Short Communication

Molecular detection of *Oceanobacillus iheyensis* in sand of Brazilian beaches

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Oceanobacillus iheyensis strain HTE831 was isolated from deep sea mud (1,050 m) from the Iheya Ridge near Okinawa, Japan, in 1998 (Lu et al., 2001). This organism is an alkaliphilic and extremely halotolerant *Bacillus*-related species. Its genome has been completely sequenced and the presence of genes required for adaptation to these extreme environments was demonstrated (Takami et al., 2002). Many proteins potentially associated with roles in the regulation of intracellular osmotic pressure and pH homeostasis were shown to be coded in its 3.6 Mb genome (Takami et al., 2002). Later on, Takaki et al. (2004) identified six kinds of new insertion sequences, a group II intron, and an incomplete transposon in the genome of strain HTE831. However, surprisingly, no other strains belonging to this species were described in the literature until 2006, when Barbosa et al. studied the Gram-positive spore-forming community found associated with plants in tropical soils. Halotolerant and facultatively alkaliphilic spore-forming Gram-positive bacteria were isolated from the rhizosphere and non-rhizosphere soils of *Blutaparon portulacoides* (St. Hill.) Mears (Am-

aranthaceae). This plant first colonized embryo dunes and backshores of Southwestern Atlantic Ocean beaches (Farias and Flores, 1989).

Different isolates were characterized phenotypically and analyzed by 16S rRNA gene sequencing and members of the species *O. iheyensis* were recovered (Barbosa et al., 2006a). Macroscopically, colonies of strains L4, L18, S1, S4, S14 and S18 are circular, creamy white, and slightly transparent. They are all strictly aerobic, catalase positive, nitrate negative, Voges-Proskauer (VP) negative, casein positive and starch hydrolysis negative. These six strains are able to grow in LB medium containing 0.5 to 20% NaCl and on pH varying from 5.7 to 9.5. They all formed a monophyletic branch with *O. iheyensis* strain HTE831 based on their 16S rRNA gene sequences (DQ058363–DQ058368; Barbosa et al., 2006a). Therefore, on the basis of the results of this investigation, different *O. iheyensis* strains isolated from *B. portulacoides* became available for further studies.

Hence, it is now reasonable to consider that strains of *O. iheyensis* are not restricted to extreme environments and may be spread in different habitats in the world. The study of *O. iheyensis* strains may lead us to a better understanding of life in saline and/or alkaline environments. New strains of *O. iheyensis* may also offer a potential source for the discovery of bioactive compounds with industrial value, such as antibiotics, biosurfactants and exopolysaccharides (Margesin and Schinner, 2001). However, no selective medium is

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Table 1. Bacterial strains used in this study and specificity of PCR system based on 16S rRNA gene of *Oceanobacillus iheyensis*.

Species	Strains	PCR product (486 bp)	Growth conditions ^a	Origin/Reference
<i>Oceanobacillus iheyensis</i>	S1, S4, S14, S18	+	LB/NaCl 10%, 32°C	Non-rhizosphere soil; Barbosa et al. (2006a)
	L4, L18	+	LB/NaCl 10%, 32°C	washed roots of <i>Blutaparon portulacoides</i> ; Barbosa et al. (2006a)
<i>O. picturae</i>	L6, L15	–	LB/NaCl 10%, 32°C	washed roots of <i>Blutaparon portulacoides</i> ; Barbosa et al. (2006a)
<i>Halobacillus</i> sp.	L7	–	LB/NaCl 10%, 32°C	washed roots of <i>Blutaparon portulacoides</i> ; Barbosa et al. (2006a)
<i>H. blutaparonensis</i>	M9	–	LB/NaCl 10%, 32°C	macerated roots of <i>Blutaparon portulacoides</i> ; Barbosa et al. (2006b)

^a LB medium consists of tryptone 1% and yeast extract 0.5% supplemented with 10% NaCl.

available for the isolation of *O. iheyensis* strains and biochemical identification is time consuming. Therefore, we describe in this study a PCR detection technique based on the 16S rRNA gene that can successfully be used for the identification of strains suspected to belong to *O. iheyensis*. Furthermore, this approach can also be used for the detection of this species directly in the environment, at least in sand samples.

