***Paenibacillus turicensis* sp. nov., a novel bacterium harbouring heterogeneities between 16S rRNA genes**

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A Gram-positive, facultatively anaerobic, rod-shaped, endospore-forming bacterium (strain MOL722^T) was characterized using phenotypic and molecular methods. Fatty acid analysis and biochemical examination indicated that the isolate belongs to the Gram-positive bacteria with low DNA G+C content, probably to the genus *Paenibacillus*. Direct sequencing of the 16S rRNA gene showed ambiguities, suggesting heterogeneity. Cloned 16S rDNA yielded seven different sequences varying at 15 positions, with one being an insertion. The isolate shares 98.5% sequence similarity with *Paenibacillus* sp. P15-9, but less than 94% similarity with other paenibacilli and bacilli. Phylogenetic analysis with different treeing methods revealed that strain MOL722^T, together with *Paenibacillus* sp. P15-9, forms a novel monophyletic clade within the genus *Paenibacillus*. Based on phenotypic and phylogenetic evidence, it is proposed that the unknown bacterium is classified as the novel species *Paenibacillus turicensis* sp. nov., the type strain of which is strain MOL722^T (= DSM 14349^T = NCCB 100011^T).

Keywords: *Paenibacillus turicensis*, 16S rDNA, 16S rRNA heterogeneities

INTRODUCTION

Based on phylogenetic data from 16S rDNA sequences, Ash *et al.* (1993), separated the so-called group 3 bacilli as the new genus *Paenibacillus*. Since then, the genus has grown from 11 to over 30 species. Paenibacilli have been isolated from a variety of sources including soil, water, the plant rhizosphere, food and diseased insect larvae, but not from clinical specimens (Daane *et al.*, 2002). Strain MOL722^T was isolated from the valve of a cerebrospinal fluid shunt of a 48-year-old man. The Gram-positive rod could not be identified by using routinely performed diagnostic methods, whereas sequencing of the 5' end of the 16S rDNA indicated moderate similarities (< 92%) to members of the genus *Paenibacillus*. We decided to investigate this strain by biochemical and molecular means.

Characterization of 16S rRNA gene sequences has become a common method for identification and

classification of bacteria. The molecular approach has also been used extensively for bacterial phylogeny (Woese *et al.*, 1990) and analysis of bacterial diversity (Bosshard *et al.*, 2000; Fox *et al.*, 1980). Many studies proposing novel species or higher-level groups include 16S rDNA sequencing (Ash *et al.*, 1993; Daane *et al.*, 2002; Chung *et al.*, 2000; Stackebrandt *et al.*, 1997). Although not intensively studied, intraspecies and even intrastrain 16S rRNA gene heterogeneities are a common phenomenon. Clayton *et al.* (1995) reported unexpected high levels of intraspecific variation (within and between strains) of prokaryotic 16S rRNA sequences deposited in GenBank. The variations were more widespread than predicted from sequencing errors. Several publications deal with intraspecific polymorphisms in 16S rRNA genes (Bricker, 2000; Cilia *et al.*, 1996; Liefting *et al.*, 1996; Martínez-Murcia *et al.*, 1999; Ninet *et al.*, 1996; Nubel *et al.*, 1996; Pettersson *et al.*, 1998; Reischl *et al.*, 1998). Remarkable effects on phylogeny and biodiversity estimates have been reported (Cilia *et al.*, 1996; Clayton *et al.*, 1995).

Full-length sequencing of PCR-amplified 16S rDNA from strain MOL722^T resulted in a mixture of sequences starting at position 1138 (*Escherichia coli*

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The GenBank accession numbers for the 16S rDNA sequences of strain MOL722^T are AF378694–AF378700.

numbering; Brosius *et al.*, 1978). Therefore, multiple clones of 16S rDNA were sequenced and revealed an insertion of one nucleotide at position 1138 in one type of 16S rDNA within this strain. In total, seven different 16S rRNA genes were determined, with 15 variable nucleotide positions.

