Bacillus vallismortis sp. nov., a Close Relative of Bacillus subtilis, Isolated from Soil in Death Valley, California

MICHAEL S. ROBERTS,1,4 L. K. NAKAMURA,2 AND FREDERICK M. COHAN3

Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824-1101; Microbial Properties Research, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604; and Department of Biology, Wesleyan University, Middletown, Connecticut 06459-0170

Five Bacillus strains isolated from Death Valley soil were shown to belong to a previously unidentified species, for which we propose the name Bacillus vallismortis. The type strain is strain DV1-F-3 (= NRRL B-14890). On the basis of previously published restriction digestion data, B. vallismortis is most closely related to Bacillus subtilis. At this time B. vallismortis can be distinguished from B. subtilis only by differences in whole-cell fatty acid compositions, DNA sequences, and levels of reassociation of genomic DNA.

The species most closely related to Bacillus subtilis are easily distinguished by differences in their DNA sequences. B. subtilis, Bacillus mojavensis, Bacillus atrophaeus, Bacillus amyloliquefaciens, and Bacillus licheniformis can be distinguished by genomic hybridization data (11, 12, 16); these species form distinct DNA sequence clusters for shared genes (11, 12, 16), and as a result of sequence divergence, levels of transformation among these species are low (14, 16, 21). However, the sequence differences among these species are accompanied by very few phenotypic differences. B. subtilis can be distinguished phenotypically from B. mojavensis only by its fatty acid composition (16) and from B. atrophaeus only by its pigmentation (12). B. subtilis can be distinguished from B. amyloliquefaciens by only three phenotypic traits (9, 11) and from the more distantly related organism B. licheniformis by only five phenotypic traits (9). The dearth of diagnostic phenotypic characteristics in this group suggests there may be species which are closely related to B. subtilis that have not been discovered yet (16). Indeed, two close relatives of B. subtilis (B. atrophaeus and B. mojavensis) have been discovered in only the last 6 years (12, 16).

A recent survey of genetic variation among soil isolates having B. subtilis-like phenotypes revealed an unexpected cluster of strains very closely related to B. subtilis (15). On the basis of the results of a restriction digestion analysis of the gyrA, pdc, and rpoB genes, a cluster of five strains obtained from Death Valley formed a monophyletic group that was most closely related to B. subtilis (Fig. 1). In this paper we present evidence that these Death Valley group strains are members of a previously unidentified Bacillus species.

MATERIALS AND METHODS

Bacterial strains. The five strains previously identified as members of the Death Valley group (15) are listed in Table 1. These strains were isolated from soil obtained at four collection sites in Death Valley (15). A prototrophic, rifampin-susceptible derivative of B. subtilis 168, designated strain 1A2 (= NRRL B-14819), was obtained from the Bacillus Genetic Stock Center. The Agricultural Research Service Culture Collection at the National Center for Agricultural Utilization Research provided the following type strains: B. amyloliquefaciens NRRL B-14393 (= ATCC 23350), B. atrophaeus NRRL NRS-215, Bacillus lentimorbus NRRL B-2522 (= ATCC 14707), B. licheniformis NRRL NRS-1264 (= ATCC 14580), B. mojavensis NRRL B-14608, Bacillus popilliae NRRL B-2309 (= ATCC 14706), and B. subtilis NRRL NRS-744 (= ATCC 6051). (The prefix NRRL indicates that a strain is maintained in the Agricultural Research Service Culture Collection; each NRRL designation includes the prefix B., which identifies organisms that were obtained directly from individuals or were isolated at the National Center for Agricultural Utilization Research, or the prefix NFS-, which identifies strains obtained from the N. R. Smith Bacillus collection.)

Selection for spontaneous rifampin-resistant mutants. We obtained rifampin-resistant (Rifr; encoded by rpoB) mutants of each strain (which were used as donors in transformation experiments) by using previously described methods (16).

