Phylogenetic Status of *Sporosarcina ureae*

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The phylogenetic position of *Sporosarcina ureae* was reexamined by "comparative cataloging" of its 16S ribosomal ribonucleic acid. The data were analyzed in the context of similar information for *Micrococcus luteus* (*Sarcina lutea*), *Bacillus pasteurii*, *B. subtilis*, and *B. stearothermophilus*. It is concluded that *S. ureae* is best classified as a member of the genus *Bacillus* in the same subgroup as is *B. pasteurii*.

The taxonomic position of *Sporosarcina ureae* has been historically controversial: in the seventh edition of *Bergey's Manual* (2), it is assigned to the genus *Sarcina* in the family *Micrococcaceae*, whereas in the most recent edition it is placed in the family *Bacillaceae* (3). The controversy originates from the fact that the vegetative cells of *S. ureae* resemble those of species in the genus *Micrococcus* in their shape, cell wall composition, and mode of division, but produce endospores (5) and possess a deoxyribonucleic acid (DNA) having a guanine plus cytosine content typical of that of the family *Bacillaceae* (1). Detailed study of these spores (6, 9, 14, 16) has shown that their size, shape, chemical composition, heat resistance, ultrastructure, and germination properties resemble those of *Bacillus* spores. Biochemically, *S. ureae* resembles species of the genus *Bacillus*, especially *B. pasteurii* (10). Jensen and Stenmark (7), characterizing the control of the 3-deoxy-D-arabino heptulosonate 7-phosphate synthetase, showed unequivocally that the control in *S. ureae* resembles that in the genus *Bacillus* but not in the genus *Micrococcus*. In addition, nucleic acid hybridization studies using ribonucleic acid (RNA) from *S. ureae* and DNA from various bacterial sources indicate that *S. ureae* is more closely related to the family *Bacillaceae* than to the family *Micrococcaceae* (8).

In the present study, comparative oligonucleotide cataloging of 16S ribosomal RNA (rRNA) is used to examine the phylogenetic position of *S. ureae*. The results indicate unequivocally that *S. ureae* is closely related to the genus *Bacillus*. In particular, *S. ureae* is found to be more closely related to *B. pasteurii* than are other species of the genus *Bacillus* examined to date. Hence, *S. ureae* is probably best regarded as a member of the subgroup of the genus *Bacillus* which contains *B. pasteurii* rather than as a separate genus within the family *Bacillaceae*.

MATERIALS AND METHODS

S. ureae ATCC 6473 was purchased from the American Type Culture Collection, as was *B. pasteurii* ATCC 11859. *Micrococcus luteus* (*Sarcina lutea* UIMD 701) was obtained from the Microbiology teaching laboratory at the University of Illinois.

Organisms were labeled with $^{32}\text{PO}_4$ in a low-phosphate medium, i.e., yeast extract-peptone medium from which most of the phosphate had been removed by precipitation as magnesium-ammonium phosphate (11). To facilitate growth of *S. ureae* and *B. pasteurii*, this medium was supplemented with urea (final concentration, 0.3 M) and adjusted to a final pH of 8.5 (4, 5, 8). Otherwise, procedures for labeling the organisms and extracting and purifying the RNA were as described elsewhere (15, 19; K. J. Pechman, Ph.D. thesis, Univ. of Illinois, Urbana, 1975).

Labeled 16S rRNA was characterized in terms of its oligonucleotide fingerprint as described previously (13, 17). Basically, this procedure is as follows. A fraction of ^{32}P -labeled 16S rRNA is digested with T1 ribonuclease, and the resulting products are separated by two-dimensional paper electrophoresis. Oligonucleotide spots on the resulting electrophoretogram (whose sequences are not obvious from their positions) are then sequenced, by a combination of "secondary" and "tertiary" enzymatic procedures, producing a sufficient variety of overlapping digestion products in each case to permit the deduction of sequence (17, 18).

RESULTS

Table 1 presents the 16S rRNA oligonucleotide catalogs for the three organisms *M. luteus*, *S. ureae*, and *B. pasteurii*. The presence or absence of these oligonucleotides in the 16S rRNA of *B. subtilis* and *B. stearothermophilus* (18) is also indicated to provide additional perspective. Only oligonucleotides (pentamer and larger) are listed, since the smaller ones, which tend to occur in multiple copies, have no significance here (12). Table 2 summarizes the oligonucleotide coincidences found among the various organisms.