METHODS

Bacterial strain and culture. Strain MOL722^T was isolated from the valve of a cerebrospinal fluid shunt of a 48-year-old man with hydrocephalus that he developed after an accident. A second control sample was sterile. The isolate was cultivated on different standard media under oxic and anoxic conditions (see phenotypic characteristics in Results and Discussion). Trypticase soy broth was used for determining the pH optimum for growth, the sodium chloride tolerance, lysozyme tolerance and doubling time. The strain was maintained in skim milk at -70°C .

Phenotypic characteristics. The morphological properties were examined by light and transmission electron microscopy. Biochemical reactions were determined by using the API 20E and API 50CHB systems according to the manufacturer's instructions (API bioMérieux) and by an in-house identification scheme for rapidly and aerobically growing Gram-positive rods (von Graevenitz & Funke, 1996). Cellular fatty acids were analysed with the MIDI system (Microbial Identification) as described previously (von Graevenitz *et al.*, 1994). Metabolic acids were identified by GLC on a GC 8000 (CE Instruments).

Genotypic analysis

DNA extraction and PCR. DNA was extracted from a single colony using the PrepMan reagent according to the manufacturer's protocol (Applied Biosystems). For blotting, high-

molecular-mass chromosomal DNA was extracted using standard methods. The 16S rRNA gene was amplified using primers 1 and 12 (Table 1). The temperature and duration for denaturation, annealing and extension were 90°C for 15 s, 50°C for 30 s and 70°C for 45 s. Forty cycles were performed using AmpliTaq DNA polymerase LD (Applied Biosystems).

Cloning. PCR products obtained with primers 1 and 12 were loaded on an agarose gel and bands of the correct size were excised and purified (Gel extraction kit, Qiagen). After standard A-tailing, the PCR products were ligated into the vector pCR2.1 (Stratagene) and the plasmids were used to transform competent cells of *E. coli* TOP10F' according to the manufacturer's instructions. Plasmid DNA was prepared from white colonies as described previously (Berghammer & Auer, 1993). Plasmids containing inserts of the correct size were sequenced with 12 different primers (Table 1) using the BigDye kit and an automatic DNA sequencer (model 310; Applied Biosystems). The primers, except numbers 6 and 7, were derived from Lane (1991). Most of them were modified to have approximately the same melting temperature and are specific for the domain *Bacteria*. Primer 6 was described previously (Fischer-Romero *et al.*, 2000); primer 7 is the reverse-complement of primer 6.

Blotting. High-molecular-mass DNA was digested with *Hind*III and *Kpn*I, loaded on a 0.8% agarose gel and blotted using standard methods. Digoxigenin-labelled probes for hybridization were DIG-Bact-8s, DIG-MOL722-GTAA and DIG-MOL722-TTC (Table 1). Hybridization was performed at 48°C for 4 h. Detection was done colorimetrically using NBT and BCIP.

Phylogenetic analysis. The 16S rDNA sequences of strain MOL722^T (homologous to *E. coli* positions 8–1542; Brosius *et al.*, 1978) were aligned with representative sequences (accessions and collection numbers of the respective strains

Table 1. Primers for amplification and sequencing of total 16S rDNA and probes for Southern blot hybridization

Primers 1 and 12 were used for amplification; primers 1–12 were utilized for sequencing. Positions are given relative to the *E. coli* numbering (Brosius *et al.*, 1978).

