Characterization of Some *Bacillus* Strains Obtained from Marine Habitats Using Different Taxonomical Methods

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Abstract: Eleven bacterial isolates were obtained from marine soil samples; collected from Red Sea, Jeddah, Saudi Arabia. The obtained isolates were compared to determine their differential characteristics and taxonomic positions. The isolates were compared with respect to their morphological, physiological, nutritional requirements and biochemical characteristics. They were all Gram positive, rod shape, spore forming, mesophilic, neutralophilic and moderate halophilic bacteria. By using the identification key and the characteristic tables, five isolates belonged to genus *Bacillus*, three of them were identified as *B. licheniformis* (27.3 %), one isolate as *B. subtilis* (9.1 %), one isolate as *B. circulans* (9.1 %). Four isolates identified as *Paenibacillus dendritiformis* (36.4 %), and two isolates showed intermediate characteristics between *B. circulans* and *P. dendritiformis* (18.2 %). Comparison of whole cell protein patterns of the all isolates using SDS polyacrylamide gel electrophoresis showed differences in their protein patterns that confirmed the previous classification.

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1. Introduction

Genus *Bacillus* represents a heterogenous group which included many important species (Blackwood et al., 2004; Liu et al., 2013). Most are widely distributed saprophytes, but some are pathogenic, including *B. anthracis*, *B. cereus*, *B. circulans*, *B. licheniformis*, and *B. subtilis* (Logan and Turnbull, 2003). Moreover, many of *Bacillus* isolates have the ability to produce different products such as antibiotics, enzymes, amino acids and other important industrial carbohydrates (Yoon et al., 2003). They used extensively in the textile, pharmaceutical and cosmetics industries and also in bioremediation (Banat et al., 2000). Species of the genus *Bacillus* were isolated from diverse habitats (Heyrman et al., 2005; Shivaji et al., 2006; Ko et al., 2006) and saline environments such as soils and aquatic habitats (Arahal and Ventosa, 2002).

These species exhibit different aspects in morphology, physiology, nutritional requirements, biochemical characteristics, and DNA base composition. The taxonomy of this genus, as other bacteria classification, includes two sets of studies: a study based on identifying phenotypic traits, and the other based on the genetic study (Vandamme et al., 1996; Dickinson et al., 2004). In fact, comparison of the electrophoretic whole-cell protein patterns proved to be useful in the evaluation of the relationships between some isolates (Swiecicka et al., 2002).

Polyphasic taxonomy have made many changes in the classification of bacteria, led to reclassification of different bacterial species (Yoon et al., 2004) and helped to reach to a good classification of newly discovered genera (Loganathan and Nair, 2004; Jeon et al., 2005). Applying modern taxonomic methods led to reclassification of some species belonging to genus *Bacillus* where they had been moved to other genera such as *Alicyclobacillus* (Wisotzkey et al., 1992) *Paenibacillus* (Ash et al., 1993), and *Aquibacillus* (Amoozegar et al., 2014).

Since this genus plays a great role in ecosystem development, it needs additional taxonomical studies to clarify its heterogeneity. The aim of the present study was to isolate and characterize different spore forming bacteria, obtained from marine habitats.

2. Material and Methods

Bacterial isolation:

The isolates were obtained from salty environments including the surface and 15cm depth of Red Sea soil (Jeddah, Saudi Arabia) using selective isolation method (Logan et al., 2000). About 20 soil samples were collected in 250ml sterile bottles and 1g from each soil sample was suspended in 20ml artificial seawater, heated to 80°C for 10 min and used for bacterial isolation on Tryptone Soya Agar (CM 0131 Oxoid), prepared with artificial seawater (Yoon et al., 2001) and $MnCl_2.4H_2O$ (10 ml/l) was added to enhance endospores production (Fahmy, 1978). The bacterial isolates were purified and maintained at 4ºC on the previous medium.

