Janibacter hoylei sp. nov., *Bacillus isronensis* sp. nov. and *Bacillus aryabhattai* sp. nov., isolated from cryotubes used for collecting air from the upper atmosphere

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Three novel bacterial strains, PVAS-1^T, B3W22^T and B8W22^T, were isolated from cryotubes used to collect air samples at altitudes of between 27 and 41 km. Based on phenotypic characteristics, chemotaxonomic features, DNA–DNA hybridization with the nearest phylogenetic neighbours and phylogenetic analysis based on partial 16S rRNA gene sequences (PVAS-1^T, 1196 nt; B3W22^T, 1541 nt; B8W22^T, 1533 nt), the three strains were identified as representing novel species, and the names proposed are *Janibacter hoylei* sp. nov. (type strain PVAS-1^T =MTCC 8307^T =DSM 21601^T =CCUG 56714^T), *Bacillus isronensis* sp. nov. (type strain B3W22^T =MTCC 7902^T =JCM 13838^T) and *Bacillus aryabhattai* sp. nov. (type strain B8W22^T =MTCC 7755^T =JCM 13839^T).

Evidence for the occurrence of micro-organisms in the upper atmosphere at altitudes of 17–85 km (Bruch, 1967; Greene *et al.*, 1964; Lysenko, 1979), obtained using a meteorological rocket (Imshenetsky *et al.*, 1978; Lysenko, 1979), a specially designed direct-flow sampler (Greene *et al.*, 1964; Rogers & Meier, 1936) and cryosamplers sent up on a balloon (Harris *et al.*, 2002; Wainwright *et al.*, 2003; Shivaji *et al.*, 2006), has established unequivocally that life forms are present in the upper atmosphere. To date, only a few studies have been published on the quantity and nature of the micro-organisms in the upper atmosphere (Bruch, 1967; Greene *et al.*, 1964; Lysenko, 1979; Rogers & Meier, 1936; Harris *et al.*, 2002). In these earlier reports, the emphasis was on detection of micro-organisms rather than their identification. Wainwright *et al.* (2003) reported the presence of two bacterial species (*Bacillus simplex* and *Staphylococcus pasteurii*) and a fungus (*Engyotontium album*), whereas Shivaji *et al.* (2006) identified four novel species of *Bacillus*, *Bacillus aerius*, *B. aerophilus*, *B. stratosphericus* and *B. altitudinis*, from cryogenic tubes used to collect air samples at altitudes of 24, 28 and 41 km.

In this paper, a polyphasic taxonomic approach was used to characterize 12 bacterial strains isolated from cryotubes that were used to collect air at altitudes of between 27 and 41 km, during a balloon flight. Three of the 12 bacterial isolates, strains PVAS-1^T, B3W22^T and B8W22^T, represent three novel species of the genera *Bacillus* (B3W22^T and B8W22^T) and *Janibacter* (PVAS-1^T).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains PVAS-1^T, B3W22^T and B8W22^T are DQ317608, EF114311 and EF114313.

Polar lipid profiles of the novel strains and related type strains and results of UV-sensitivity experiments are available as supplementary material with the online version of this paper.

Collection of air from 24 km and above

The balloon with the cryosampler payload used for collecting high-altitude air samples (altitude 20–41.4 km) was launched on 20 April 2005 from the National Scientific Balloon Facility of the Tata Institute of Fundamental Research at Hyderabad, India. The cryogenic sampler, comprising a 16-cryoprobe assembly, is similar to one used previously (Wainwright *et al.*, 2003; Shivaji *et al.*, 2006). All 16 cryosampling tubes were autoclaved at 120 °C for 4 h and integrated into the payload in a tissue-culture laboratory which was UV-sterilized overnight. The cryotubes were also checked for sterility after the assembly. For this purpose, three cryotubes were disconnected from the assembly, opened in a laminar flow hood, rinsed with sterile phosphate buffer (0.1 M, pH 7.2) and plated on Luria–Bertani (LB) agar and incubated at 37 °C. No colonies had appeared after 1 month, indicating that the cryotubes were sterile. Therefore, the isolates reported in Table 1 should have originated from the air samples collected at the given altitudes. Cryotubes 1, 3, 5, 7, 9, 12, 13 and 15 were examined at the Centre for Cellular and Molecular Biology, Hyderabad, while cryotubes 2, 4, 6, 8, 10 and 14 were similarly studied at the National Centre for Cell Sciences, Pune. Care was taken that the two laboratories followed similar protocols, and there was frequent interaction and discussion between the two groups to ensure homogeneity of procedures and interpretation.