Primer	Sequence (5' → 3')	Position
1. Bact-8s-20	AGAGTTTGATCMTGGCTCAG	8–27
2. Bact-339a-17	GCTGCCTCCCGTAGGAG	355–339
3. Bact-341s-17	CCTACGGGAGGCAGCAG	341–357
4. Bact-536a-18	GTATTACCGCGGCTGCTG	536–519
5. Bact-519s-18	CAGCAGCCGCGTAATAC	519–536
6. Bact-787a-20	GGACTACHAGGGTATCTAAT	806–787
7. Bact-787s-20	ATTAGATACCCTDGTAGTCC	787–806
8. Bact-907s-20	AAACTCAAAGGAATTGACGG	907–9267
9. Bact-1098a-17	GGGTTGCGCTCGTTGCG	1114–1098
10. Bact-1098s-17	CGCAACGAGCGCAACCC	1098–1114
11. Bact-1389s-18	CTTGTACACACCGCCCGT	1389–1406
12. Bact-1525a-17	AAGGAGGTGATCCARCC	1541–1525
DIG-Bact-8s	AGAGTTTGATCMTGGCTCAG*	8–27
DIG-MOL722-TTC	AGCAATTCGGTTGGGCACT*	1130–1148
DIG-MOL722-GTAA	AGCAAGTAAGGTTGGGCAC*	1130–1147

* Probes are digoxigenin-labelled at the 5' end.

are shown in Fig. 4) of all validly published paenibacilli and other taxa obtained from the GenBank database using PILEUP from the Wisconsin GCG package (Devereux *et al.*, 1984). Phylogenetic trees with bootstrap analysis were constructed with the neighbour-joining, UPGMA, Fitch–Margoliash and maximum-parsimony methods using the programs SEQBOOT, DNADIST, NEIGHBOR, FITCH, KITSCH, DNAPARS and CONSENSE of the PHYLIP program package (Felsenstein, 1989). The trees were rooted using *E. coli* (accession no. J01859) as an outgroup. In addition, the sequences were fitted into an alignment of about 6500 full or partial primary structures provided with the ARB software package (Strunk *et al.*, 1999) as described elsewhere (Bosshard *et al.*, 2002).

RESULTS AND DISCUSSION

Phenotypic characteristics

Phenotypic characteristics of strain MOL722^T and other selected paenibacilli are shown in Table 2. Cells of strain MOL722^T were Gram-positive, motile, spore-forming rods, approximately 1.5–3 × 0.5–1 µm in size. The isolate produced ellipsoidal endospores in swollen sporangia in the terminal or subterminal region of the cell (Fig. 1). Strain MOL722^T was facultatively anaerobic and grew on sheep-blood, chocolate and Brucella agars. Growth was also observed in thio-glycollate, Todd–Hewitt and trypticase soy broth. In Luria–Bertani medium, very weak growth was observed, and no growth occurred on MacConkey agar. On sheep-blood agar, the non-haemolytic colonies were greyish-white with a convex elevation and regular margins. Growth was found to be optimal at 37–42 °C, with a range from 15 to 48 °C. In contrast, the optimum growth temperature for the majority of *Paenibacillus* species is 28–30 °C (Shida *et al.*, 1997a). However, only recently, several novel *Paenibacillus* species have been described with temperature optima around 37–40 °C (Chung *et al.*, 2000; Shida *et al.*, 1997b; Tcherpakov *et al.*, 1999; Yoon *et al.*, 1998). The doubling time at optimal pH and temperature was 45 min as measured by OD₆₀₀.

In contrast to almost all other *Paenibacillus* species (Shida *et al.*, 1997a), strain MOL722^T was negative for catalase. Oxidase was also negative. The strain was susceptible to vancomycin but resistant to penicillin. Acid production as shown in Table 2 was tested using the API 50CH system. In contrast to other paenibacilli (Daane *et al.*, 2002), trehalose was not fermented. Although the T-values were low, the strain was identified as *Bacillus circulans* (T = 0.61, %id = 94.5) or *Bacillus coagulans* (T = 0.63, %id = 3.2) by the API 50CH system. This illustrates the general problem of (mis-)identification by biochemical means for species not included in a given database. The results of cellular fatty acid analysis are shown in Table 3. A predominance of anteiso-C_{15:0} is typical for paenibacilli; however, these are the highest reported values of C_{16:0}, C_{14:0} and iso-C_{14:0} for members of this genus (Shida *et al.*, 1997a). End products of glucose fermentation were acetate and lactate only.