Morphological and physiological characteristics:

Microscopic studies, colony and cell morphology, and Gram staining were determined (Bonde, 1981; Cote and Gherna, 1994). All media that were used for physiological and biochemical tests have been done according to Gordon et al. (1973). Other physiological characteristics were performed with the API 20E (bioMérieux). Determining nutritional requirements for the isolates were carried out on different media (Smibert and Krieg 1994).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS- PAGE):

SDS- PAGE of whole-cell proteins was performed for some isolates (No. 1, 3, 4, 6 and 10) according to described methods (Jackman, 1985; Kanzawa et al., 1995). Freshly cells (0.1g) were suspended in 1.0ml of 0.0625 M Tris-HC1 buffer (pH 6.8) containing 2% sodium dodecyl sulfate, 5% of 2-mercaptoethanol, and 10% glycerol and the mixture was heated at 100°C for 10 min. Then, samples were centrifuged at 10,000 rpm for 10 min and stored at -20°C until used. Electrophoresis was performed in 10% acrylamide gels, and bands were visualized by coomassie blue staining (Laemmli 1970). Standard proteins (phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α –lactalbumin) were used to compare proteins patterns.

3. Results

All bacterial isolates were moderate halophiles (NaCl range from 0% to 15%), mesophilic with temperature range from 10°C to 55°C (optimum 30°C), and neutralophilic with pH range from 5 to 10.5 (optimum 7). All isolates were spore-formers, rod-shaped and Gram-positive. For all the isolates; catalase, hydrolysis of starch and tyrosine, and fermentation of glucose were positive. However, hydrolysis of cellulose, deamination of phenylalanine, gas production from glucose, arginine dihydrolase, lysine and ornithine decarboxylase, production of H_2S, tryptophane desaminase, urease production, indole, and acid production from sugars recorded negative results. The isolates showed many different characters as recorded in Table 1. One isolate (isolate No. 1) has long chains of cells (Figure 1), and some of them have palisade arrangement of cells such as the isolate No. 5 (Figure 2). Other characteristic features were noticed, some isolates have both swollen and nonswollen sporangia in the same microscopic field (Figure 3). Furthermore, there is a difference in the position of spores between strains, some isolates had terminal spores (Figure 4), but spore position in all others was central to sub-terminal.

The isolates were classified according to their nutritional needs in two distinct groups; four isolates No. 1, 5, 8 and 10 grew well on nutrient agar (or in broth) without any addition and seven isolates required specific needs such as magnesium chloride only or with either yeast extract or sodium chloride (isolates No. 2, 3, 4, 6, 7, 9 and 11). Further classification of the first group, according to some physiological characteristics indicated that three isolates (No. 1, 5 and 10) were identified as *Bacillus licheniformis*, and the isolate No. 8 was identified as *B. subtilis*. The second group was further subdivided on the same bases into three subgroups; one isolate (No. 4) was identified as *B. circulans*, four isolates (No. 2, 3, 9 and 11) as *Paenibacillus dendritiformis*, and two isolates (No. 6 and 7) shared intermediate characteristics between *B. circulans* and *P. dendritiformis*. The identification was made regarding to differences in their biochemical characteristics and according to the key used for identification of spore forming bacteria (Reva et al., 2001). In order to compare the tested isolates with some other known species of the same genus, special lists of defined species were used (Claus and Berkeley, 1986; Tcherpakov et al., 1999).

The representative isolates (No. 1, 3, 4, 6 and 10) of these taxonomical groups were compared with respect to their whole-cell proteins obtained after SDS-polyacrylamide gel electrophoresis. The banding patterns obtained for isolates No. 1 and 10, were nearly similar, and barely distinguishable (Figure 5), indicating their affiliation within a single species. These two isolates also displayed identical biochemical and physiological features, and were distinguished only on morphological bases. Also we notice that the banding pattern of isolate No. 6 appeared to be quite distinct from all the others and shared bands with isolates No. 3 and 4, which correlated well with their classification according to other taxonomical characterization used in this study.