Purification of genomic DNA. For DNA preparation, the type strains of B. popilliae and B. lentimorbus were cultured in J medium by using the protocol of Gordon et al. (6); all other strains were cultured in brain heart infusion medium (Difco). Genomic DNA for use in transformation experiments was obtained from a spontaneous Rifr mutant of each donor strain, as described previously (3). The procedure used to prepare highly purified DNA for reassociation experiments has been described previously (13).

Transformation. Using the protocol of Cohan et al. (3), we induced strains to become competent for transformation in liquid cultures. Each strain was then transformed to rifampin resistance with genomic DNA from its own Rifr mutant (homologous transformation) and with DNAs from Rifr mutants of other strains (heterologous transformation). We calculated transformation frequencies by determining the fraction of cells from each recipient culture that were transformed to rifampin resistance, after we corrected for mutation. Sexual isolation (i.e., resistance to transformation) between a recipient and a test donor was quantified by determining the ratio of the frequency of homologous transformation to the frequency of heterologous transformation (14, 21).

DNA-DNA reassociation and G+C content determinations. The renaturation rates of genomic fragments from pairs of strains were determined spectrophotometrically with a model 250 UV spectrophotometer (Gillford Systems, CIBA-Corning Diagnostics Corp., Oberlin, Ohio) equipped with a model 2527 thermor Programmer (13). The equation of De Ley et al. (4) was used to calculate the extent of DNA-DNA reassociation.

The G+C content was estimated by the thermal melting procedure described by Mandel and Marmur (10). Escherichia coli DNA with a G+C content of 51 mol% was used for comparison.

Phenotypic characterization. Physiological, morphological, and metabolic characteristics (except fatty acid composition) were determined as described previously (6, 13). The whole-cell fatty acid composition was determined by using the MIDI system of Sasser (18) and organisms that had been grown for 24 h at 28°C on Trypticase soy agar. We determined the fatty acid compositions of the five members of the Death Valley group and compared these compositions with data for related species obtained previously (16). B. lentimorbus and B. popilliae were not characterized because their phenotypes were known to be very different from the phenotypes of B. subtilis-like organisms (2).

RESULTS

Sexual isolation between the Death Valley group and B. subtilis. Each Death Valley group strain failed to show any evidence of transformation toward rifampin resistance, whether the donor DNA was from the recipient’s own Rifr mutant or from a Rifr mutant of B. subtilis 1A2; that is, the frequency of appearance of rifampin-resistant colonies when donor DNA was added was about the same as the frequency of appearance under mutation alone (data not shown). Because of this apparent lack of competence in the Death Valley group strains, sexual isolation between the Death Valley group and
other taxa could be studied only when Death Valley group strains were used as donors.

We investigated sexual isolation between the Death Valley group and *B. subtilis* by using *B. subtilis* 1A2 as the recipient. In homogamic transformation experiments, *B. subtilis* 1A2 was transformed at a frequency of $6.64 \times 10^{-3}$. The frequency of transformation was lower than this value when any of the five Death Valley group strains was used as the donor; strain 1A2 was transformed by Death Valley group strains at an average frequency of transformation of $4.24 \times 10^{-3}$ (Table 2). The average level of sexual isolation (ratio of homogamic transformation frequency to heterogamic transformation frequency) between the Death Valley group strains and *B. subtilis* 1A2 was $1.57$ (i.e., the sexual isolation value $[\log_{10}$ transformed]) for the five Death Valley group strains was $0.20 \pm 0.05$ [mean $\pm$ standard error]; $10^{1.20} \pm 1.57$ (Table 2). The Death Valley group strains were not significantly heterogamous in their levels of sexual isolation from the recipient in an analysis of variance ($F_{5,24} < 1$). The extents of sexual isolation between the Death Valley group strains and strain 1A2, although modest, were highly significant ($t = 4.09, P < 0.01$; one-tailed $t$ test for the difference between the mean log$_{10}$-transformed sexual isolation value, $0.20 \pm 0.05$, and zero). In contrast, previous results have shown that the recipient which we used, *B. subtilis* 1A2, is not sexually isolated from *B. subtilis* strains; the log$_{10}$-transformed values of sexual isolation between strain 1A2 and other *B. subtilis* strains range from 0.02 to 0.10, with a mean of 0.06 $\pm$ 0.02, which is not significantly different from zero (16).