Detection of viable bacteria from air collected in the cryotubes

All the work related to detection of microbes in air samples from the cryotubes was carried out in a room with very little human traffic and inside a laminar flow hood available exclusively for the handling of the probes. The probes were transferred to the laminar flow hood one at a time and, prior to the transfer, the surface of the cryotubes was cleaned and sterilized with alcohol. All instruments used to unscrew the outlet valve of the cryotube such as spanners and screwdrivers were surface-sterilized with alcohol and flame-heated prior to use. The outlet valve of the cryotube was then connected to a series of two Millipore filtration units using sterile tubing and the air was sequentially filtered through a 0.45 μm filter (Millipore cat. no. HAWP 04700) and a 0.22 μm filter (Millipore cat. no. GSWP 04700) under aseptic conditions in the laminar flow hood. The 0.45 μm and 0.22 μm filters were then used for detection of bacteria. For this purpose, each filter was cut into quarters and one of the quarters was transferred to a nutrient agar plate (0.5 % peptone, 0.3 % beef extract, 0.5 % NaCl and 1.5 % agar, w/v) and incubated at 15 °C. After about 10 days, if no growth was observed, the filter was transferred to an LB agar plate (1 % tryptone, 0.5 % yeast extract, 1.0 % NaCl and 2.0 % agar, w/v; pH 7.2) or an oligotrophic LB agar plate (1/10 LB) and incubated at 15 °C for a further 15 days. Subsequently, if no colonies

Table 1. Bacteria isolated from cryotubes used for collecting air at altitudes of 20–41.4 km

With the exception of strain PVAS-1^T, which was isolated from an air sample, all the strains were isolated from washes of the cryotubes.

Cryotube	Altitude (km)	Temperature (°C)	Air mass (g)	Isolated bacteria			
				Strain	Nearest phylogenetic neighbour	Accession no.	16S rRNA gene sequence similarity (%)
1	20–24	−50	140	None	–		–
2	40–41.4	−20		None	–		–
3	24–27	−40	65	None	–		–
4	24–27	−40		PVAS-8	*Stenotrophomonas rhizophila* e-p10^T	AJ293463	99.0
5	27–30	−35	55	B3W22^T	*Bacillus silvestris* HR3-23^T	AJ006086	99.5
6	27–30	−35	55	None	–		–
7	30–35	−25	25	None	–		–
8	30–35	−25		PVAS-4	*Acinetobacter radioresistens* BA50	FJ263931	100.0
9	35–40	−20	13	B5W22-1	*Micrococcus flavus* LW4^T	DQ491453	99.2
				B5W22-2	*Streptomyces maritimus* BD26^T	AF233338	99.0
10	35–40	−20		PVAS-5	*Stenotrophomonas rhizophila* e-p10^T	AJ293463	99.2
				PVAS-6	*Acinetobacter calcoaceticus* MTCC 9488	FM210755	99.0
				PVAS-10	*Bacillus pumilus* BSH-4	EF488975	99.0
11	40–41.4	−20		PVAS-1^T	*Janibacter anophelis* CCUG 49715^T	AY837752	98.0
				PVAS-2	*Methylobacterium chloromethanicum* CM4^T	CP001298	99.0
				PVAS-3	*Methylobacterium chloromethanicum* CM4^T	CP001298	99.0
12, 13, 15	40–41.4	−20	2.5	B8W22^T	*Bacillus megaterium* IAM 13418^T	D16273	99.7
14	40–41.4	−20	–	None	–		–

appeared, the filters were incubated on LB agar at 25 °C for up to a month. It should be noted that, when hydrophobic 0.45 μm (Millipore cat. no. HVHP 04700) and 0.22 μm (cat. no. GVHP 04700) filters were used for filtering the air, a thin layer of medium of the same composition was applied to the surface of the plates before the filters were placed on the surface of the medium.

Two of the remaining quarters of the filter were transferred to minimal salts agar. This medium contained (per 100 ml) 20 ml of a 3.39 % (w/v) sodium dihydrogen phosphate solution, 20 ml 1.5 % (w/v) potassium phosphate, 20 ml 0.25 % (w/v) NaCl, 20 ml 0.5 % (w/v) ammonium chloride and 18 ml Millipore water. The final concentration of agar was 2 %. The medium was also supplemented with 2 ml sterile 20 % glucose and 0.2 ml sterile 0.1 M $MgSO_4$ solution; the pH was adjusted to 7. This medium was used for the cultivation of organisms which require low nutrients. Blood agar [2.3 % (w/v) peptone, 0.1 % (w/v) corn starch, 0.5 % (w/v) NaCl and 1.5 % (w/v) agar, to which 5 % (v/v) defibrinated blood was added (after autoclaving and cooling the medium)] was used for the cultivation of organisms which require rich media. Plates of minimal salt agar and blood agar were incubated at 30 °C. One quarter of the filter was also incubated at 30 °C on Sabouraud agar (1.0 % enzymic digest of casein, 4 % glucose and 1.5 % agar, w/v) to facilitate fungal growth (data not shown).

Of the 15 cryotubes from which air was sampled, only a single novel bacterial colony was detected, in cryotube 11, corresponding to air collected at an altitude of 40–41.4 km (Table 1). When placed on minimal salts agar, the filter paper from cryotube 11 showed a single cream-coloured colony after 24 days of incubation at 30 °C, and it was designated strain PVAS-1^T.