Thus, strain MOL722^T can be differentiated from other paenibacilli by its negative catalase, its inability to ferment trehalose, its higher temperature optimum for growth and its relatively high contents of C_{16:0}, C_{14:0} and iso-C_{14:0} fatty acids.

Genotypic analysis

To investigate the genealogical classification of the isolate, comparative 16S rRNA gene sequence analysis was performed. Direct sequencing failed in both directions from position 1144 onward (position 1138, *E. coli* numbering; Brosius *et al.*, 1978). From this nucleotide on, the sequence was ambiguous, as if two different sequences were interleaved. Later, sequencing of cloned 16S rDNA revealed an insertion of one nucleotide in a stretch of three and four variable positions, respectively, on one type of the gene, leading to a sequence shift.

Sequencing of various clones containing 16 rRNA genes revealed the presence of several rRNA operons. Besides the above-mentioned highly variable region at positions 1141–1144 (types A and B), a second highly variable region was found at positions 195–198 (types 1–3). In total, 15 variable positions were detected by comparing plasmid insert sequences (Table 4). Ambiguities that occurred in only one clone were interpreted as possible errors of the polymerase during amplification and are therefore not taken into account. In pairwise comparisons, two to 12 transitions and transversions are equivalent to sequence differences of 0.15 to 0.77% within the total 16S rRNA gene. The variable nucleotide positions detected correspond to rapidly evolving nucleotides in the regions V1, V2, V6, V7, V9 and the stem of helix 50 (according to Neefs *et al.*, 1993). The potential secondary structures of the two highly variable regions (positions 195–198 and 1141–1144) in biologically active rRNAs are shown in Fig. 2. All differences at positions 195–198 in helix 10 (V2) are of the transition type U:A ↔ U:G and vice versa (Fig. 2a). In rRNAs, non-canonical pairs U:G are frequently characteristic features (Gutell *et al.*, 1994). The differences at positions 1141–1144 (V7) are located in the terminal loop of helix 43 (Fig. 2b). Thus, the exchanges do not interfere significantly with secondary structure formation.

Southern blot hybridization experiments (Fig. 3) were designed to verify the presence of different 16S rDNA sequences in the genome of strain MOL722^T. For restriction, two enzymes (*Hind*III and *Kpn*I) were used that cut once in the 16S rDNA. The probe sequences are shown in Table 1. Probe DIG-Bact-8s generated eight (*Hind*III) and six (*Kpn*I) hybridization signals (Fig. 3a, lanes 2 and 3). Of the eight bands from the digestion with *Hind*III, two are double bands, which were interpreted with care, i.e. six to eight different rRNA genes. The probes DIG-MOL722-TTC and DIG-MOL722-GTAA are two newly designed probes directed against the variable regions of clone types A and B at positions 1141–1144. With these probes, three

Table 2. Phenotypic characteristics of strain MOL722^T and other *Paenibacillus* species