**DNA-DNA reassociation.** Table 3 shows the levels of DNA-DNA reassociation between the Death Valley group strains and the type strains of the following taxa: *B. amyloliquefaciens*, *B. atropphaeus*, *B. lentimorbus*, *B. licheniformis*, *B. mojavensis*, *B. popilliae*, and *B. subtilis*. This list includes all of the species that are, according to 16S RNA sequence data (1), at least as

---

**TABLE 1. Bacillus strains compared in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source*</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>DV1-A-1 (= NRRL B-14892)</td>
<td>1</td>
<td>Isolated from sand dune with mesquite tree, 36° 39’ N, 117° 5’ W</td>
</tr>
<tr>
<td>DV1-F-3 (= NRRL B-14890)</td>
<td>1</td>
<td>Isolated from sand dune with mesquite tree, 36° 39’ N, 117° 5’ W</td>
</tr>
<tr>
<td>DV4-D-3 (= NRRL B-14893)</td>
<td>1</td>
<td>Isolated from alluvial fan with creosote bushes, 36° 18’ N, 116° 54’ W</td>
</tr>
<tr>
<td>DV7-C-1 (= NRRL B-14894)</td>
<td>1</td>
<td>Isolated from arroyo in alluvial fan with desert holly bushes, 36° 21’ N, 115° 51’ W</td>
</tr>
<tr>
<td>DV8-1.7-4 (= NRRL B-14891)</td>
<td>1</td>
<td>Isolated from alluvial fan with desert holly bushes, 36° 17’ N, 116° 54’ W</td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em> NRRL B-14393 (= ATCC 23350)</td>
<td>2</td>
<td>L. L. Campbell strain F from J. Fukonoto, from soil</td>
</tr>
<tr>
<td><em>B. atropphaeus</em> NRRL NRS-213</td>
<td>2</td>
<td>N. R. Smith, “<em>B. subtilis</em> var. riger,” from Colorado soil</td>
</tr>
<tr>
<td><em>B. lentimorbus</em> NRRL B-2522 (= ATCC 14707)</td>
<td>2</td>
<td>Isolated from diseased Japanese beetle grub</td>
</tr>
<tr>
<td><em>B. licheniformis</em> NRRL NRS-1264 (= ATCC 14580)</td>
<td>2</td>
<td>R. E. Gordon from T. Gibson 46</td>
</tr>
<tr>
<td><em>B. mojavensis</em> NRRL B-14085</td>
<td>2</td>
<td>Isolated by F. M. Cohan from soil, Mojave Desert, Calif.</td>
</tr>
<tr>
<td><em>B. popilliae</em> NRRL B-2309 (= ATCC 14706)</td>
<td>2</td>
<td>Isolated from commercial anti-Japanese beetle spore dust</td>
</tr>
<tr>
<td><em>B. subtilis</em> strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRRL NRS-744 (= ATCC 6051)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1A2 (= NRRL B-14819)</td>
<td>3</td>
<td>J. Lederberg</td>
</tr>
</tbody>
</table>

---

* 1, F. M. Cohan, Department of Biology, Wesleyan University, Middletown, Conn.; 2, L. K. Nakamura, National Center for Agricultural Utilization Research, Peoria, Ill.; 3, D. R. Zeigler and D. H. Dean, Bacillus Genetic Stock Center, Ohio State University, Columbus.
* 5 All Death Valley group strains have been deposited in the Agricultural Research Service Culture Collection at the National Center for Agricultural Utilization Research, Peoria, Ill.
* T = type strain.