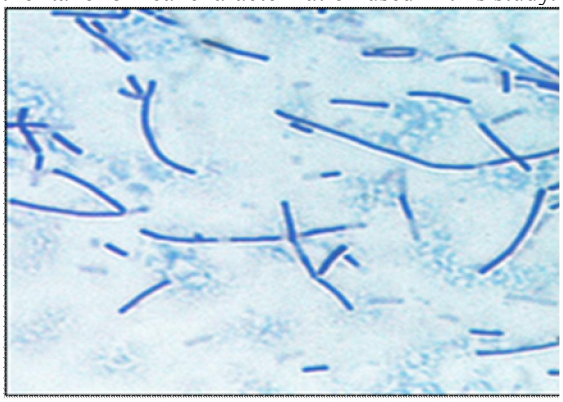

Figure 1. Rods and long chains of the isolate No. 1 under Light Microscope (x1000)

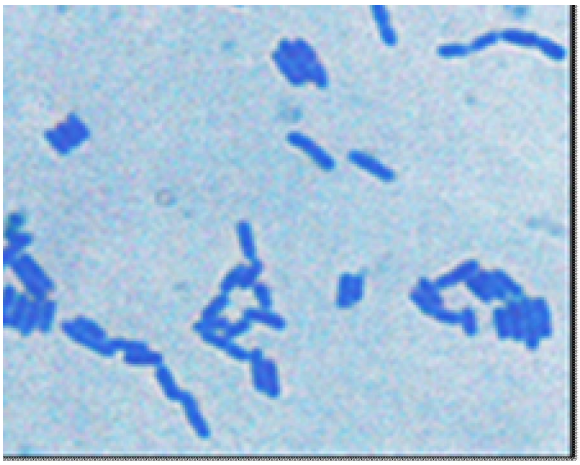

Figure 2. Rods and palisade shape of the isolate No. 5 under Light Microscope (x1000)

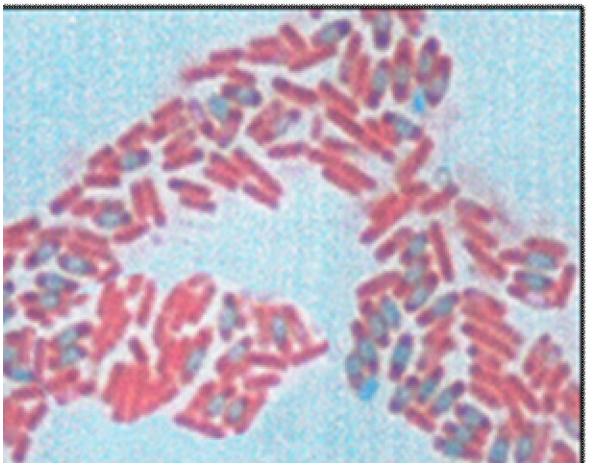

Figure 3. Endospores and vegetative cells of the isolate No. 10 under Light Microscope (x1000)

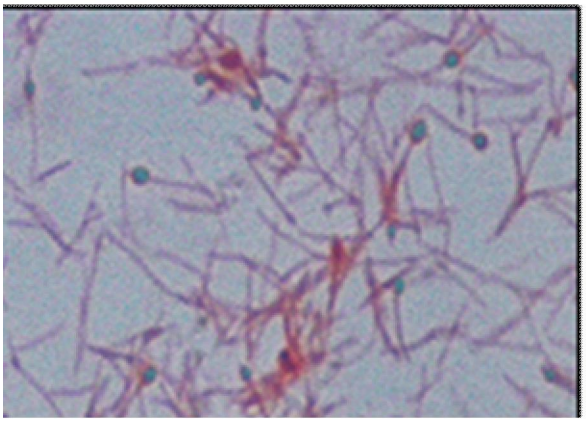

Figure 4. Terminal endospores and vegetative cells of the isolate No. 4 under Light Microscope (x1000)

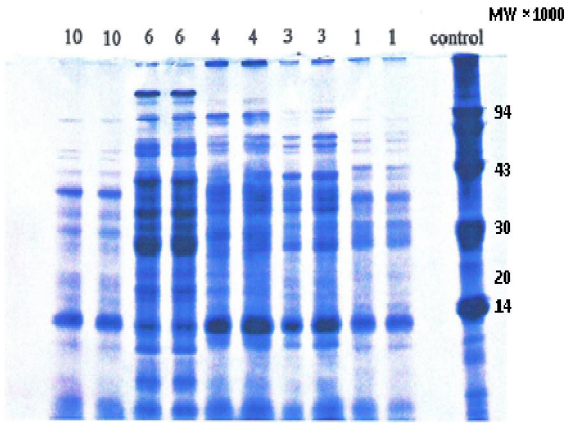

Figure 5. SDS - polyacrylamide gel electrophoresis for the isolates (No. 1,3, 4, 6, and 10) with control