Taxa: 1, MOL722^T (data from this study); 2, *P. alvei*; 3, *P. pabuli*; 4, *P. polymyxa*; 5, *P. validus* (data for taxa 2–5 from Daane *et al.*, 2002; Shida *et al.*, 1997a, b); 6, *P. naphthalenovorans* (Daane *et al.*, 2002); 7, *P. jamilae* (Aguilera *et al.*, 2001); 8, *P. koreensis* (Chung *et al.*, 2000); 9, *P. campinasensis* (Yoon *et al.*, 1998); 10, *P. dendritiformis* (Tcherpakov *et al.*, 1999); 11, *P. thiaminolyticus* (Shida *et al.*, 1997a). All strains were negative for H₂S production and tryptophan deaminase (not reported for taxa 7–11) and lysine decarboxylase (not reported for taxa 7, 9–11). None of the strains produced acid from erythritol, L-xylose, dulcitol, D-xylose, D-fucose, D-arabitol, L-arabitol, 2-ketogluconate or 5-ketogluconate (not reported for taxa 8–11). All strains produced acid from maltose and sucrose, but not from D-tagatose (not reported for taxa 9–11). +, Positive; –, negative; v, variable; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Gram reaction	V	NR	+	NR	NR	+	V	+	V	–	NR
Anaerobic growth	+	+	V	+	–	–	+	+	+	+	+
Temperature for growth (°C)											
Optimum	37–42	28	28–30	30	28–35	30–37	30	38–40	40	37	28
Range	15–48	NR	NR	NR	NR	NR	30–40	10–50	10–45	20–45	NR
pH for growth											
Optimum	7	NR	NR	NR	NR	NR	7	NR	10	NR	NR
Range	5.5–9.5	NR	NR	NR	NR	NR	5–12	NR	7.5–10.5	NR	NR
Growth at/with:											
10 °C	–	–	+	–	–	–	–	NR	+	–	NR
50 °C	–	–	–	–	+	–	NR	NR	–	–	–
pH 5.6	+	–	+	+	NR	NR	+	NR	–	+	–
3 % NaCl	+	–	+	+	–	–	–	NR	+	+	V
5 % NaCl	+	–	–	–	–	–	–	NR	+	+	V
0.001 % Lysozyme	+	+	–	+	NR	NR	+	+	NR	NR	–
0.1 % Lysozyme	+	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Catalase	–	+	+	+	+	+	+	+	+	+	+
Oxidase	–	NR	–	–	–	NR	–	+	–	+	+
ONPG	+	+	+	+	–	–	–	NR	NR	NR	NR
Arginine dihydrolase	–	–	–	–	–	–	NR	+	NR	NR	NR
Ornithine decarboxylase	–	–	–	–	–	–	NR	+	NR	NR	NR
Citrate utilization	–	–	–	–	–	V	–	–	NR	–	+
Urease	–	+	–	–	+	+	NR	–	–	+	–
Indole production	–	+	–	–	–	–	NR	–	NR	+	+
Voges–Proskauer	+	–	–	+	–	+	+	–	NR	NR	NR
Gelatin hydrolysis	–	+	–	+	–	–	+	NR	–	NR	NR
Nitrate reduction	–	–	V	V	V	V	+	+	NR	–	+
Aesculin hydrolysis	+	+	+	+	+	–	+	+	–	NR	NR
Acid production from:											
Glycerol	–	+	+	+	+	–	+	NR	NR	NR	NR
D-Arabinose	–	–	+	–	–	–	–	NR	NR	NR	NR
L-Arabinose	+	–	+	+	–	–	+	+	NR	–	V
Ribose	+	+	+	+	+	–	+	–	NR	NR	NR
D-Xylose	+	–	+	+	+	–	+	–	NR	–	–
Adonitol	–	+	–	–	–	–	–	–	NR	NR	NR
Methyl β-xyloside	+	–	+	+	–	–	–	NR	NR	NR	NR
Galactose	+	–	+	+	+	+	+	+	NR	NR	NR
D-Glucose	+	–	+	+	+	+	+	+	NR	+	+
D-Fructose	+	–	+	+	+	V	+	NR	NR	NR	NR
D-Mannose	+	–	+	+	+	+	+	NR	NR	NR	NR
L-Sorbose	–	–	–	–	–	V	–	NR	NR	NR	NR
Rhamnose	–	–	+	–	–	–	–	+	NR	NR	NR
Inositol	–	–	+	–	+	–	–	+	NR	NR	NR
Mannitol	–	–	+	+	+	+	+	+	NR	–	V
Sorbitol	–	–	–	–	–	–	–	+	NR	NR	NR
Methyl α-D-mannoside	–	–	–	–	–	–	V	NR	NR	NR	NR

Table 2 (cont.)