**TABLE 2. Levels of sexual isolation when *B. subtilis* 1A2 was the recipient and Death Valley group strains were the donors**

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Transformation frequency*</th>
<th>Sexual isolation value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death Valley group strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DV1-A-1</td>
<td>$-2.39 \pm 0.12$</td>
<td>0.22 $\pm 0.13$</td>
</tr>
<tr>
<td>DV1-F-3$^c$</td>
<td>$-2.54 \pm 0.10$</td>
<td>0.36 $\pm 0.15$</td>
</tr>
<tr>
<td>DV4-D-3</td>
<td>$-2.36 \pm 0.09$</td>
<td>0.18 $\pm 0.16$</td>
</tr>
<tr>
<td>DV7-C-1</td>
<td>$-2.34 \pm 0.10$</td>
<td>0.16 $\pm 0.17$</td>
</tr>
<tr>
<td>DV8-1.7-4</td>
<td>$-2.24 \pm 0.06$</td>
<td>0.06 $\pm 0.15$</td>
</tr>
<tr>
<td>Mean$^d$</td>
<td>$-2.37 \pm 0.05$</td>
<td>0.20 $\pm 0.05$</td>
</tr>
</tbody>
</table>

---

* Transformation frequencies were log$_{10}$ transformed as described by Cohan et al. (3). The values are the mean $\pm$ standard error for each strain, based on five experimental trials.
* Factor by which the recipient’s transformation frequency was reduced in heterogamic transformation compared with the frequency at which the recipient was transformed by its own Rifr mutant’s DNA. Sexual isolation values were log$_{10}$ transformed. The values are the mean $\pm$ standard error for each strain, based on five experimental trials. The recipient was transformed by its own Rifr mutant’s DNA at an average log$_{10}$-transformed frequency of $-2.18 \pm 0.14$ ($6.64 \times 10^{-3}$) in five experimental trials.
* Mean $\pm$ standard error for five Death Valley group strains.
* $P < 0.01$ as determined by a one-tailed $t$ test of the difference between the experimental value and zero.
closely related to \( B. subtilis \) as \( B. licheniformis \) is. The strains of the Death Valley group strains exhibited high levels of DNA-DNA reassociation with one another (85.6 to 100%) but much lower levels of reassociation (10.2 to 35.4%) with the type strains of the other taxa.

**Phenotypic characterization.** The Death Valley group strains could not be distinguished from \( B. subtilis \) or \( B. mojavensis \) by any of the physiological, morphological, or metabolic characteristics shown in Table 4, and very few of these characteristics distinguished these isolates from the other species. A positive oxidase test was the only trait that differentiated the Death Valley group strains from \( B. atrophaeus \). The inability to ferment lactose differentiated the Death Valley group strains from \( B. amyloliquefaciens \). Utilization of propionate, anaerobic growth, and a G+C content of 46 mol% separated the Death Valley group strains from \( B. licheniformis \). Table 4 shows that 15 traits distinguished the Death Valley group strains from \( B. lentimorbus \) and \( B. popilliae \). Other distinguishing characteristics of \( B. lentimorbus \) and \( B. popilliae \) are insecticidal activity, formation of swollen sporangia, and an inability to grow in nutrient broth (2).

All of the organisms listed in Table 4 (except \( B. lentimorbus \))

