been proposed as Arthrobacter gangotriensis sp. nov. and Arthrobacter kerguelensis sp. nov. [42].

We also report the isolation at a growth temperature of 50 °C of a Delftia sp. strain from a hot spring algal mat (Long Pu, China) with activity towards BuH and DU. To our knowledge, no hydantoinase from this genus has been reported previously. However, thermophilic hydantoinsases have been described. A thermophilic \(\beta\)-hydantoinase from the moderate thermophile Bacillus steaethermophilus SD-1 was shown to have pH and temperature optima of approximately 8.0 and 65 °C, respectively [22]. The most thermophilic hydantoinase reported to date (\(T_{\text{opt}} = 80^\circ\text{C}\)) is derived from the hyperthermophilic archaeon, Methanococcus jannaschii; isolated from a submarine hydrothermal vent [43]. Thermostable hydantoinsases have also been isolated from a mesophilic Bacillus sp. AR9 [23] and a moderate thermophilic B. steaethermophilus N51122A, with optimal temperatures between 60 and 70 °C [24].

\(\alpha\)-Hydantoinase activity has been reported in Burkholderia pickettii [44], towards L-5-mercaptoethyl-hydantoin in Enterobacter cloacae [45], and in several different Flavobacterium species [46,47]. Bacterial isolates recovered from South African soil and belonging to these genera were also found in our study.

We also report the detection of hydantoinase activity in bacterial genera where this enzyme has not been reported previously. These include Stenotrophomonas sp. N1, Microbacteriaceae sp. K3, Staphylococcus sp. H7, Acinetobacter sp. K5, Delftia sp. I24 and an unidentified isolate I20 (possibly Streptomyces; Dr. Eberspacher, personal communication).

The aim of this study was the recovery of microorganisms with the ability to cleave hydantoins. The standard condition for principal isolation of microorganisms was medium 1 (pH 7.0) at 30 °C. Apart from Delftia sp. I24, all bacterial isolates were recovered under standard conditions. However, isolates originating from saline and cold environments (Mongolia and Antarctica, respectively) were not isolated under conditions exactly reflecting their environmental origins. After subsequent tests for their ability to grow under saline and/or cold conditions, these strains were designated as psychrophilic or moderately halophilic, as appropriate.

4. Conclusion

We conclude, on the basis of our isolation data and published literature that: (i) hydantoinase-positive bacterial isolates are very widely dispersed, both geographically and with respect to environmental conditions, and (ii) among culturable aerobes, hydantoinsases are predominantly found in certain genera (Pseudomonas, Ochrobactrum, Bacillus, Arthrobacter). We have also
described microorganisms for which no hydantoinase activity was reported previously.

With respect to the natural function of hydantoinases, it may be significant that the majority of the isolates recovered were derived from samples with a high organic load, such as compost soil and guano. These findings support the hypothesis that hydantoinases are involved in catabolic pathways to access hydantoins and hydantoin-like molecules as metabolic substrates.

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