The bacterial strains used in this study, their origins and growth characteristics are presented in Table 1. All *Oceanobacillus* and *Halobacillus* strains were grown in LB medium (tryptone 1%, yeast extract 0.5%) supplemented with 10% NaCl and incubated for 24–48 h at 32°C. Bacterial cultures were stored aerobically in LB/NaCl 10% slants at room temperature and at –80°C in LB/NaCl 10% medium containing 20% glycerol.

The study areas were located north of Rio de Janeiro State in the Parque Nacional da Restinga de Jurubatiba (22°17'S and 41°41'W), south of Rio de Janeiro City on Copacabana Beach (22°58'S and 43°10'W), Arraial do Cabo (Praia da Reserva—22°57'S and 42°1'W) and south of Bahia State in Itacaré (Praia da Camboa and Praia da Engenhoca—14°16'S and 38°59'W). Sand samples from the different beaches were collected near the ocean (wet sand; 0 to 20 cm of depth) in sterile flasks. In the laboratory, the sand samples were used either directly for DNA extraction or 2 g of each sample were mixed with 38 ml of LB medium supplemented with 15% NaCl. These flasks were incubated for 48 h at 32°C. After the incubation period, the mixture was used for total microbial community DNA extraction and also for the isolation of bacterial strains onto LB agar supplemented with 10% NaCl. The plates

were incubated for 3 days at 32°C. The different isolates were characterized phenotypically as described by Barbosa et al. (2006a).

Genomic DNAs were extracted from all bacterial strains by using the protocol described by Seldin and Dubnau (1985). The total microbial community DNA was extracted directly from the sand samples and also from the enrichment cultures using FastPrep Spin kit for soil DNA (BIO 101 Systems, CA, USA). DNA preparations were visualized after electrophoresis in a 0.8% agarose gel in 1 × TBE buffer (Sambrook et al., 1989) and DNA concentration was measured in a GenQuant apparatus (Pharmacia Biotech, Cambridge, England).

The *Oceanobacillus iheyensis* HTE831 16S rRNA gene sequence available in GenBank (<http://www.ncbi.nlm.nih.gov/>) was compared to different halophilic/halotolerant bacterial 16S rRNA gene sequences by using Clustal X (Thompson et al., 1997). Those sequences were chosen based on the phylogenetic tree presented by Barbosa et al. (2006a). After alignment, two sequences were manually found as potential specific regions for *O. iheyensis* (Table 2): a 15-mer forward primer—OCEANO 203F: 5'-TCATCTCCTGATGAG-3'—and a 20-mer reverse primer—O689R: 5'-CTCTTCTGCACTCAAGTTCC-3'. Both primers were further analyzed by BLAST-N (Altschul et al., 1997) to search for homologous nucleotide sequences in the GenBank database.

The conditions used for amplification with both primers designed in this study were one cycle of 94°C (30 s) and 25 cycles of 94°C (1 min), 53°C (1 min 30 s) and 72°C (1 min 30 s). A final extension step was run for 5 min at 72°C and the reaction tubes were then cooled

Table 2. Sequence alignment of *Oceanobacillus iheyensis* specific regions of the 16S rRNA gene sequence with some closely related sequences, showing target regions for the design of specific primers.