---

**TABLE 3. Levels of DNA-DNA reassociation between genomes of isolates**

<table>
<thead>
<tr>
<th>Strain</th>
<th>% DNA-DNA reassociation with</th>
</tr>
</thead>
<tbody>
<tr>
<td>DV1-A-1</td>
<td>(100)$^a$</td>
</tr>
<tr>
<td>DV1-F-3$^T$</td>
<td>(100)</td>
</tr>
<tr>
<td>DV4-D-3</td>
<td>97.5</td>
</tr>
<tr>
<td>DV7-C-1</td>
<td>100</td>
</tr>
<tr>
<td>DV8-1.7-4</td>
<td>100</td>
</tr>
<tr>
<td>( B. amyloliquefaciens ) NRRL B-14393$^T$</td>
<td>22.4</td>
</tr>
<tr>
<td>( B. atrophaeus ) NRRL NRS-213$^T$</td>
<td>16.0</td>
</tr>
<tr>
<td>( B. lentimorbus ) NRRL B-2522$^T$</td>
<td>30.0</td>
</tr>
<tr>
<td>( B. licheniformis ) NRRL NRS-1264$^T$</td>
<td>29.5</td>
</tr>
<tr>
<td>( B. mojavensis ) NRRL B-14698$^T$</td>
<td>32.3</td>
</tr>
<tr>
<td>( B. popilliae ) NRRL B-2309$^T$</td>
<td>26.9</td>
</tr>
<tr>
<td>( B. subtilis ) NRRL NRS-744$^T$</td>
<td>25.9</td>
</tr>
</tbody>
</table>

$^a$ The reassociation values are the averages of two determinations; the maximum difference found between determinations was 7%.

$^b$ The values in parentheses indicate that, by definition, the reassociation value was 100%.

---

**TABLE 4. Phenotypic comparison of the Death Valley group strains, \( B. subtilis \), \( B. atrophaeus \), \( B. mojavensis \), \( B. amyloliquefaciens \), \( B. licheniformis \), \( B. lentimorbus \), and \( B. popilliae \)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Death Valley group strains</th>
<th>( B. subtilis ) NRRL NRS-744$^T$</th>
<th>( B. atrophaeus ) NRRL NRS-213$^T$</th>
<th>( B. mojavensis ) NRRL B-14698$^T$</th>
<th>( B. amyloliquefaciens ) NRRL B-14393$^T$</th>
<th>( B. licheniformis ) NRRL NRS-1264$^T$</th>
<th>( B. lentimorbus )$^a$</th>
<th>( B. popilliae )$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at pH 5.7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth in the presence of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5% NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7% NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10% NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.001% Lysozyme</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Oxidase activity</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Acetymethylcarbinol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Maximum growth temp (°C)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>55</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Minimum growth temp (°C)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Acid produced from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Propionate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduced to nitrite</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein decomposition</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80 decomposition</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>43</td>
<td>43</td>
<td>42</td>
<td>43</td>
<td>43</td>
<td>46</td>
<td>38</td>
<td>41</td>
</tr>
</tbody>
</table>

$^a$ Data from reference 2.

$^b$ –, negative reaction; +, positive reaction; w, weak reaction; ND, not determined.
### Table 5. Cellular fatty acid compositions of the Death Valley group strains, B. amyloliquefaciens, B. atrophaeus, B. licheniformis, B. mojavensis, and B. subtilis