During the filtration process, plates containing fungal and bacterial media were left open in the laminar flow hood throughout the operation to check the sterility of the hood. These plates were incubated at 25 °C (for fungi) or 37 °C (bacteria) for 7 days prior to use, to confirm the sterility of the plates. Colonies were not detected on any of these plates, indicating that the sampling was done under sterile conditions.

Detection of viable bacteria from washes of empty cryotubes

It seemed possible that some bacteria might have remained at the bottom of the tubes or attached to their polished walls and thus escaped detection. To check this possibility, all 15 empty cryotubes were injected with 200 ml sterile phosphate buffer (0.1 M, pH 7.2) and agitated at 22 °C for 6 h in a shaker. The liquid was then removed using sterile tubing and a syringe and filtered through a 0.22 μm Millipore filter (47 mm diameter). These filters were each cut aseptically into four sectors and each sector was placed on a plate containing one of four different media, LB agar, minimal salts agar, blood agar and Sabouraud agar, and incubated under the conditions described above. The plates were observed for the appearance of colonies over a period of 1 month.

From the 15 cryotube wash samples, 12 bacterial colonies were detected (Table 1). Nine (PVAS-2, -3, -4, -5, -6, -8 and -10 and B5W22-1 and B5W22-2) showed more than 98 % 16S rRNA gene sequence similarity to reported species (Table 1). Therefore, further attempts were not made to characterize them to the species level.

Morphological, biochemical and chemotaxonomic characteristics

Morphological, growth and biochemical studies of viable colonies were performed using standard methods (Holding & Collee, 1971; Smibert & Krieg, 1994). LB agar was used for growth and maintenance and for the determination of phenotypic and chemotaxonomic characteristics of all strains except PVAS-2 and -3. Strains PVAS-2 and -3 were grown on a medium containing 0.25 % (w/v) K_2HPO_4, 0.225 % (w/v) NaH_2PO_4, 0.05 % (w/v) $(NH_4)_2SO_4$, 0.02 % (w/v) $MgSO_4 . 7H_2O$ and 0.1 % (v/v) methanol. The shape, size and motility of the strains were ascertained using a Leitz Diaplan phase-contrast microscope with an oil-immersion objective (× 100). The sensitivity of the cultures to antibiotics was determined by using antibiotic discs (HiMedia). Utilization of various carbon compounds as sole carbon sources was tested in mineral liquid medium supplemented with 0.2 % filter-sterilized carbon source as described previously (Shivaji *et al.*, 2006). Fatty acid and lipid composition (Sato & Murata, 1988; Kiran *et al.*, 2004), DNA G+C content (Shivaji *et al.*, 1992, 2005) and DNA–DNA hybridization (Tourova & Antonov, 1987; Shivaji *et al.*, 1992) were determined according to standard procedures. Analyses of polar lipids, respiratory quinones and *meso*-diaminopimelic for PVAS-1^T were carried out by the Identification Service of the DSMZ (Braunschweig, Germany). Isoprenoid quinones were also extracted according to the method of Collins *et al.* (1977), separated by HPLC and identified as described previously (Reddy *et al.*, 2003). Peptidoglycan was prepared and analysed according to the method described by Komagata & Suzuki (1987).

Detailed phenotypic and chemotaxonomic characteristics (listed in Tables 2, 3 and 4 and in the species descriptions) were compared with those of *Bacillus silvestris* DSM 12223^T, *Bacillus megaterium* MTCC 428^T, *Janibacter anophelis* CCUG 49715^T, *Janibacter terrae* DSM 13876^T, *Janibacter melonis* DSM 16063^T, *Janibacter limosus* DSM 11140^T and *Janibacter corallicola* DSM 18906^T.

Cells of strain PVAS-1^T are Gram-positive, coccoid, non-endospore-forming and non-motile and occur singly or in clumps. Some biochemical characteristics are listed in Table 2. *meso*-Diaminopimelic acid is the diagnostic diamino acid in the cell-wall peptidoglycan and iso-$C_{16:0}$

Table 2. Physiological properties that differentiate strain PVAS-1^T and the type strains of other species of the genus *Janibacter*

Strains: 1, *Janibacter hoylei* sp. nov. PVAS-1^T (data from this study); 2, *Janibacter anophelis* DSM 18333^T; 3, *Janibacter terrae* DSM 13876^T; 4, *Janibacter limosus* DSM 11140^T (unless indicated, data in columns 2–4 from Kämpfer *et al.*, 2006); 5, *Janibacter melonis* DSM 16063^T (Yoon *et al.*, 2004); 6, *Janibacter corallicola* DSM 18906^T (Kageyama *et al.*, 2007). All strains were negative for growth at 40 °C, utilization of *N*-acetylglutamic acid, haemolysis, motility, methyl red test and H_2S production and positive for catalase, starch hydrolysis and gelatin hydrolysis. All strains tolerate up to 6 % NaCl. +, Positive; −, negative; w, weakly positive; d, delayed reaction; TSBA, tryptic soy broth agar; CASO agar, casein-peptone-soymeal peptone agar.