Bacterial species (accession number)	Primer OCEANO 203F: TARGET 5'→3' (187–202)					Primer O689R: TARGET 5'→3' (666–685)						
	TCA	TCT	CCT	GAT	GAG	CTC	TTC	TGC	ACT	CAA	GTT	CC
<i>O. iheyensis</i> HTE831 (BA000028)	.	.	.	.	.	.	.	.	.	.	.	.
<i>Oceanobacillus</i> sp. GSP76 (AY553092.1)	.	.	.	.	.	.	.	.	.	.	.	.
<i>Oceanobacillus</i> sp. 72 (AY553088.1)	.	.	.	.	.	.	.	.	.	.	.	.
<i>Oceanobacillus</i> sp. 67 (AY553084.1)	.	.	.	.	.	.	.	.	.	.	.	.
<i>Oceanobacillus</i> sp. 5 (DQ190427.1)	.	.	.	.	.	.	.	.	.	.	.	.
S1 (DQ058366.1)	.	.	.	.	.	.	.	.	.	.	.	.
S4 (DQ058367.1)	.	.	.	.	.	.	.	.	.	.	.	.
S14 (DQ058368.1)	.	.	.	.	.	.	.	.	.	.	.	.
S18 (DQ058365.1)	.	.	.	.	.	.	.	.	.	.	.	.
L4 (DQ058364.1)	.	.	.	.	.	.	.	.	.	.	.	.
L18 (DQ058363.1)	.	.	.	.	.	.	.	.	.	.	.	.
<i>O. picturae</i> (AJ315060.1)	CTT	.TG	.A	.A	.GA	.	.	.T	.	.	.C	.T
<i>O. oncorrhynchi</i> (AB188089.1)	CT	.A	.	.	.GA	.	.	.	.	.	.C	.T
<i>O. profundus</i> (DQ298074.2)	.	.	.A	.G	.	.	.	.T	.	.	.C	.T
<i>O. chironomi</i> (DQ298074.2)	.T	.C	.	.G	.AA	.	.	.T	.	.	.C	.T
<i>Virgibacillus halodenitrificans</i> (AB021186.1)	.	.A	.	.	.N	.	.	.T	.	.	.C	.T
<i>V. proomii</i> (AJ012667.1)	.TC	.TG	.A	.A	.G	.	.	.T	.	.	.C	.T
<i>V. pantothenicus</i> (AB039331.1)	.G	.G	.A	.C	.	.	.	.T	.	.	.C	.
<i>V. carmonensis</i> (AJ316302.1)	.T	.A	.A	.G	.AGA	.	.	.T	.	.	.C	.T
<i>V. necropolis</i> (AJ315056.1)	.T	.A	.A	.G	.AGA	.	.	.T	.	.	.C	.T
<i>V. dokdonensis</i> (AY822043.1)	G.G	.G	.A	.C	.CA	.	.	.T	.	.	.C	.T
<i>V. koreensis</i> (AY616012.1)	.AC	.	.A	.G	TTT	.	.	.T	.	.	.	.
<i>V. marismortui</i> (AJ009793.1)	.G	.TG	.A	.A	.C	.	.	.T	.	.	.C	.T
<i>V. olivae</i> (DQ139839)	T.G	.TG	.A	.A	.C	.	.	.T	.	.	.C	.T
<i>V. salexigens</i> (Y11603.1)	.TG	—A	.A	—C	A..	.C	.	.T	.	.	.C	.T
<i>Amphibacillus</i> sp. (AY121432.1)	.T	.TA	.	.A	.G	.	.	.T	.	.	.	.T
<i>Halobacillus salinus</i> (AF500003.1)	C.T	.G	.A	.A	.GA	.	.	.T	C.	.	.	.
<i>H. litoralis</i> (X94558)	.T	.G	.A	.A	.GA	.	.	.T	C.	.	.	.

Only the oligonucleotides different from the given sequences are indicated. N represents A, T, G or C; —represents a gap. Numbers in parenthesis correspond to *E. coli* 16S rRNA gene sequence numbers (Brosius et al., 1981).

to 4°C. The 25 µl PCR reaction mix contained 10 mmol L⁻¹ Tris HCl, 50 mmol L⁻¹ KCl, 2.5 mmol L⁻¹ MgCl₂, 0.1% Triton X-100, 1/50 (v/v) dimethyl sulfoxide (DMSO), 0.4 mmol L⁻¹ dNTPs, 0.4 µmol L⁻¹ of each primer, 1.25 U of *Taq* DNA polymerase and 1 µl (about 50 ng) of the DNA extract. Negative controls (without DNA) were run in all amplifications. The PCR products

were analyzed by electrophoresis in a 1.4% agarose gel in 1 × TBE buffer.