4. Discussions

Although few systematic studies have examined *Bacillus* species from marine environments (Ki et al., 2009), there are significant studies indicate that marine-derived *Bacillus* strains have been proved to be a very promising source for natural product leads (Liu at al., 2014). These studies have been demonstrated the presence of a variety of species which have the ability to produce several important compounds, such as antibiotics, enzymes, and some secondary metabolites that have biological activities (He et al., 2013; Lentini et al., 2007; Mondol et al., 2013). Isolation *Bacillus* strains from marine habitat does not restrict on soil samples, some species were isolated from marine invertebrates and sea water (Ivanova et al., 1999). Pasteurization the bacterial suspensions at 80°C for 10 min is one of the effective method to select for *Bacillus* species (Pichinoty et al., 1983; Finlay at al., 2000). Species that were obtained from the present study which were *B. licheniformis* and *B. subtilis* were reported to have been detected from marine environments (Ivanova et al., 1999), *B. circulans* (Das et al., 2008), and some marine isolates belong to the genus *Paenibacillus* (Ettoumi et al., 2009).

Recently, the systematic of *Bacillus* species has been widely used modern taxonomy methods which includes studying in addition to phenotypic characterization, some genotypic and/or phylogenetic studies. For example, Ivanova et al. (1999) used fatty acid composition and antibiotic sensitivity patterns to determine the taxonomic affiliation of some *Bacillus* marine isolates. The results from Berber (2004) indicated that whole-cell protein profiles using SDS-PAGE combined with computerized analysis of cellular protein profiles provide an effective approach to investigate of taxonomic relationships within *Bacillus* species. Moreover, phylogenetic tree based

on 16S sequencing of marine *Bacillus* isolates were used to clarify their systematic position (Siefert et al., 2000; Swaathy et al., 2014).

Table 1. Distinctive phenotypic properties of the tested bacterial isolates

Character tested	1	2	3	4	5	6	7	8	9	10	11
Cell shape	R (LC)	R (PA)		R	R (PA)	R			R (PA)		
Spores shape	Oval										
Position of spores	T	C/T		T	C/T			T	C/T		
Swollen sporangia	-	-/+	-/+	+	-/+	+	+	-	-/+	-/+	-/+
Anaerobic growth	+	+	+	+	+	+	+	-	+	+	+
Growth temperature range (°C)	10-55	10-40		10-50	10-50	10-40		10-50	10-40	10-55	10-40
pH range	5-10.5	6-10.5		6-10		6-9		6-10	6-10.5	5-10.5	6-10.5
NaCl (%) range	0-15	2-15		2-10	0-10	2-10		0-7	2-15	0-15	2-15
Oxidase	+	+	+	-	+	+	+	+	+	+	+
Hydrolysis of:											
Gelatin	+	+	+	-	+	+	-	+	+	+	+
Casein	+	+	+	-	-	+	-	+	+	+	+
Nitrate reduction	+	-	-	-	+	-	-	+	-	+	-
Use of citrate	-	-	-	-	+	+	-	+	-	-	-

*R: rods; LC: Long chains; PA: Palisade arrangement, T: Terminal spore; C/T: Central to sub-terminal spore, +: Positive; -: Negative