Characteristic	1	2	3	4	5	6
Growth at 37 °C on TSBA	+	+	w	−	+	+
Growth at 37 °C on CASO agar*	+	w	+	−	+	−
Growth with NaCl at:						
8 %*	+	+	+	+	−	+
10 %*	+	+	w	w	−	+
Utilization of:						
D-Malate*	−	−	w	+	w	+
Sodium benzoate*	−	−	+	+	w	w
Sodium citrate*	−	−	−	−	w	−
Sodium formate*	−	−	w	−	w	−
Sodium succinate*	−	−	w	w	w	+
D-Gluconate	−	+	+	+	−	+
D-Glucose	−	+	+	+	w	−
Inositol	−	+	+	w	−	+
Maltose	−	+	d	+	w	+
D-Mannose	−	+	+	w	w	+
Monomethyl succinate	+	−	w	+	−	−
Putrescine	−	−	+	+	−	−
Sucrose	−	+	+	+	w	+
Trehalose	−	+	d	+	+	−
D-Galactose	−	−	−	−	+	+
Casein*	+	+	+	+	−	−
Tween 80*	−	+	−	−	+	−
Aesculin*	−	−	−	w	+	−
Voges–Proskauer reaction*	w	−	+	w	+	−

*Data generated for this study by the Identification Services of the DSMZ.

(50.4 %), iso-$C_{18:0}$ (5.1 %), 10-methyl $C_{17:0}$ (10.9 %), $C_{17:1}\Delta 9c$ (12.1 %) and $C_{18:1}\Delta 9c$ (6.0 %) are the predominant fatty acids. Mycolic acids are absent. The predominant isoprenoid quinone present is MK-8(H_4). The DNA G+C content is 72.8 mol%. These characteristics and phylogenetic analysis of the 16S rRNA gene sequence indicated that PVAS-1^T is a member of the genus *Janibacter*.

Cells of strains B3W22^T and B8W22^T are Gram-positive, rod-shaped, endospore-forming and catalase-positive, with $C_{15:0}$, $C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{14:0}$, anteiso-$C_{16:0}$ and $C_{16:1}\Delta 9c$ as the predominant fatty acids (Tables 3 and 4). The polar lipid profile of strain B3W22^T contains phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS) and one unidentified lipid, whereas that of strain B8W22^T has only PG and PE. MK-6, MK-7 and MK-8 are the menaquinones in strain B3W22^T whereas, in B8W22^T, only MK-7 was present (Table 3). The DNA G+C contents of the two strains are 38–40 mol%. These characteristics indicated that strains B3W22^T and B8W22^T are members of the genus *Bacillus* (Tables 3 and 4).

Phylogenetic analysis

The 16S rRNA gene was amplified from genomic DNA, purified and sequenced as described previously (Shivaji *et al.*, 2000; Pidiyar *et al.*, 2004). To ascertain the phylogenetic affiliation of the novel strains, the almost-complete 16S rRNA gene sequences of the bacterial isolates were manually corrected and aligned using CLUSTAL_X (Thompson *et al.*, 1994). Phylogenetic trees were constructed based on neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods using MEGA 3.1 (Kumar *et al.*, 2004). Evolutionary distances were determined with Kimura's two-parameter model (Kimura, 1980). Bootstrap analysis (Felsenstein, 1993) was performed for 1000 replications. Reference sequences were retrieved from GenBank under the accession numbers indicated on the trees.

Phylogenetic analysis of the 16S rRNA gene sequence of strain PVAS-1^T (1196 bp) indicated that its closest relatives were *Janibacter anophelis* CCUG 49715^T (98 % similarity) and *Janibacter terrae* DSM 13953^T (98 %) (Lang *et al.*, 2003). Both the neighbour-joining tree (Fig. 1) and the maximum-parsimony tree (not shown) revealed that strain PVAS-1^T clustered most closely with these strains. DNA–DNA relatedness of PVAS-1^T with *Janibacter terrae* DSM 13953^T was 29.8 % and with *Janibacter anophelis* DSM 18333^T was 13.7 %, and the reciprocal reactions in both cases gave values of about 9–10 % . Thus, strain PVAS-1^T represents a novel species of the genus *Janibacter*, for which the name *Janibacter hoylei* sp. nov. is proposed.

Strains B3W22^T and B8W22^T are related at the 16S rRNA gene sequence level (91 % similarity), but the sequences differ by more than 2.5 %, indicating that they probably represent different species. BLASTN analysis indicated that the nearest phylogenetic neighbour of B3W22^T is *Bacillus silvestris* H3-23^T (99.5 % similarity), with which it forms a clade in the phylogenetic tree, and it is separated from *Caryophanon latum* DSM 14151^T and *Caryophanon tenue* DSM 14152^T, with which it exhibits about 97 and 96 % similarity, respectively (Fig. 2). The members of the genus *Caryophanon* are characterized by the presence of slightly curved to straight trichomes, and are thus clearly different from B3W22^T.