The PCR product of the 16S rRNA gene obtained from the sand sample (Praia da Reserva) was cloned using the pGEM-T easy vector according to the instructions of the manufacturer (Promega, Madison, WI, USA). After transformation of *Escherichia coli*

JM109 competent cells, clones were picked and the presence of inserts of the correct size was verified by PCR using M13f (5'-GTAAACGACGGCCAG-3') and M13r (5'-CAGGAAACAGCTATGAC-3') primers. Selected clones were sequenced using M13f and M13r primers by an ABI Prism 3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA). The 16S rRNA gene sequences of selected representative clones obtained in this study from the sand sample (Praia da Reserva) were compared to the GenBank database by using the algorithm BLAST-N to identify the most similar sequences, and then aligned with different species of *Oceanobacillus* recovered from the same database by using the software Clustal X.

For the determination of the detection limit of the PCR amplification system, the sand from Praia da Reserva was air-dried and transferred (5 g) to microcosms consisting of plastic 10 ml-pots, as previously described in Vollú et al. (2003). *O. iheyensis* strain S4 was grown in LB supplemented with 10% NaCl at 32°C for 24–48 h and then ten-fold serial dilutions of the culture were plated onto LB agar and incubated for 3 days for determining the colony forming units (CFU). The microcosms were inoculated in duplicate by carefully pipetting washed cells (twice in 10% saline) of strain S4 on top (10^1 , 10^2 , 10^3 , 10^4 and 10^5 CFU ml⁻¹), establishing a sand moisture content of 10%. The inoculated sand was homogenized using a sterile spatula and, after 24 h, total microbial community DNA was extracted using a FastPrep Spin kit for soil DNA as described before, and used further for PCR analysis.

In a previous study, six strains (L4, L18, S1, S4, S14 and S18; Barbosa et al., 2006a) were isolated from *Blutaparon portulacoides* usually found in Southwestern Atlantic Ocean beaches, and it was clearly demonstrated that these strains formed a monophyletic branch with *O. iheyensis*, indicating that this species is more ubiquitous than previously thought (Lu et al., 2001). For the molecular detection of this species in other environments (at least in sand) and for helping with the taxonomic identification of possible *O. iheyensis* isolates, 16S rRNA gene-based primers were designed and tested for specificity in this study. 16S rRNA gene sequences belonging to six isolates previously identified as *O. iheyensis*, to the type strain (HTE831) of this species and to representatives of other species of the genera *Oceanobacillus*, *Virgibacillus*, *Halobacillus*, and *Amphibacillus* sp. were retrieved from GenBank and compared. After the alignment of these se-

quences, two regions were revealed as potentially specific for *O. iheyensis* (Table 2). Therefore, one 15-mer (forward) and the other 20-mer (reverse) primers were selected based on these regions—OCEANO 203F: 5'-TCATCTCCTGATGAG3' and O689R: 5'-CTCT-TCTGCACTCAAGTTCC-3'. These forward and reverse primers selected were further analyzed to determine their specificity for the species *O. iheyensis* by using all 16S rRNA gene sequences deposited in the GenBank and BLAST. The result showed that the first hits belonged to the type strain of *O. iheyensis* and to other *O. iheyensis* isolates. Only the remaining hits belonged to other halophilic/halotolerant spore-forming bacteria. Moreover, the specificity of the primers was further tested by using pure-culture DNAs from the six *O. iheyensis* strains isolated from *B. portulacoides* (Barbosa et al., 2006a), two strains of *O. picturae* and two strains of the related genus *Halobacillus* (Table 1). Using the conditions described here for the PCR reaction, only the strains of *O. iheyensis* showed the expected 486 bp-product (Fig. 1).

