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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Keywords: *Bacillus*; salty environment; gel electrophoresis; Jeddah

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 MnCl₂·4H₂O (10 ml/l) was added to enhance endospores production (Fahmy, 1978). The bacterial isolates were purified and maintained at 4°C on the previous medium.
Morphological and physiological characteristics:

Microscopic studies, colony 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-HCl 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 H2S, 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)
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 et 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 et 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

<table>
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<tr>
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<td>R (PA)</td>
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<td>Spores shape</td>
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<td>Position of spores</td>
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<td>Swollen sporangia</td>
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<td>Anaerobic growth</td>
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<td>Growth temperature range (°C)</td>
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*R*: rods; *LC*: Long chains; *PA*: Palisade arrangement; *T*: Terminal spore; *C/T*: Central to sub-terminal spore; *+: Positive; *-*: Negative

References


