Reclassification of bioindicator strains \textit{Bacillus subtilis} DSM 675 and \textit{Bacillus subtilis} DSM 2277 as \textit{Bacillus atrophaeus}

Dagmar Fritze and Rüdiger Pukall

On the basis of high DNA–DNA reassociation values and confirmatory automated RiboPrint analysis, two aerobic spore-forming strains hitherto allocated to \textit{Bacillus subtilis} and used as bioindicators (DSM 675, hot-air sterilization control; DSM 2277, ethylene oxide sterilization control) are reclassified as \textit{Bacillus atrophaeus}.

Keywords: sterilization control, ‘\textit{Bacillus globigii}’, red strain, ‘\textit{Bacillus subtilis} var. \textit{niger}’, \textit{Bacillus atrophaeus}

Strains of the species \textit{Bacillus subtilis} are used in a variety of applications, an important one being sterilization control. Strains of this species produce spores of specific resistance to, for example, dry heat or ethylene oxide and are thus proposed for testing the effectiveness of such methods for sterilization (Kelsey, 1967; Russell \textit{et al}., 1992; US Pharmacopeia, 1995; CEN–European Committee for Standardization, 1997a, b). \textit{B. subtilis} DSM 675, originally designated as the ‘red strain’, was especially suited for routine use because of its distinctly coloured colonies.

Modern taxonomic methods have led to numerous reclassifications and rearrangements of strains, species and genera. This has been particularly true for the genus \textit{Bacillus}, which has undergone a wide range of taxonomic developments in recent years. Most of these investigations are usually based on type strains; only rarely are additional strains of the species in question included. Thus, strains of practical importance, e.g. test and control strains, are often not taken into account.

The long history of strain DSM 675, the ‘red strain’

In 1900, Migula described the species ‘\textit{Bacillus globigii}’. When Smith \textit{et al}., (1952) re-examined a number of strains received under this name, they had to allocate all of them to other more established species. Strains with traits corresponding to the original description were transferred to \textit{Bacillus licheniformis}, because the original description of ‘\textit{B. globigii}’ by Migula was judged to be synonymous with that for \textit{B. licheniformis}. Those strains not corresponding to the original description were allocated to \textit{Bacillus circulans}, \textit{Bacillus pumilus} and ‘\textit{B. subtilis} var. \textit{niger}’. Two strains from the Bacon Laboratories (the ‘red strain’ and the ‘brown strain’) were allocated to the latter species and were designated as NRS-1221A and NRS-1221B, respectively. In the same work, the authors concurrently reduced ‘\textit{Bacillus niger}’ from species to variety because they had found no discriminatory property, other than pigmentation, between \textit{B. subtilis}, ‘\textit{Bacillus atrophaeus}’ and ‘\textit{B. niger}’.

This property was shown to be susceptible to culture conditions (e.g. cultivation on media containing glucose or cultivation at a high incubation temperature). Clarifying the situation, Smith \textit{et al}., (1952) stated (p. 83) that ‘the characterization of \textit{B. subtilis} serves for ‘\textit{B. subtilis} var. \textit{niger}’ by adding the words substrate blackened to the description of the growth on mediums containing tyrosine’.

Later, Gordon \textit{et al}., (1973) found ‘varieties’ unsatisfactory and subsumed them under \textit{B. subtilis} knowing that this was a ‘lumped’ group; this group, with the arrival of better tests and methods, could then be taken apart again and ‘good’ species described. Indeed, since then, a number of new species have been separated from the species \textit{B. subtilis sensu stricto} and validly published (Priest \textit{et al}., 1987; Nakamura, 1989; Roberts \textit{et al}., 1994, 1996; Nakamura \textit{et al}., 1999).