To estimate the sensitivity of the PCR amplification system for the detection of *O. iheyensis* in the environment, PCR reactions were performed using DNA extracted directly from the sand samples and from the samples pre-enriched in LB medium supplemented with 15% NaCl. No PCR product was obtained when the DNA extracted directly from the different beaches was used as a template. Conversely, when the DNA obtained from the enrichment cultures was used, the expected product could be visualized in Restinga de Jurubatiba and Praia da Reserva sand samples (Fig. 1). This fact could indicate that the population of *O. iheyensis* is low in these two sand samples (below the limit of detection for the PCR technique) or also very low or non-existent in the other beaches. To confirm the sequence of the PCR product, it was cloned in pGEM-T and seven clones were sequenced. All of them were affiliated with *O. iheyensis*, showing similarities varying from 99 to 99.8% (GenBank—accession numbers EU159894–EU159900).

To determine the detection limit of the PCR amplification system for *O. iheyensis* cells in sand, an experiment was performed with different inoculum densities of strain S4 applied to sand obtained in Praia da Reserva in microcosms. Cell inoculum densities exceeding 10^6 cells per g of sand were needed for the prompt detection via PCR, whereas uninoculated sand did not produce a PCR amplification product (Fig. 2). In previ-

Fig. 1. Agarose gel (1.4%) showing PCR products from DNAs obtained from different *O. iheyensis* strains.

(1) L12; (2) M17; (3) S4 and from the sands of; (4) Restinga de Jurubatiba (enriched); (5) Restinga de Jurubatiba (without enrichment); and (6) Praia da Reserva (enriched). Lanes (7) *O. picturae* strain L9; (8) *Halobacillus* sp. strain L6; (9) *H. blutaparonensis* strain M9 and (10) without DNA correspond to negative controls.

ous reports, Rosado et al. (1996) introduced either vegetative cells or spores of *Paenibacillus durus* at different concentrations (10^2 to 10^6 per g of dry soil) in Flevo silt loam soil and Vollú et al. (2003) introduced vegetative cells (10^2 to 10^6 per g of dry soil) of *P. macerans* in Cerrado soil (dark red latosol with clayey texture) for short-term experiments aimed at studying the sensitivity of the PCR detection method based on the 16S rRNA gene developed for *P. durus* and *P. macerans*, respectively. Inoculum exceeding 10^2 (*P. durus*) and 10^3 (*P. macerans*) cells or spores per g of soil were detected via PCR in both studies. The same level of specificity was obtained by Briglia et al. (1996) for the detection of *Mycobacterium chlorophenolicum* in soil. Although the limit of sensitivity obtained in our study was low when compared to those studies, probably because of the different physicochemical characteristics of the soils used, the applicability of the method is evident for the detection of *O. iheyensis*. Further studies on the method developed here should be conducted in other environments, in order to enable natural detection of *O. iheyensis* populations. Primers specific for other spore-forming bacteria, such as *Clostridium* sp. (Van Dyke and McCarthy, 2002) and *Bacillus* sp. (Wu et al., 2006) have already been developed and are being used for this purpose.

Finally, to determine the applicability of our system for the identification of isolates supposed to belong to *O. iheyensis*, various colonies with different morphologies and only one colony with characteristics typical of *O. iheyensis*—circular, creamy white, and slightly

Fig. 2. Detection limit of the PCR amplification system for *O. iheyensis* (primers OCEANO 203F and O689R) using the sand from Praia da Reserva inoculated with different cell concentrations.

Different lanes correspond to: (1) Kb ladder (Promega); (2) DNA of *O. iheyensis* strain S4 (pure culture); (3, 4) DNA from sand inoculated with S4 (10^7 CFU ml^{-1}); (5, 6) DNA from sand inoculated with S4 (10^6 CFU ml^{-1}); (7, 8) DNA from sand inoculated with S4 (10^5 CFU ml^{-1}).

transparent; casein positive and starch hydrolysis negative—were chosen from the LB plus 10% NaCl inoculated plates with the sand obtained from the three beaches in Rio de Janeiro and the two beaches in Bahia, for further amplification with primers OCEANO 203F and O689R. Only the strain (denominated AC10) isolated from Praia da Reserva in Arraial do Cabo and

presenting characteristics typical of *O. iheyensis* showed the expected amplification product (data not shown). Hence, the method developed not only detects *O. iheyensis* in sand, but it can also be used in taxonomic studies for the quick identification of strains suspected to be *O. iheyensis*.