TABLE 1. Tabulation of characteristic T1 ribonuclease-generated oligonucleotide sequences for *Micrococcus luteus*, *Sporosarcina ureae*, and *Bacillus pasteurii*"

Oligonucleotide	<i>M. luteus</i>	<i>S. ureae</i>	<i>B. pasteurii</i>	<i>B. subtilis</i>	<i>B. stearothermophilus</i>	Oligonucleotide	<i>M. luteus</i>	<i>S. ureae</i>	<i>B. pasteurii</i>	<i>B. subtilis</i>	<i>B. stearothermophilus</i>
Pentamer						Octamer					
CCCCG	1	0	0	-	+	CCAACCCG	0	0	1	-	-
CACCG	1	0	0	-	-	ACAAACCG	0	1	1	+	-
CCACG	0	1	1	+	-	AACACCAG	0	1	1	+	+
CCCAG	2	1	1	+	+	CAAACCCG	0	1	0	-	-
ACCCG	0	1	1	+	-						
CAACG	1	1	1	++	+	CCACACUG	1	1	1	+	+
ACACG	1	1	1	+	+	CUCAACCG	0	1	0	+	+
ACAAG	1	0	0	-	+	ACAUCCCG	0	0	1	-	-
						ACCCCAUG	1	0	0	-	-
UCCCG	1	1	1	+	+						
CCCUG	0	0	1	-	-	CCCUUCAG	0	1	0	-	-
UCACG	1	0	0	-	-	CCCCUAG	0	1	1	+	-
UACCG	1	1	1	+	+	CCUAUCAG	1	0	0	-	-
CUCAG	1	1	1	+	+	UCAACUCG	1	0	0	-	-
ACUCG	1	0	0	-	-	CUUAACCG	0	0	1	-	-
ACCUG	0	2	2-1	+	+	AUACCCUG	1	1	1	+	+
UACAG	0	1	1-2	-	-	CUACAAUG	1	1	1	+	+
UCAAG	1	0	0	- ^b	- ^b						
CUAAG	0	1	1	+	+	AAUCCUG	1	0	0	-	-
UAACG	0	0	1	+	+						
CAAUG	0	1	0	+	+	UUCCUUCG	0	0	1	-	-
AUCAG	1	1	1	+	+	AUUUAUCG	1	0	0	-	-
ACUAG	1	0	0	-	-	AAUUAUUG	1	1	1	+	+
AAUCG	0	1	1	+	+						
ACAUG	1	1	0	-	-						
AUACG	2	0	0	-	-	Nonamer					
AACUG	0	1	0	-	-	CAACCCUCG	1	0	0	-	+
UAAAG	1	1	1	++	+	CUACACACG	0	1	1	+	+
AUAAG	1	0	0	-	+	UACACACCG	1	1	1	+	+
AAAUG	0	1	1	+	+	UAACACCCG	1	1	1	+	+
						CUCACCAAG	1	1	0	+	+
CUCUG	0	1	1	+	++	ACAUCCAG	0	1	0	-	-
CCUUG	1-2	0	0	+	++						
UCAUG	0	1	0	-	-	CAACCCUUG	0	0	1	+	-
CUAUG	0	0	1	-	-	ACUCCUACG	1	1	1	+	+
UAUCG	0	1	1	+	-	CUUACCAAG	0	0	1	-	-
ACUUG	0	1	0	-	+	CACUCUAAG	0	0	1	+	-
AUCUG	1	0	0	-	-	CUAACUACG	1	1	1	+	+
UAUAG	0	0	1	-	-	CUAAUACCG	0	1	1	+	+
UUAAG	3	1-2	1	++	+	AAUCCACG	0	1	1	+	+
UAAUG	1	1	1	-	-	AUAACUCCG	0	1	1	+	+
AUUAG	2	1	1	+	++	(AU, AAU, C, C)AG	0	0	1	-	-
AAUUG	1	1	1	+	+						
						CCCCUUAUG	1	1	1	+	+
UCUUG	1	2	2	+	+						
						(CC, U)UUUAAG	0	1	1	-	-
UUUUG	1	0	0	-	-	AUUAAUUCG	1	0	0	-	-
Hexamer						UUUAAUUCG	0	1	1	+	+
CCCACG	0	1	1	-	+						
CACAAG	1	1	1	+	+						