References

1. Amoozegar, M. A., Bagheri, M., Didari, M., Mehrshad, M., Schumann, P., Spröer, C., Sánchez- Porro, C., and Ventosa, A. (2014) *Aquibacillus halophilus* gen. nov., sp. nov., a moderately halophilic bacterium from a hypersaline lake, and reclassification of *Virgibacillus koreensis* as *Aquibacillus koreensis* comb. nov. and *Virgibacillus albus* as *Aquibacillus albus* comb. nov., *Int. J. Syst. Evol. Microbiol.* 64:3616-3623.
2. Arahal, D. R. and Ventosa, A. (2002). Moderately halophilic and halotolerant species of *Bacillus* and related genera, *in*: Berkeley, R., Heyndrickx, M., Logan, N. and De Vos, P. (eds) *Applications and Systematics of Bacillus and Relatives*, p. 83–99, Oxford: Blackwell.
3. Ash, C., Priest, F.G. and Collins, M. D. (1993) Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test: Proposal for the creation of a new genus *Paenibacillus*, *Antonie Van Leeuwenhoek* 64:253-260.
4. Banat, I. M., Makkar, R. S. and Cameotra, S. S. (2000) Potential commercial applications of microbial surfactants, *Appl. Microbiol. Biotechnol.* 53 (5): 495–508.
5. Berber I. (2004) Characterization of *Bacillus* species by numerical analysis of their SDS-PAGE protein profiles, *J. Cell Mol. Biol.* 3: 33-37.
6. Blackwood, K. S., Turenne, C. Y., Harmsen, D. and Kabani1, A. M. (2004) Reassessment of sequence–based targets for identification of *Bacillus* species, *J. Clin. Microbiol.* 42 (4): 1626-1630.
7. Bonde, G. J. (1981) *Bacillus* from marine habitats: Allocation to phena established by numerical techniques, *in*: Berkeley, R. C. W. and Goodfellow, M. (eds) *The Aerobic Endospore–forming Bacteria: Classification and Identification*, p. 181-215, London: Academic Press.
8. Claus, D. and Berkeley, R. C. W. (1986) The Genus *Bacillus* Cohn 1872, *in*: Sneath, P. H. A., Mair, N. S., Sharpe, M. E. and Holt, J. G. (eds) *Bergey's Manual of Systematic Bacteriology*, vol. 2, p. 1105-1140, Baltimore: Williams & Wilkins.
9. Cote, R. J. and Gherna, R. L. (1994) Nutrition and Media, *in*: Gerhardt, P., Murray, R. G. E., Wood, W. A. and Krieg, N. R. (eds) *Methods for General and Molecular Bacteriology*, Washington D. C.: American Society for Microbiology.
10. Das, P. Mukherjee, S. and Sen, R. (2008) Antimicrobial potential of a lipopeptide biosurfactant derived from a marine *Bacillus circulans*, *J. Appl. Microbiol.* 104: 1675–1684.
11. Dickinson, D. N., La Duc, M. T., Satomi, M., Winefordner, J. D., Powell, D. H. and Venkateswaran, K. (2004) MALDI–TOFMS compared with other polyphasic taxonomy