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References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.*, **25**, 3389–3402.
- Barbosa, D. C., von der Weid, I., Vaisman, N., and Seldin, L. (2006a) Halotolerant spore forming gram positive bacterial diversity associated with *Blutaparon portulacoides* (St. Hill) Mears, a pioneer species in Brazilian coastal dunes. *J. Microbiol. Biotechnol.*, **16**, 193–199.
- Barbosa, D. C., Bae, J.-W., von der Weid, I., Vaisman, N., Nam, Y.-D., Chang, H.-W., Park, Y.-H., and Seldin, L. (2006b) *Halobacillus blutaparonensis* sp. nov., a moderately halophilic bacterium isolated from *Blutaparon portulacoides* roots in Brazil. *J. Microbiol. Biotechnol.*, **16**, 1862–1867.
- Briglia, M., Eggen, R. I. L., de Vos, W. M., and van Elsas, J. D. (1996) Rapid and sensitive method for the detection of *Mycobacterium chlorophenolicum* CPC-1 in soil based on 16S rRNA gene-targeted polymerase chain reaction. *Appl. Environ. Microbiol.*, **62**, 1478–1480.
- Brosius, J., Dull, T. J., Sleeter, D. D., and Noller, H. F. (1981) Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J. Mol. Biol.*, **148**, 107–127.
- Farias, M. E. and Flores, F. E. V. (1989) Effect of salinity on *Blutaparon portulacoides* (A. St.-Hil.) Mears (Amaranthaceae): Relation between photosynthetic rate, sodium content, water economy, and growth at the foliar level. *Rev. Brasil. Biol.*, **48**, 155–164.
- Lu, J., Nogi, Y., and Takami, H. (2001) *Oceanobacillus iheyensis* gen. nov., sp. nov., a deep-sea extremely halotolerant and alkaliphilic species isolated from a depth of 1050 m on the Iheya Ridge. *FEMS Microbiol. Lett.*, **205**, 291–297.
- Margesin, R. and Schinner, F. (2001) Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles*, **5**, 73–83.
- Rosado, A. S., Seldin, L., Wolters, A. C., and van Elsas, J. D. (1996) Quantitative 16S rDNA-targeted polymerase chain reaction and oligonucleotide hybridization for the detection of *Paenibacillus azotofixans* in soil and the wheat rhizosphere. *FEMS Microbiol. Ecol.*, **19**, 153–164.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Seldin, L. and Dubnau, D. (1985) DNA homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans* and other nitrogen fixing *Bacillus* strains. *Int. J. Syst. Bacteriol.*, **35**, 151–154.
- Takaki, Y., Matsuki, A., Chee, G. J., and Takami, H. (2004) Identification and distribution of new insertion sequences in the genome of the extremely halotolerant and alkaliphilic *Oceanobacillus iheyensis* HTE831. *DNA Res.*, **11**, 233–245.
- Takami, H., Takaki, Y., and Uchiyama, I. (2002) Genome sequence of *Oceanobacillus iheyensis* isolated from the Iheya Ridge and its unexpected adaptive capabilities to extreme environments. *Nucleic Acids Res.*, **30**, 3927–3935.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **25**, 4876–4882.
- Van Dyke, M. I. and McCarthy, E. J. (2002) Molecular biological detection and characterization of *Clostridium* populations in municipal landfill sites. *Appl. Environ. Microbiol.*, **68**, 2049–2053.
- Vollú, R. E., dos Santos, S. C. C., and Seldin, L. (2003) 16S rRNA targeted PCR for the detection of *Paenibacillus macerans*. *Let. Appl. Microbiol.*, **37**, 415–420.
- Wu, X.-Y., Walker, M. J., Hornitzky, M., and Chin, J. (2006) Development of a group-specific PCR combined with ARDRA for the identification of *Bacillus* species of environmental significance. *J. Microbiol. Methods*, **64**, 107–119.