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Methyl α -D-glucoside	—	—	+	+	+	V	V	NR	NR	NR	NR
N-Acetylglucosamine	+	+	+	—	—	—	—	+	NR	NR	NR
Amygdalin	+	—	+	+	—	—	+	—	NR	NR	NR
Arbutin	+	+	+	+	—	—	+	NR	NR	NR	NR
Salicin	+	—	+	+	—	—	+	—	NR	NR	NR
Cellobiose	+	—	+	+	—	—	+	NR	NR	NR	NR
Lactose	+	—	+	+	—	—	+	+	NR	NR	NR
Melibiose	+	+	+	+	+	V	+	NR	NR	NR	NR
Trehalose	—	+	+	+	+	+	+	+	NR	NR	NR
Inulin	—	—	+	+	—	—	V	—	NR	NR	NR
Melezitose	—	—	+	+	—	—	—	NR	NR	NR	NR
D-Raffinose	+	+	+	+	+	—	+	+	NR	NR	NR
Starch	+	+	+	+	+	—	+	NR	NR	+	+
Glycogen	+	—	+	+	+	—	+	NR	NR	NR	NR
Xylitol	—	—	—	—	—	V	—	NR	NR	NR	NR
β -Gentiobiose	+	+	+	+	—	—	V	NR	NR	NR	NR
D-Turanose	—	—	+	+	+	V	+	NR	NR	NR	NR
L-Fucose	—	—	+	—	—	—	NR	NR	NR	NR	NR
Gluconate	—	—	+	—	—	—	—	NR	NR	NR	NR

Fig. 1. Transmission electron micrographs of a vegetative cell (a), an endospore in a swollen sporangium (b) and an endospore (c) of isolate MOL722^T from a 3 day culture on sheep-blood agar. Bar, 0.4 μ m.

to five fragments were detected in the restriction patterns (Fig. 3b, c). However, they hybridized only to equally sized fragments. From these experiments, it is not evident how many 16S rRNA genes of type A or B are present in the novel strain. A more precise investigation of the rRNA operons would require much more detailed analysis. Nevertheless, from the Southern blot analysis, it is concluded that strain MOL722^T contains at least six to eight different rRNA operons, which is in good agreement with cloning experiments, where seven different sequences were found.

rRNA sequences are, in general, believed to show low variability within species (Woese, 1987). However, Clayton *et al.* (1995) reported unexpected high levels of

intraspecific variation (within and between strains) of prokaryotic 16S rRNA sequences deposited in GenBank. Twenty-six per cent of sequence pairs (two deposited sequences from one species) exhibited differences of more than 1%. In bacteria, rRNA operons are present in numbers of one to 15 (Klappenbach *et al.*, 2001). Few studies address the question of heterogeneity among multiple 16S rRNA operons; the reported differences range from 0 to 5% (Bricker, 2000; Cilia *et al.*, 1996; Liefting *et al.*, 1996; Mylvaganam & Dennis, 1992; Ninet *et al.*, 1996; Nubel *et al.*, 1996; Pettersson *et al.*, 1998). In *Paenibacillus polymyxa*, for example, a maximum of eight variant positions were found in a fragment of 347 bp by pairwise comparison of the six sequences, equivalent to a 2.3% sequence difference (Nubel *et al.*,

Table 3. Cellular fatty acid composition of strain MOL722^T and several other species of the genus *Paenibacillus*

Values are percentages of total fatty acids. Data for reference species were taken from Daane *et al.* (2002), Shida *et al.* (1997a) and Tcherpakov *et al.* (1999). ND, Not detected.