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Fatty acid no.</th>
<th>Death Valley group strains (n = 5)</th>
<th>B. amyloliquefaciens (n = 3)*</th>
<th>B. atrophaeus (n = 5)*</th>
<th>B. licheniformis (n = 5)*</th>
<th>B. mojavensis (n = 22)*</th>
<th>B. subtilis (n = 5)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0 iso</td>
<td>1</td>
<td>1.07 ± 0.02*</td>
<td>2.46 ± 0.69*</td>
<td>1.44 ± 0.14</td>
<td>1.31 ± 0.44</td>
<td>0.98 ± 0.24</td>
<td>1.13 ± 0.24</td>
</tr>
<tr>
<td>15:0 iso</td>
<td>2</td>
<td>24.60 ± 1.37</td>
<td>30.50 ± 5.93</td>
<td>15.02 ± 2.55</td>
<td>28.87 ± 2.69</td>
<td>22.33 ± 3.19</td>
<td>29.27 ± 4.64</td>
</tr>
<tr>
<td>15:0 anteiso</td>
<td>3</td>
<td>37.5 ± 0.77</td>
<td>36.48 ± 7.88</td>
<td>51.36 ± 1.08</td>
<td>37.75 ± 2.58</td>
<td>42.51 ± 1.67</td>
<td>40.19 ± 3.98</td>
</tr>
<tr>
<td>16:1 cis9 alcohol</td>
<td>4</td>
<td>0.45 ± 0.04</td>
<td>0.62 ± 0.87</td>
<td>1.16 ± 0.20</td>
<td>0.91 ± 0.44</td>
<td>0.69 ± 0.29</td>
<td>0.23 ± 0.35</td>
</tr>
<tr>
<td>14:0 iso</td>
<td>5</td>
<td>4.06 ± 0.39</td>
<td>4.40 ± 0.75</td>
<td>3.10 ± 0.61</td>
<td>4.42 ± 1.72</td>
<td>2.56 ± 0.41</td>
<td>2.36 ± 0.34</td>
</tr>
<tr>
<td>16:1 cis5</td>
<td>6</td>
<td>0.64 ± 0.03</td>
<td>2.14 ± 0.11</td>
<td>1.72 ± 0.11</td>
<td>1.53 ± 0.24</td>
<td>1.74 ± 0.40</td>
<td>1.52 ± 0.45</td>
</tr>
<tr>
<td>16:0</td>
<td>7</td>
<td>2.71 ± 0.42</td>
<td>4.52 ± 0.50</td>
<td>1.99 ± 0.32</td>
<td>3.91 ± 0.53</td>
<td>2.05 ± 0.41</td>
<td>3.14 ± 0.40</td>
</tr>
<tr>
<td>17:1 cis7 iso</td>
<td>8</td>
<td>1.55 ± 0.40</td>
<td>1.67 ± 0.61</td>
<td>1.99 ± 0.44</td>
<td>1.23 ± 0.33</td>
<td>3.45 ± 0.62</td>
<td>1.72 ± 0.42</td>
</tr>
<tr>
<td>17:0 iso</td>
<td>9</td>
<td>14.43 ± 0.66</td>
<td>9.01 ± 1.34</td>
<td>4.97 ± 0.59</td>
<td>6.99 ± 1.06</td>
<td>8.92 ± 1.46</td>
<td>9.59 ± 1.56</td>
</tr>
<tr>
<td>17:0 anteiso</td>
<td>10</td>
<td>12.07 ± 1.09</td>
<td>7.06 ± 2.69</td>
<td>14.83 ± 2.36</td>
<td>11.30 ± 0.93</td>
<td>12.53 ± 1.89</td>
<td>9.38 ± 0.95</td>
</tr>
</tbody>
</table>

* Data from reference 16.

* Mean ± standard deviation.

### Discussion

A previous survey (15) of sequence diversity in close relatives of B. subtilis showed that the Death Valley group Bacillus strains form a distinct, monophyletic group that is most closely related to B. subtilis (Fig. 1). This previous study showed that the Death Valley group can be distinguished from other taxa on the basis of the results of restriction digestion analyses of the gyrA, polC, and rpoB genes.

In this study we found that in principle, the Death Valley group can be distinguished from its closest relative, B. subtilis, on the basis of weak sexual isolation in transformation experiments and B. popilliae, which were not tested) fermented cellobiose, fructose, galactose, maltose, mannose, raffinose, L-rhamnose, ribose, salicin, sorbitol, sucrose, and trehalose; melibiose was not fermented by these organisms (data not shown).

The principal fatty acids found in the Death Valley group strains, B. amyloliquefaciens, B. atrophaeus, B. licheniformis, B. mojavensis, and B. subtilis included 12-methyltridecanoic (14:0 iso), 13-methyltetradecanoic (15:0 iso), 12-methyltetradecanoic (15:0 anteiso), 14-methylpentadecanoic (16:0 iso), n-hexadecanoic (16:0), 15-methylpentadecanoic (17:0 iso), 14-methylhexadecanoic (17:0 anteiso), cis-5-hexadecanoic (16:1 cis5), and cis-15-methyl-17-hexadecanoic (17:1 cis7 iso) acids. cis-9-Hexadecenoic was also found.