DNA–DNA hybridization studies indicated that the relatedness between B3W22^T and *Bacillus silvestris* DSM

12223^T is only 29 % (reciprocal reaction 37 %). Furthermore, these two strains exhibit phenotypic and chemotaxonomic differences (Tables 3 and 4) that imply that B3W22^T represents a novel species; the name proposed is *Bacillus isronensis* sp. nov.

In the phylogenetic tree, strain B8W22^T forms a robust clade with its nearest phylogenetic neighbour *Bacillus megaterium* IAM 13418^T (Fig. 2), with which it shows 99.7 % similarity at the 16S rRNA gene sequence level. Despite this high similarity, it is observed that, at the whole genome level, the DNA–DNA relatedness between B8W22 and *Bacillus megaterium* MTCC 428^T is only 35 % (reciprocal reaction 43 %), indicating that B8W22^T could be assigned to a novel species. Furthermore, B8W22^T and *Bacillus megaterium* MTCC 428^T differ in their phenotypic and chemotaxonomic characteristics (Tables 3 and 4), implying that B8W22^T does indeed represent a novel species, for which the name proposed is *Bacillus aryabhattai* sp. nov. Similarity of B8W22^T to other *Bacillus* species at the 16S rRNA gene sequence level was less than 95 %.

Sensitivity to UV radiation

Cultures were grown to an OD_{660} of 1.0 at 30 °C and the sensitivity to UV radiation was determined by exposure to a UV lamp (UV-B, 15 W64; Sankyo Denki) as described previously (Shivaji *et al.*, 2006). Cultures of *Bacillus silvestris* DSM 12223^T, *Bacillus megaterium* MTCC 428^T, *Micrococcus luteus* MTCC 106^T, *Janibacter limosus* DSM 11140^T and *Janibacter terrae* DSM 13876^T were used as controls in these experiments. The results indicate that strains B3W22^T, B8W22^T and PVAS-1^T are more resistant to UV irradiation than their nearest phylogenetic neighbours (Supplementary Table S1, available in IJSEM Online).

Conclusions

The bacterial strains identified are unlikely to be laboratory contaminants, because no such cultures were handled in the laboratory. The control plates that were exposed to the air flow of the laminar flow hood during the entire air filtration procedure did not show any colonies. Furthermore, all possible precautions were taken to rule out contamination of the cryotubes throughout the assembly and deployment stages of the experiment; it is unlikely that they came from the cryotube assembly facility and survived the transit through the stratosphere, because the control cryotubes were completely sterile. It has been argued that micro-organisms can travel across interplanetary space during routine meteoritic exchanges between the Earth and Mars (Gladman *et al.*, 1996; Mileikowsky *et al.* 2000; Nicholson *et al.*, 2000). Furthermore, it is possible that micro-organisms reach the stratosphere from the Earth as a result of volcanic eruptions, the updraft caused by blue lightning strikes (Pasko *et al.*, 2002), thunderstorms and forest fires (Fromm *et al.*, 2004) and gravitophotophoresis (Rohatschek, 1996). Finally, in 1982, Hoyle and Wickramasinghe proposed the theory of 'Panspermia' (Hoyle & Wickramasinghe, 1986, 1993, 1999).

Description of *Janibacter hoylei* sp. nov.

Janibacter hoylei (hoy′le.i. N.L. gen. n. *hoylei* of Hoyle, named after Sir Fred Hoyle, the famous English astronomer).

Colonies on LB agar are creamish, entire, round and 1–2 mm in diameter. Cells stain Gram-positive and show oxidative metabolism; they are non-motile, non-endospore-forming cocci, 0.4–0.7 μm in diameter, that occur singly or in clumps. Good growth (visible colonies with a diameter of 1 mm) occurs after 2 days of incubation on nutrient agar at 25–30 °C. Grows in LB broth at 20–40 °C and at pH 5–10, with optimum growth at 30 °C and pH 9. Grows in the presence of 5 % (w/v) NaCl in LB broth and exhibits weak growth in LB broth containing 10 % NaCl. Resistant to UV radiation. Catalase- and oxidase-positive; H_2S is not produced. Voges–Proskauer negative, indole-negative and reduces nitrate to nitrite. Results of carbon source utilization tests are shown in Table 2. Does not produce acid from alcohols or the following sugars: D-glucose, D-fructose, D-galactose, lactose, maltose, cellobiose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-ribose, D-sorbitol, trehalose and D-xylose. *meso*-Diaminopimelic acid is the diagnostic diamino acid in the cell-wall peptidoglycan. The main fatty acids present are iso-$C_{16:0}$, 10-methyl $C_{17:0}$, $C_{18:1}\Delta9c$ and $C_{17:1}\Delta9c$. The predominant isoprenoid quinone present is MK-8(H_4). In whole-cell hydrolysates, ribose and glucose are present, but arabinose, galactose and mannose are absent. Mycolic acids are absent. The DNA base composition of the type strain is 72.8 mol% G+C.