TABLE 1—Continued

Oligonucleotide	<i>M. luteus</i>	<i>S. ureae</i>	<i>B. pasteurii</i>	<i>B. subtilis</i>	<i>B. stearothermophilus</i>	Oligonucleotide	<i>M. luteus</i>	<i>S. ureae</i>	<i>B. pasteurii</i>	<i>B. subtilis</i>	<i>B. stearothermophilus</i>
AAACCG	0	1	1	+	+	Decamer					
AACAAG	0	1	1	+	—	AAACUCAAG	0	1	1	+	+
CAAACG	0	0	1	—	—						
UCCACG	0	1	0	+	+	CUUAACCCCG	1	0	0	—	—
CUAACG	1	1	0	+	+	(AU,AC)AUCAUG	0	1	0	—	—
CACUAG	1	0	0	—	—	UCUAAUACCG	1	0	0	—	—
ACACUG	0	1	0	—	—	UAACCCUUG	0	1	0	—	—
UAAACG	1	1	1	+	+	CCACUCUAUG	0	1	0	—	—
AAUACG	1	1	1	+	+						
ACUAAG	1	0	0	—	—	UUU(CU)CUUUG	0	0	1	—	—
AAACUG	1	1	1	+	+						
AUACAG	0	1	0	—	—	Undecamer					
						ACCAUUCCACG	1	0	0	—	—
UUCCCG	1	1	1	+	+	AACCUUACCAG	0	1	1	+	+
CCUUCG	1	0	0	—	+						
UCUCAG	1	0	0	—	—	CCUAACCCUUG	1	0	0	—	—
UCCAUG	1	0	1	—	—	CCAUCAUUCAG	0	1	0	—	—
UCACUG	0	1	1	—	—	CCUAAUACAUG	0	1	1	+	+
UACCUG	1	0	0	—	—	CUUAAACACAUG	1	0	0	—	—
AUCCCG	0	1	0	—	—	UACCUUACCAG	0	1	0	—	—
AUCCUG	1	1	1	+	+						
CAUUAG	0	1	1	+	+	UACCUCAUUAG	0	0	1	—	—
UAAUCG	1	1	1	+	+						
UAAACG	1	1	1	+	—	[CCCCCUUUU]AG	0	1	0	—	—
AUACUG	1	0	0	—	—						
						UAACCCUUUUG	0	0	1	—	—
UUUCCG	1	1	1	+	—	UUUCCCCUUG	1	0	0	—	—
CUUUCG	1	0	0	—	—						
(UC)UCUG	1	0	0	—	—	≥Duodecamer					
UCAUUG	0	1	1	+	+	AACCUUACCAAG	1	0	0	—	—
						ACUAA[CUC]AAAG	1	0	0	—	—
UUUUCG	0	1	1	+	—						
Heptamer						[CAA,CCCUUAA]UG	0	1	0	—	—
AACACCG	1	0	0	—	—	CCCUUAAACUCUG	1	0	0	—	—
CAAACAG	0	1	1	—	+						
						UCAAUCAUCAUG	1	1	1	+	+
CACUCCG	0	1	1	+	+	CAAUCUUACAAUG	0	1 ^c	1	—	—
CCUACCG	1	0	0	—	—						
CAACUCG	1	1	1	+	+	AUUUCUCCCUUCG	0	1 ^c	0	—	—
CAACCUG	0	1	1	—	+						
UAAACAG	1	1	1	+	+	CUAAUCCCCAAAAG	1	0	0	—	—
UACAACG	1 ^c	0	0	—	—						
CACAAUG	1	0	0	—	—	UAAA[CCUCUUU]CAG	1	0	0	—	—
UAAAAAG	0	1	0	—	—						
						[UAA,UCCA]U[CU,CUU]C G	0	1	0	—	—
CUUCACG	1	0	0	—	—						
UUCCAG	0	1	1	—	—	AUAUACCU- UUCCCUUG	0	1	0	—	—
CAUUCAG	0	0	1	+	+	CCAAUCCACAAAAUCG	0	1	0	—	—
UUCACAG	1	0	0	—	—	CUAAUCCCAUAAAACCG	0	0	1	—	—

TABLE 1—Continued

Oligonucleotide	<i>M. luteus</i>	<i>S. ureae</i>	<i>B. pasteurii</i>	<i>B. subtilis</i>	<i>B. stearothermophilus</i>	Oligonucleotide	<i>M. luteus</i>	<i>S. ureae</i>	<i>B. pasteurii</i>	<i>B. subtilis</i>	<i>B. stearothermophilus</i>
UAACCUG	1	0	0	+	—	Post-transcriptionally modified ^f					
ACUCAUG	1	0	0	—	—						
ACCUUAG	0	0	1	—	—	AUG	1	0	0	—	—
AACUCUG	0	0	1	—	—	GCCG	1	0 ^c	1	+	+
CAUUAAG	1	1	1	+	—	C ^c CCG	1	1	1	+	+
UACAAUG	1	0	0	—	—	CAACG	1	1	0 ^c	+	+
UAAUACG	1	1	1	+	+	UAACAAG	1	1	1	+	+
AUAUCAG	1	0	0	—	—	UCA ^c ACCACG	0	1	1	+	+
AAACUUG	0	0	1	—	—	U ^c CAAAUCAUCAUG	0	0	1	—	—
UACCUUG	0	1	1	+	—	5'-Termini					
UUAUCCG	1	0	0	—	—	pU _{1,3} UUUACG	1	0	0	—	—
UUCCAUG	1	0	0	—	—	pU[CUU]AUG	0	1	0	—	—
AAUAUUG	1	0	0	—	—	pUAUUAUG	0	0	1	—	—
CUUUCUG	0	1	1	—	—	3'-Termini					
						AUCACCU[CCUUU]U _{OH}	1	1	1	+	+