Significant differences in the levels of several fatty acids could be used to differentiate the Death Valley group strains from other taxa (Table 5). Specifically, differences in the levels of 16:0 iso, 16:1 cis5, 17:0 iso, and 17:0 anteiso distinguished the Death Valley group strains from B. subtilis; differences in the levels of 5 of the 10 fatty acids found distinguished the Death Valley group strains from B. amyloliquefaciens; B. atrophaeus and B. mojavensis could be distinguished from the Death Valley group strains by significant differences in 6 of the 10 fatty acids found (not the same ones); and the Death Valley group strains and B. licheniformis could be distinguished by significant differences in two fatty acids.

We performed a principal-component analysis (17) on the fatty acid phenotypes to determine whether the Death Valley group strains form a phenotypic cluster that is distinct from other taxa. As shown in Fig. 2, the first three principal components of fatty acid variation distinguished the Death Valley group strains as members of a separate phenotypic cluster.

**FIG. 2.** Principal-component analysis based on the 10 fatty acids in Table 5. The Death Valley group is a distinct phenotypic cluster when pairs of the first three principal components are used. Symbols: +, Death Valley group strains; ○, B. subtilis strains; □, B. licheniformis strains; ■, B. amyloliquefaciens strains; ▲, B. mojavensis strains; ●, B. atrophaeus strains. The eigenvalues for the first three principal components are 3.54, 2.47, and 2.09, respectively. The first three principal components (PC for i = 1,3) are the following functions of fatty acid phenotypes: PC1 = 0.20 FA1 + 0.40 FA2 + 0.24 FA3 + 0.24 FA5 + 0.34 FA6 + 0.24 FA7 + 0.34 FA8 + 0.24 FA9 + 0.34 FA10; PC2 = 0.50 FA1 + 0.07 FA2 + 0.07 FA3 + 0.44 FA4 + 0.21 FA5 + 0.44 FA6 + 0.15 FA7 + 0.15 FA8 + 0.15 FA9 – 0.74 FA10; and PC3 = 0.16 FA1 – 0.41 FA2 + 0.25 FA3 + 0.08 FA4 + 0.46 FA5 – 0.31 FA6 + 0.20 FA7 - 0.43 FA8 – 0.03 FA9 + 0.45 FA10. Where FAi is the percentage of the ith fatty acid (see Table 5). Each principal component was standardized to have a mean of zero and variance equal to the corresponding eigenvalue.
iments. Transformation of a B. subtilis recipient by the Death Valley group strains resulted in a mean sexual isolation value (ratio of homogamic to heterogamic transformation frequencies) of 1.57; this value corresponds to a 36% reduction in transformation compared with homogamic transformation.

Previous studies have shown that sexual isolation in Bacillus transformation experiments is largely determined by the extent of sequence divergence at the gene transformed and that sexual isolation is closely predicted as a log-linear function of sequence divergence (14, 21), as follows: log10 ($\rho$) = 18.87$\pi$ − 0.23, where $\pi$ is the donor-recipient sequence divergence value at the transformed gene (rpoB) and $\rho$ is the sexual isolation value. While these previous studies did not include the Death Valley group strains, the observed sexual isolation between the Death Valley group strains and B. subtilis ($\rho$ = 1.57) is reasonably close to the value predicted from this equation ($\rho$ = 1.93), given 2.7% divergence at rpoB (15, 21). This close fit supports the hypothesis that sexual isolation between the Death Valley group and B. subtilis strains is caused by sequence divergence between these groups.

While the modest sexual isolation between the Death Valley group and B. subtilis appears to be real, the magnitude of isolation between these groups is probably not large enough for this trait to be used reliably as a diagnostic characteristic. This is in contrast to what is found with more divergent taxa, which exhibit greater levels of sexual isolation. For example, B. mojavensis was first discovered because its strains consistently showed sexual isolation from B. subtilis (3, 16).