Nakamura (1989) re-examined the black-pigment-producing strains of \textit{B. subtilis} and, on the basis of pigment production (on two different media) and DNA hybridization studies, he was able to discriminate between three groups of strains. Group 3 did not produce any pigment on either medium and included the type strain of \textit{B. subtilis}. Group 2 was a pigment-forming variant but still belonged to \textit{B. subtilis sensu stricto}.
patterns for all of the strains involved were generated with B

The present study reveals high DNA–DNA homology values between the two strains and the type strain of B. atrophaeus (DSM 7264T) and low hybridization values with B. subtilis DSM 10T. In addition, RiboPrint patterns for all of the strains involved were generated and compared with each other and with other Bacillus type strains. Strains DSM 675 and DSM 2277 showed a close association with B. atrophaeus, and a separation from B. subtilis was confirmed (the similarity coefficients of the RiboPrint patterns were approximately 0.92 and 0.94, respectively; see Fig. 1).

Table 1. Bacillus strains investigated in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>DSM no.</th>
<th>History</th>
<th>Other collection nos</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>DSM 675</td>
<td>← BMTU ← ATCC ← N. R. Smith (1221A, ‘B. subtilis var. niger’) ← Frederik S. Bacon Laboratories, Watertown, Massachusetts, 1947 (‘Bacillus globigii’, ‘red strain’) ← C. R. Phillips, Fort Detrick, USA ← Elisabeth McCoy</td>
<td>ATCC 9372, NCIB 8058, CIP 77.18 NRS 1221A, IFO 13721, NCDO 738</td>
</tr>
<tr>
<td>B. atrophaeus</td>
<td>DSM 7264T</td>
<td>← NRRL ← NRS-213 (‘B. subtilis var. niger’)</td>
<td>NRRL–NRS 213T, ATCC 49337T</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>DSM 10T</td>
<td>← ATCC ← H. J. Conn, strain Marburg</td>
<td>NRS 744T, ATCC 6051T, CCM 2216T, NCIB 3610T, NCTC 3610T, IFO 12210T</td>
</tr>
</tbody>
</table>

Table 2. Percentage DNA–DNA similarity

The DNA–DNA similarity values are the means of at least two determinations.

<table>
<thead>
<tr>
<th>Strain</th>
<th>DSM 2277</th>
<th>DSM 675</th>
<th>DSM 7264T</th>
<th>DSM 10T</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSM 2277</td>
<td>–</td>
<td>87</td>
<td>98</td>
<td>30</td>
</tr>
<tr>
<td>DSM 675</td>
<td>–</td>
<td>–</td>
<td>88</td>
<td>32</td>
</tr>
<tr>
<td>B. atrophaeus</td>
<td>DSM 7264T</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>DSM 10T</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

ND, Not determined.

stricto according to the high DNA–DNA similarity values between groups 2 and 3. Both groups (2 and 3) represent the species B. subtilis. Group 1, which produced a brownish-black pigment on one medium and a brown pigment on the other, showed low levels of DNA hybridization with groups 2 and 3. Thus, group 1 was described as the new species Bacillus atrophaeus. Twenty-one of the 25 strains in this group had previously been designated as ‘B. subtilis var. niger’.

Unfortunately, neither ‘B. subtilis var. niger’ DSM 675 (or any of its equivalents in other collections) nor ‘B. subtilis var. niger’ DSM 2277 was included in this study. To reveal the taxonomic position of these important sterilization control strains, spectroscopic DNA–DNA hybridizations (Huß et al., 1983) and automated RiboPrint (Qualicon) analyses (Bruce, 1996) were performed on all relevant strains (Table 1, Table 2, Fig. 1).
Thus, both sterilization control strains DSM 675 and DSM 2277, previously named ‘B. globigii’, ‘B. niger’, ‘B. subtilis var. niger’ and, finally, B. subtilis, have to be reclassified as members of the species B. atrophaeus. Species descriptions of B. subtilis and B. atrophaeus are not affected by this reclassification, as Smith et al. (1952) had classified the ‘red strain’ as ‘B. subtilis var. niger’ after its substrate blackening of media containing tyrosine. Nakamura (1989) described the soluble pigment as ‘brownish black’ or ‘dark brown’ and stated that ‘except for the colour of the soluble pigment, all of the strains were indistinguishable by the standard characterization method; i.e. they exhibited the traits typical of B. subtilis’.

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References