Fatty acid	MOL722 ^T	<i>Paenibacillus</i> spp.
C _{14:0}	8.9	0.2–3.7
C _{15:0}	ND	ND–3.5
C _{16:0}	25.4	1.5–24.7
iso C _{14:0}	8.5	ND–7.9
iso C _{15:0}	5.8	0.2–14.4
iso C _{16:0}	4.1	1.3–27.1
iso C _{17:0}	ND	0.2–7.1
anteiso C _{15:0}	41.4	34.5–81.0
anteiso C _{17:0}	ND	1.0–30.2
C _{16:1} ω11	ND	ND–5.2
iso C _{17:1} ω10	ND	ND–1.7

1996) and, in the archaeon *Haloarcula marismortui*, even a 5% difference has been reported (Mylvaganam & Dennis, 1992). These findings document that heterogeneities between 16S rRNA genes seems to be a common phenomenon and, that for species identification, 16S rDNA analysis has to be interpreted with care.

Phylogenetic analysis

Comparative database analysis using the fastA algorithm (Pearson & Lipman, 1988) with the six cloned full-length 16S rRNA genes suggested that strain MOL722^T belongs to the genus *Paenibacillus*. Additionally, the signature sequences BG3 (Ash *et al.*, 1993) and PAEN515F (Shida *et al.*, 1997a) for the genus *Paenibacillus* were also found within the 16S rDNA sequences of our strain. Levels of 16S rDNA sequence similarity were 98.5% to *Paenibacillus* sp.

Fig. 2. Proposed secondary structures of the two variable regions of strain MOL722^T 16S rRNA. The variable positions are framed. (a) Helix 10 in the V2 region (Neefs *et al.*, 1993), with the three different sequences possible at positions 195–198. (b) Helix 43 in the V7 region, with the two observed terminal loops at positions 1141–1144. Numbers correspond to positions in the sequence of clone type A.

isolate P15-9 and between 93.7% (*Paenibacillus illinoisensis* and *P. polymyxa*) and 87.2% (*Paenibacillus alvei*) to other paenibacilli. However, the strain also showed relatively high similarities to other bacilli, e.g. 93.7% to *Bacillus chitinolyticus*, 93% to *Bacillus ehimensis* and 91.5% to *Bacillus vortex*. Database searches with these *Bacillus* species suggest that the next relatives of *B. chitinolyticus*, *B. ehimensis* and *B. vortex* are members of the genus *Paenibacillus*. This indicates that these three species may be misclassified as *Bacillus* species and are probably indeed members of *Paenibacillus*. After multiple sequence alignment, the similarities were 99.2% to *Paenibacillus* sp. isolate P15-9 and between 93.5% (*P. illinoisensis*) and 89.1% (*Paenibacillus alginolyticus*) to the other paenibacilli. This is, to the knowledge of the authors, the first time that intragenus identities have been found to be below the value reported by Shida *et al.* (1997b)

Table 4. Variable 16S rDNA positions between MOL722^T clones

Numbers correspond to positions in the sequence of clone type A. ND, Not determined.

Clone type	73	89	195–198	209	223	1012	1141–1144	1270	1535
A1	A	A	AAAG	C	A	G	GTAA	A	C
A2	A	A	AGAG	C	G	G	GTAA	G	C
A3a	A	A	GAGA	C	A	G	GTAA	A	T
A3b*	ND	ND	GAGA	T	A	G	GTAA	A	C
B1	A	G	AAAG	C	A	A	TTC–	G	C
B2	G	G	AGAG	C	A	G	TTC–	G	C
B3	A	G	GAGA	T	A	A	TTC–	G	T

* This clone contained only a partial sequence as insert.

Fig. 3. Southern blot. Chromosomal DNA from strain MOL722^T was digested with *Hind*III (lanes 2) and *Kpn*I (lanes 3). Lanes 1 contain markers, lane 4 in (c) is a PCR product of the 16S rDNA of strain MOL722^T. Fragments were separated by agarose gel electrophoresis and blots were hybridized with probe DIG-Bact-8s (a), DIG-MOL722-TTC (b) or DIG-MOL722-GTAA (c). Arrows indicate double bands and bands smaller than 0.5 kb, which are of low intensity. Bands of low intensity but larger than 2.0 kb were interpreted as partially digested fragments.