The Death Valley group strains exhibit levels of DNA-DNA reassociation of less than 35% with the type strains of previously described Bacillus species, but the levels of intra- and intergroup reassociation are 85% or more. These data confirm the cohesiveness of the Death Valley group and establishes this group as a genetically distinct taxon.

The Death Valley group differs significantly from all of its closest relatives in whole-cell fatty acid composition. A principal-component analysis of fatty acid composition showed that the Death Valley group strains form a phenotypic cluster that is distinct from all related taxa (Fig. 2). Thus, fatty acid composition provides a phenotypic diagnostic characteristic for distinguishing the Death Valley group.

Although several physiological, morphological, and metabolic traits were considered in this study, at this time the Death Valley group can be distinguished phenotypically from B. subtilis only on the basis of whole-cell fatty acid composition. Thus, the Death Valley group fits a pattern for other close relatives of B. subtilis; namely, close relatives of B. subtilis that can be distinguished on the basis of sequence divergence are generally distinguished by very few physiological, morphological, or metabolic characteristics (3, 5, 7–9, 11, 12, 14–16, 19, 20).

Description of Bacillus vallis-mortis sp. nov. Bacillus vallis-mortis (val.lis.mor’tis. L. n. vallis, valley; L. fem. n. mors, death; M. L. gen. fem. n. vallis-mortis, of Death Valley). Vegetative cells are bacilli that are 0.8 to 1.0 $\mu$m wide by 2.0 to 4.0 $\mu$m long (as determined by measurements obtained from photomicrographs) and occur singly and in short chains. Motile. Gram positive. Forms ellipsoidal spores centrally and paracentrally in unsulloned sporangia.

Agar colonies are opaque, smooth, circular, entire, and 1.0 to 2.0 mm in diameter after incubation for 2 days at 28°C. Catalase and oxidase are produced. Acrobic. Acetyl-CoA carbinoil is produced. The pH in Voges-Proskauer broth after 2 days ranges from 5.4 to 5.7. Nitrate is reduced to nitrite. Starch and casein are hydrolyzed. Citrate is utilized, but propionate is not utilized. Hydrogen sulfide, indole, and dihydroxyacetone are not produced. Egg yolk lecithin and urea are not decomposed. Tween 80 is decomposed. The pH in litmus milk is alkaline; casein is digested.

Phenylalanine and tyrosine are not decomposed.

Acid but no gas is produced from L-arabinose, cellobiose, fructose, glucose, galactose, lactose, mannoti, mannoiti, L-rhamnose, ribose, salicin, sorbitol, sucrose, trehalose, and xylose. Lactose and melibiose are not fermented.

The optimum growth temperature ranges from 28 to 30°C, the maximum growth temperature is about 50°C, and the minimum growth temperature ranges from 5 to 10°C. Growth occurs at pH 5.7 and in the presence of 10% NaCl. Inhibited by 0.001% lysozyme.

The thermal denaturation temperature for DNA of the type strain is 86.9°C, and the G+C content determined from this value is 43 mol%.

The description given above is virtually identical to the descriptions of B. atrophaeus, B. mojavensis, and B. subtilis. B. vallis-mortis can be differentiated from these previous described species by DNA reassociation data, by data from re- striction digestion analyses of selected genes, and by fatty acid analysis data.

Isolated from desert soil.

The type strain is strain DV1-F-3, which has been deposited in the Agricultural Research Service Culture Collection as strain NRRL B-14890.

ACKNOWLEDGMENTS

Soil from Death Valley National Monument was collected under permit A9015 issued by the U.S. National Park Service.

This research was supported by National Institutes of Health grant GM39501, U.S. Environmental Protection Agency grant R82-1388-100, and research grants from Wesleyan University.

REFERENCES

gence on sexual isolation in Bacillus. Genetics 134:401–408.