The type strain is PVAS-1^T (=MTCC 8307^T =DSM 21601^T =CCUG 56714^T), isolated from an air sample collected at an altitude of 40–41.4 km using a cryosampler.

Description of *Bacillus isronensis* sp. nov.

Bacillus isronensis (is.ro.nen′sis. N.L. masc. adj. *isronensis* arbitrary name pertaining to ISRO, the acronym of the Indian Space Research Organization, which largely funded the studies in which the type strain was isolated).

Colonies on nutrient agar are white, entire, round and 3–4 mm in diameter. Cells produce round terminal endospores and are motile. Grows at 5–37 °C and pH 6–10. Does not grow at 42 °C or at pH 4 or 11. Tolerates up to 5.8 % NaCl. Resistant to UV radiation. Grows on peptone. Positive for oxidase, lipase, gelatinase, starch hydrolysis, caseinase, tryptophan deamination, nitrate reduction and indole production. Produces acid from cellobiose and utilizes a number of sugars, amino acids and other carbon compounds as sole carbon sources

Table 3. Physiological properties that differentiate strains B3W22^T and B8W22^T and their nearest phylogenetic neighbours

Strains: 1, *Bacillus isronensis* sp. nov. B3W22^T; 2, *Bacillus silvestris* DSM 12223^T; 3, *Bacillus aryabhattai* sp. nov. B8W22^T; 4, *Bacillus megaterium* MTCC 428^T. All four strains have Gram-positive, motile, rod-shaped cells that produce endospores, grow at 20–37 °C and pH 6–10, tolerate 5.8 % NaCl and grow in peptone broth and are positive for catalase and β-galactosidase but do not grow at pH 4 or 11 and are negative for citrate utilization, the methyl red test and H_2S production. None of the strains produces acid from sucrose or inulin or uses dextran, citric acid, cellulose, glycogen, thioglycolate, hydroxybutyric acid, salicin, D-glucuronic acid, L-aspartic acid, sodium succinate, valeric acid, L-cysteine, L-tyrosine, L-phenylalanine, L-proline or L-histidine as sole carbon sources. All four strains utilize glycerol, *myo*-inositol, methyl α-D-galactoside, L-ornithine and L-creatinine as sole carbon sources, are resistant to discs containing colistin (10 μg) and are sensitive to discs containing norflaxacin (10 μg), tobramycin (15 μg), lomefloxacin (30 μg), amikacin (30 μg), roxithromycin (15 μg), ciprofloxacin (30 μg), nitrofurantoin (300 μg), cefoperazone (75 μg), vancomycin (30 μg), lincomycin (15 μg), cephotaxime (30 μg), kanamycin (30 μg), novobiocin (30 μg), chloramphenicol (30 μg), ampicillin (25 μg), tetracycline (30 μg), bacitracin (10 μg), gentamicin G (30 μg), polymyxin B (50 μg), oleandomycin (15 μg), spectinomycin (100 μg), rifampicin (30 μg), erythromycin (15 μg) and carbenicillin (100 μg). +, Positive; −, negative; V, variable; W, weak; S, sensitive; R, resistant.

Characteristic	1	2	3	4
Isolation source	Upper atmosphere	Forest soil	Upper atmosphere	Soil
Colony diameter (mm)	3–4	3–4	5–8	6–8
Colony shape	Entire, round	Entire, round	Entire, round, flat	Entire, round, flat
Colony colour	White	White	Peach	White/cream
Endospores	Round, terminal	Round, terminal	Ellipsoidal, central	Ellipsoidal, central
Growth at/in:				
5 °C	+	−	−	−
10 °C	+	+	+	−
1.5 M NaCl	V	+	+	+
2 M NaCl	−	+	+	+
2.5 M NaCl	−	+	−	+
Temperature for growth (°C)				
Optimum	28	20–30	28	37
Maximum	37	40	37	40
Oxidase	+	−	+	+
Urease	−	−	+	+
Lipase (Tween 80 hydrolysis)	+	−	−	−
Gelatinase	+	−	+	+
Phosphatase	−	−	+	−
Aesculin hydrolysis	−	−	+	+
Starch hydrolysis	+	−	+	+
Lysine decarboxylase	−	−	+	+
Arginine decarboxylase	−	−	−	+
Arginine dihydrolase	−	−	−	+
Caseinase	+	−	+	+
Tryptophan deamination	+	−	+	−
Reduction of nitrate to nitrite	+	−	+	+
Indole production	+	−	−	−
Voges–Proskauer test	−	−	+	−
Ornithine decarboxylase	−	−	+	−
Malonate utilization	−	−	+	−
Acid production from:				
Fructose	V	−	+	+
Glucose, sorbitol, inositol, melibiose, mannitol, lactose, D- and L-arabinose, D- and L-xylose, D- and L-ribose, glycerol, galactose	−	−	+	+
Trehalose, maltose, adonitol, erythritol, mannose	−	−	+	−
Rhamnose	−	−	−	+
Cellobiose	+	−	+	+

Table 3. cont.