(89.9%) for members of the genus *Paenibacillus*. Although DNA–DNA reassociation is still considered the ‘gold standard’ in the present species definition, no DNA–DNA reassociation experiments were performed in this study because the similarity values to other species were low. According to Stackebrandt & Goebel (1994), DNA pairing studies need to be performed at rDNA similarities of $\geq 97\%$. Below this value, it can be assumed that the strains belong to two different species or even higher taxonomic groups. Keswani & Whitman (2001) even suggested a threshold value of 98.6% similarity for taxa where the rRNA sequences do not possess non-ultrametric properties and Drancourt *et al.* (2000) used 99% for species identification.

The phylogenetic affiliation was inferred using different programs, neighbour-joining, UPGMA, Fitch–Margoliash and maximum-parsimony, of the PHYLIP package (Felsenstein, 1989) and maximum-parsimony of the ARB package (Strunk *et al.*, 1999). All methods placed the six sequences of strain MOL722^T side by side and together with the sequence of *Paenibacillus* sp. isolate P15-9 as a monophyletic, deeply branching clade within the genus *Paenibacillus*.

Fig. 4. Phylogenetic relationship of strain MOL722^T to most validly published *Paenibacillus* species and some other aerobic, rod-shaped, endospore-forming bacteria based on 16S rRNA gene sequences. The branching pattern was generated by the neighbour-joining method (with Jukes–Cantor distance correction). A total of 1430 aligned bases between positions 41 and 1423 (*E. coli* numbering; Brosius *et al.*, 1978) were used for this analysis. Numbers indicate bootstrap values greater than 50 (of 100 cycles). Sequence accession numbers are in parentheses. Bar, 0.1 nucleotide substitutions per site.

The phylogram in Fig. 4 shows the detailed evolutionary relationship of strain MOL722^T and most validly described paenibacilli as deduced by the neighbour-joining method.

Based on all of the above results, it is concluded that strain MOL722^T represents a novel species of *Paenibacillus*, for which the name *Paenibacillus turicensis* sp. nov. is proposed. It is assumed that this novel isolate was probably a contaminant in the specimen and does not have clinical significance.

Description of *Paenibacillus turicensis* sp. nov.

Paenibacillus turicensis (tu.ri.cen'sis. L. adj. *turicensis* of Turicum, the Latin name of Zürich, where the type strain was isolated).

Cells are Gram-positive, motile rods, 1.5–3 × 0.5–1 µm in size. Terminal to subterminal oval spores are formed in swollen sporangia. On sheep-blood agar, the non-haemolytic colonies were greyish-white with a convex elevation. Facultatively anaerobic, growing at 15–48 °C and pH 5.5–9.5 with optima of 37–42 °C and

pH 7. Doubling time under optimal conditions is 45 min. Grows in the presence of up to 5 % NaCl and 0.1 % lysozyme. Negative for catalase, oxidase, urease, nitrate reduction and indole production. Positive for β -galactosidase and Voges–Proskauer reaction. Starch is hydrolysed. Ferments L-arabinose, ribose, D-xylose, methyl β -xyloside, galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, D-raffinose, glycogen, β -gentiobiose and 5-ketogluconate (weak). The major fatty acids are anteiso-C_{15:0} (41 %), C_{16:0} (25 %), C_{14:0} (9 %) and iso-C_{14:0} (9 %). The bacterium contains at least six to eight 16S rRNA genes. The 16S rDNA shares 98.5 % sequence similarity with *Paenibacillus* sp. isolate P15-9 and 93.7–87.2 % similarity with other paenibacilli. The type strain is strain MOL722^T (= DSM 14349^T = NCCB 100011^T), which harbours up to 0.77 % heterogeneity between 16S rRNA genes, including, among others, an insertion of one nucleotide.

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