^a The number of copies of an oligonucleotide found in a given RNA is indicated by the numerals 0, 1, 2, etc. Where this number is somewhat uncertain, a range, e.g., 1-2, etc., is given. For *B. subtilis* and *B. stearothermophilus*, whose complete catalogs are not given herein, + and ++ denote the presence of one copy and two copies, respectively, of a given oligonucleotide in that organism; — denotes its absence. The complete catalogs for these two organisms are given elsewhere (18).

^b The absence of the given oligonucleotide is likely but not totally certain.

^c The presence of the given oligonucleotide is likely but not totally certain.

^d This oligonucleotide occurs in *B. pasteurii*; however, the initial C, i.e., UCAA... is post-transcriptionally modified in that case; see below.

^e The sequence of the given oligonucleotide is likely but not totally certain.

^f A post-transcriptionally modified base is indicated by a superscript dot.

DISCUSSION

A number of points are apparent in the data. The *M. luteus* catalog has relatively few sequences in common with the other catalogs. In particular, there is no specific resemblance between it and the *S. ureae* catalog. On the other hand, *S. ureae* clearly manifests a *Bacillus* "signature." All but one of the sequences (hexamer and larger) common to the three catalogs of bona fide species of the genus *Bacillus* are found in the *S. ureae* catalog. *S. ureae* belongs, therefore, within the family *Bacillaceae*—a

conclusion consistent with the previous evidence reclassifying it therein.

The present method, however, permits a more detailed classification of *S. ureae*. Table 2 shows clearly that *S. ureae* and *B. pasteurii* more closely resemble one another than either resembles the other species of the genus *Bacillus*. The number of sequences (hexamer and larger) common to this pair of organisms is 53, whereas the various pairings of the other *Bacillus* species yield 41 to 49 such coincidences. Moreover, *S. ureae* and *B. pasteurii* have five coincident sequences that are unique to that

TABLE 2. Summary of sequence comparisons for catalogs in Table 1^a

Sequences	<i>M. luteus</i>	<i>S. ureae</i>	<i>B. pasteurii</i>	<i>B. subtilis</i>	<i>B. stearothermophilus</i> ^b
Unique to a given organism	37 (44)	17 (19)	16 (19)	15 (20)	21 (25)
In common with all three bacilli	23 (34)	37 (54)	38 (56)	38 (56)	38 (56)
In common with:					
<i>M. luteus</i>	—	29 (43)	28 (41)	31 (45)	28 (45)
<i>S. ureae</i>	29 (43)	—	53 (77)	50 (71)	44 (63)
<i>B. pasteurii</i>	28 (41)	53 (77)	—	49 (70)	41 (59)
<i>B. subtilis</i>	31 (45)	50 (71)	49 (70)	—	45 (65)
<i>B. stearothermophilus</i>	28 (45)	44 (63)	41 (59)	45 (65)	—
Unique to a given organism and:					
<i>M. luteus</i>	—	0 (1)	1 (1)	1 (1)	1 (4)
<i>S. ureae</i>	0 (1)	—	5 (6)	0 (0)	0 (1)
<i>B. pasteurii</i>	1 (1)	5 (6)	—	2 (2)	0 (0)
<i>B. subtilis</i>	1 (1)	0 (0)	2 (2)	—	1 (1)
<i>B. stearothermophilus</i>	1 (4)	0 (1)	2 (2)	1 (1)	—

^a The tabulation includes all sequences (hexamer and larger) and all post-transcriptionally modified sequences. The numbers in parentheses represent the tabulation when sequences of length five are also included.

^b Complete data given in reference 18.

pairing (Table 2). No other pairing of the organisms described herein produces more than two such unique coincidences. Thus, it is necessary either to include *S. ureae* in the genus *Bacillus* or to exclude *B. pasteurii* from it. The latter alternative is unreasonable in that *B. pasteurii* appears at least as closely related to *B. subtilis* as is *B. stearothermophilus*.

We conclude, therefore, that the classification of *S. ureae* as a separate genus within the family *Bacillaceae* is no longer phylogenetically justifiable. Instead, it is reasonable to classify *S. ureae* in the subgroup of the genus *Bacillus* that contains *B. pasteurii*.

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REPRINT REQUESTS

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