Characteristic	1	2	3	4
Carbon-source utilization				
D-Glucose, D-galactose, rhamnose, D- and L-arabinose, melibiose, sucrose, D-mannitol, raffinose, D-mannose, D-xylose, D-sorbitol, aesculin, 2-ketogluconate, 5-ketogluconate, sodium fumarate	−	−	+	+
L-Sorbose, D-fructose, trehalose, L-xylose, lactose, maltose, propionate, starch, methyl α-D-mannoside, polyethylene glycol, sodium acetate, potassium acetate, amygdalin, xylitol, erythritol	+	−	+	+
Cellobiose, fumaric acid, sodium formate	−	−	+	−
D-Ribose	−	+	+	+
Lactic acid, adonitol, *N*-acetylglucosamine, melezitose	+	−	+	−
Dulcitol, arbutin	+	−	−	+
Inulin, methyl α-D-glucoside, L-fucose	−	−	−	+
Dextrin, malic acid	+	−	−	−
Pyruvate, methyl β-D-galactoside	+	+	−	+
Sodium gluconate, malate	+	−	W	+
α-Ketoglutaric acid	+	+	−	−
Amino acids used				
Glycine, L-leucine	+	−	+	+
L-Alanine	−	−	+	+
L-Isoleucine	+	−	W	−
L-Serine, L-threonine, L-arginine, L-glutamic acid, L-methionine	−	−	+	−
L-Lysine, L-asparagine	+	+	+	−
L-Glutamine	+	−	−	−
L-Tryptophan	+	−	+	−
Antibiotic sensitivity				
Penicillin (10 μg)	S	S	R	R
Cefuroxime (30 μg)	S	R	S	S
Cefazolin (30 μg), co-trimoxazole (25 μg)	S	S	S	R
Amoxicillin (15 μg)	R	R	S	S
Streptomycin (25 μg), nalidixic acid (30 μg)	R	S	S	S
Doxycycline (10 μg)	R	R	S	R
DNA G+C content (mol%)	40.0	39.3	38.0	37.3
Peptidoglycan type*	Lys–Glu–Ala	Lys–Glu–Ala	DAP–Ala–Glu	DAP
Menaquinone(s)	6, 7, 8	6, 7, 8	7	7
Polar lipids†	PG, PE, PS, 1UL	PG, DPG, PE, PS, 1UL	PG, PE	PG, PE

*DAP, Diaminopimelic acid.

†DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; UL, unknown lipid.

(Table 3). The type strain is sensitive to a number of antibiotics (Table 3). The fatty acids present are $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, iso-$C_{13:0}$, iso-$C_{15:0}$, anteiso-$C_{14:0}$, anteiso-$C_{16:0}$, $C_{16:1}\Delta 9c$, $C_{16:1}\Delta 11$ and $C_{18:1}\Delta 9$ (Table 4). The interpeptide bridge of the cell-wall peptidoglycan consists of Lys–Glu–Ala. The lipids present are PG, PE, PS and an unidentified lipid. MK-6, MK-7 and MK-8 are the menaquinones present. The DNA G+C content of the type strain is 40 mol%.

The type strain is B3W22^T (=MTCC 7902^T =JCM 13838^T), isolated from a cryogenic tube used for collecting an air sample from an altitude of 27–30 km.

Description of *Bacillus aryabhattai* sp. nov.

Bacillus aryabhattai (a.ry.a.bhat′ta.i. N.L. gen. n. *aryabhattai* of Aryabhatta, named after the renowned Indian astronomer of the 5th century AD).

Table 4. Total fatty acid compositions of strains B3W22^T and B8W22^T and related type strains

Strains: 1, *Bacillus isronensis* sp. nov. B3W22^T; 2, *Bacillus silvestris* DSM 12223^T; 3, *Bacillus aryabhattai* sp. nov. B8W22^T; 4, *Bacillus megaterium* MTCC 428^T. Data are percentages of total fatty acids and were obtained in this study; strains were grown on nutrient agar (1 % beef extract, 1 % peptone, 0.5 % NaCl and 1.5 % agar; pH 7.2) and incubated overnight at 30 °C.