approaches for the identification and classification of *Bacillus pumilus* spores, *J. Microbiol. Methods* 58: 1-12.
12. Ettoumi, B., Raddadi, N., Borin, S., Daffonchio, D., Boudabous, A. and Cherif, A. (2009) Diversity and phylogeny of culturable spore-forming bacilli isolated from marine sediments, *J. Basic Microbiol.* 49:S13–S23.
13. Fahmy, F. A. (1978) Untersuchungern zur Taxonomie farbstoffbildender *Bacillus* – Stämme aus Salzmarsch– und Wattböden, Ph. D. Thesis, University of Goltingen, West Germany.
14. Finlay, W. J., Logan, N.A. and Sutherland, A.D. (2000) *Bacillus cereus* produces most emetic toxin at lower temperatures, *Lett. Appl. Microbiol.* 31(5): 385–389.
15. Gordon, R. E., Haynes, W. C. and Pang, C. H. N. (1973) The genus *Bacillus*, Agriculture Handbook No. 427, Washington D. C.: United States Department of Agriculture.
16. He, S., Wang, H., Yan, X., Zhu, P., Chen, J. and Yang, R. J. (2013) Preparative isolation and purification of macrolactin antibiotics from marine bacterium *Bacillus* amyloliquefaciens using high-speed counter-current chromatography in stepwise elution mode, *J. Chromatogr. A*, 1272: 15–19.
17. Heyrman, J., Rodríguez-Díaz, M., Devos, J., Felske, A., Logan, N. A. and De Vos, P. (2005) *Bacillus arenosi* sp. nov., *Bacillus arvi* sp. nov. and *Bacillus humi* sp. nov., isolated from soil, *Int. J. Syst. Evol. Microbiol.* 55: 111–117.
18. Ivanova, E. P., Vysotskii, M. V., Svetashez, V. I., Nedashkovskaya, O. I., Gorshkova, N. M., Mikhailov, V. V., Yumoto, N., Shigeri, Y., Taguchi, T. and Yoshikawa, S. (1999) Characterization of *Bacillus* strains of marine origin, *Int. Microbiol.* 2:267-271.
19. Jackman, P. J. H. (1985) Bacterial taxonomy based on electrophoretic whole-cell protein patterns, *in*: Goodfellow, M. and Minnikin, D. E. (eds) *Chemical Methods in Bacterial Systematics*, p. 115-129, London: Academic Press.
20. Jeon, C. O., Lim, J.-M., Park, D.-J.and Kim, C.-J. (2005) *Salinimonas chungwhensis* gen. nov., sp. nov., a moderately halophilic bacterium from a solarsaltern in Korea, *Int. J. Syst. Evol. Microbiol.* 55: 239-243.
21. Kanzawa, Y., Harada, A., Takeuchi, M., Yokota, A. and Harada, T. (1995) *Bacillus curdlanolyticus* sp. nov. and *Bacillus kobensis* sp. nov., Which Hydrolyze Resistant Curdlan, *Int. J. Syst. Bacteriol.* 45 (3): 515-521.
22. Ki, J. S., Zhang, W. and Qian, P. Y. (2009) Discovery of marine *Bacillus* species by 16S rRNA and *rpoB* comparisons and their usefulness for species identification, *J. Microbiol. Methods* 77:48–57.
23. Ko, K. S., Oh, W. S., Lee, M. Y., Lee, J. H., Lee, H., Peck, K. R., Lee, N. Y. and Song, J. H. (2006) *Bacillus infantis* sp. nov. and *Bacillus idriensis* sp. nov., isolated from a patient with neonatal sepsis, *Int. J. Syst. Evol. Microbiol.* 56: 2541–2544.
24. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* (London) 227: 680-685.
25. Lentini, V., Gugliandolo, C. and Maugeri, T. L. (2007) Identification of enzyme-producing thermophilic bacilli isolated from marine vents of Aeolian Islands (Italy), *Ann. Microbiol.* 57:355–361.
26. Liu, Y., Zheng, H., Zhan, G., Qin, W., Tian, L., and Li, W. (2014) Establishment of an efficient transformation protocol and its application in marine-derived *Bacillus* strain, *Sci. China Life Sci.* 57 (6): 627-635.
27. Liu, Y., Lai, Q., Dong, C., Sun, F., Wang, L., Li, G. and Shao, Z. (2013) Phylogenetic diversity of the *Bacillus pumilus* group and the marine ecotype revealed by multilocus sequence analysis, *Plos One* 8(11):1–11.
28. Logan, N. A. and Turnbull, P. C. B. (2003) *Bacillus* and other aerobic endospore-forming bacteria, *in*:Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A. and Yolken. R. H. (eds) *Manual of Clinical Microbiology*, 8th ed., p. 445–460, Washington, DC: American Society for Microbiology.
29. Logan, N. A., Lebbe, L., Hoste, B., Goris, J., Forsyth, G., Heyndrickx, M., Murray, B. L., Syme, N., Wynn-Williams, D. D. and De Vos, P. (2000) Aerobic endospore-forming bacteria from geothermal environments in northern Victoria Land, Antarctica, and Candlemas Island, South Sandwich Archipelago, with the proposal of Bacillus fumarioli sp. nov., *Int. J. Syst. Evol. Bactriol.* 50: 1741-1753.
30. Loganathan, P. and Nair, S. (2004) *Swaminathania salitolerans* gen. nov., sp. nov., a salt-tolerant, nitrogen-fixing and phosphate-solubilizing bacterium from wild rice (Porteresia coarctata Tateoka), *Int. J. Syst. Evol. Microbiol.* 54: 1185-1190.
31. Mondol, M. A. M., Shin, H. J. and Islam, M. T. (2013) Diversity of secondary metabolites from marine *Bacillus* species: chemistry and