Fatty acid	1	2	3	4
$C_{12:0}$	0	0	0	7.4
$C_{13:0}$	0	0	0	1.0
$C_{14:0}$	0	2.5	4.2	1.2
$C_{15:0}$	51.5	57.6	46.2	5.2
$C_{16:0}$	2.8	1	0	35.7
$C_{17:0}$	4.5	3.3	0	0
$C_{18:0}$	0	0	2.8	0
iso-$C_{13:0}$	1.8	0	0	0
iso-$C_{15:0}$	3.5	3.2	1.3	1.0
anteiso-$C_{14:0}$	8.8	8.4	34.3	1.1
anteiso-$C_{16:0}$	2.8	2.5	5.7	5.4
$C_{16:1}\Delta 9c$	19.8	21.5	4.7	13.8
$C_{16:1}\Delta 11c$	2.0	0	0	0
$C_{18:1}\Delta 9c$	2.5	0	0	2.1
$C_{18:1}\Delta 11c$	0	0	0	26.6

Colonies on nutrient agar are peach-coloured, entire, round, flat and 5–8 mm in diameter. Cells produce ellipsoidal and central endospores and are motile. Growth occurs at 10–37 °C and pH 6–10. Does not grow at 42 °C or at pH 4 or 11. Tolerates up to 11.6 % NaCl. Resistant to UV radiation. Grows on peptone. Positive for phosphatase, DNase, tryptophan deamination, ornithine decarboxylase and malonate utilization (Table 3). Produces acid from various carbon sources and utilizes a number of sugars, amino acids and other carbon compounds as sole carbon sources (Table 3). The type strain is sensitive to a number of antibiotics (Table 3). The fatty acids present are $C_{14:0}$, $C_{15:0}$, $C_{18:0}$, iso-$C_{15:0}$, anteiso-$C_{14:0}$, anteiso-$C_{16:0}$ and $C_{16:1}\Delta 9c$ (Table 4). The interpeptide bridge of the cell-wall peptidoglycan consists of *meso*-diaminopimelic acid–Ala–Glu. The lipids present are PG and PE. MK-7 is the menaquinone present. The DNA G+C content of the type strain is 38 mol%.

The type strain is B8W22^T (=MTCC 7755^T=JCM 13839^T), isolated from a cryogenic tube used for collecting an air sample from an altitude of 40–41.4 km.

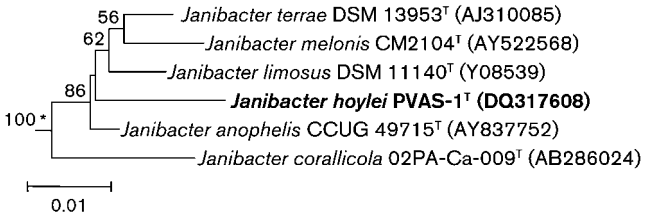

Fig. 1. Neighbour-joining phylogenetic tree reconstructed on the basis of 16S rRNA gene sequences showing the phylogenetic relationships between strain PVAS-1^T and closely related species of the genus *Janibacter*. The sequence of *Brevibacterium linens* DSM 20425^T (GenBank accession no. X77451; not shown) was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) >50 % are given at nodes. Bar, 1 substitution per 100 nucleotide positions. The asterisk (*) indicates that this was the only branch point that was conserved in the parsimony analysis.

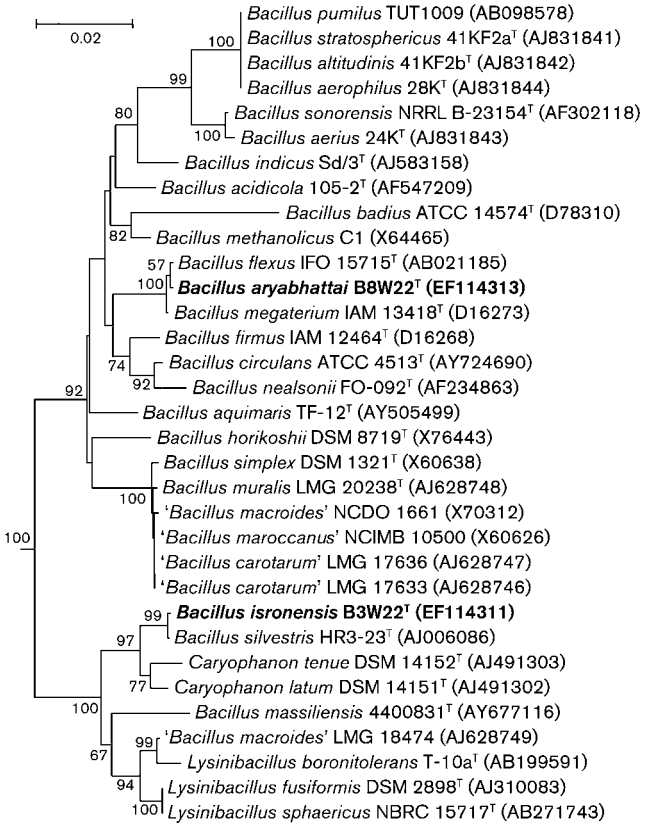

Fig. 2. Neighbour-joining phylogenetic tree reconstructed on the basis of 16S rRNA gene sequences showing the phylogenetic relationships between strains B3W22^T and B8W22^T and closely related species of the genera *Bacillus*, *Caryophanon* and *Lysinibacillus*. The sequence of *Microbacterium indicum* DSM 20030^T (GenBank accession no. AJ536198; not shown) was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) >50 % are given at nodes. Bar, 2 substitutions per 100 nucleotide positions.

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