biological activity, Mar. Drugs 11(8): 2846–2872.
32. Pichinoty, F., De Barjac, H., Mandel, M. and Asselineau, J. (1983) Description of *Bacillus azotoformans* sp. nov., *Int. J. Syst. Bacteriol.* 33: 660-662.
33. Reva, O. N., Sorokulova, I. B. and Smirnov, V. V. (2001) Simplified technique for identification of the aerobic spore-forming bacteria by phenotype, *Int. J. Syst. Evol. Microbiol.* 51: 1361-1371.
34. Shivaji, S., Chaturvedi, P., Suresh, K., Reddy, G. S., Dutt, C. B., Wainwright, M., Narlikar, J. V. and Bhargava, P. M. (2006) *Bacillus aerius* sp. nov., *Bacillus aerophilus* sp. nov., *Bacillus stratosphericus* sp. nov. and *Bacillus altitudinis* sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes, *Int. J. Syst. Evol. Microbiol.* 56: 1465–1473.
35. Siefert, J. L., Larios-Sanz, M., Nakamura, L. K., Slepecky, R. A., Paul, J. H., Moore, E. R. Fox, G. E. and Jurtshuk, J. P. (2000) Phylogeny of marine *Bacillus* isolates from the Gulf of Mexico, *Curr. Microbiol.* 41:84–88.
36. Smibert, R. M. and Krieg, N. R. (1994) Phenotypic Characterization, *in*: Gerhardt, P., Murray, R. G. E., Wood, W. A. and Krieg, N. R. (eds) *Methods for General and Molecular Bacteriology*, p. 21-40, Washington D. C.: American Society for Microbiology.
37. Swaathy, S., Kavitha, V., Pravin, A. S., Sekaran, G., Mandal, A. B. and Gnanamani, A. (2014) Phylogenetic Framework and Biosurfactant Gene Expression Analysis of Marine *Bacillus* spp. of Eastern Coastal Plain of Tamil Nadu, *Int. J. Bacteriol.* p. 1-10.
38. Swiecicka, I., Fiedoruk, K. and Bednarz, G. (2002). The occurrence and properties of *Bacillus thuringiensis* isolated from free-living animals, *Lett. Appl. Microbiol.* 34: 194–198.
39. Tcherpakov, M., Ben-Jacob, E. and Gutnick, D. L. (1999) *Paenibacillus dendritiformis* sp. nov., proposal for a new pattern-forming species and its localization within a phylogenetic cluster, *Int. J. Syst. Bacteriol.* 49:239-246.
40. Vandamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K. and Swings, J. (1996) Polyphasic Taxonomy, a Consensus Approach to Bacterial Systematics, *Microbiol. Rev.* 60 (2): 407-438.
41. Wisotzkey, J. D., Jurtshuk, P., Fox, G. E., Deinhard, G. and Poralla, K. (1992) Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov., *Int. J. Syst. Bacteriol.* 42: 263-269.
42. Yoon, J.-H., Oh, T.-K. and Park, Y.-H. (2004) Transfer of *Bacillus halodenitrificans* Denariaz et al. 1989 to the genus *Virgibacillus* as *Virgibacillus halodenitrificans* comb. nov., *Int. J. Syst. Evol. Microbiol.* 54: 2163-2167.
43. Yoon, J.-H., Oh, H.-M., Yoon, B.-D., Kang, K. H. and Park, Y.-H. (2003) *Paenibacillus kribbensis* sp. nov. and *Paenibacillus terrae* sp. nov., bioflocculants for efficient harvesting of algal cells, *Int. J. Syst. Evol. Microbiol.* 53: 295-301.
44. Yoon, J.-H., Lee, K.-C., Weiss, N., Kho, Y. H., Kang, K. H. and Park, Y.-H. (2001) *Sporosarcina aquimarina* sp. nov., a bacterium isolated from seawater in Korea, and transfer of *Bacillus globisporus* (Larkin and Stokes 1967), *Bacillus psychrophilus* (Nakamura 1984) and *Bacillus pasteurii* (Chester 1898) to the genus *Sporosarcina* as *Sporosarcina globispora* comb. nov., *Sporosarcina psychrophila* comb. nov. and *Sporosarcina pasteurii* comb. nov., and emended description of the genus *Sporosarcina*, *Int. J. Syst. Evol. Microbiol.* 51: 1079-1086.

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