Bacillus psychrophilus Larkin and Stokes 1967, a Later Subjective Synonym of *Bacillus globisporus* Larkin and Stokes 1967

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The type strain of *Bacillus psychrophilus* Larkin and Stokes 1967 and that of *Bacillus globisporus* Larkin and Stokes 1967 are very nearly identical. Therefore these names are regarded as synonyms. On the basis of the rule of priority, *B. globisporus* is the correct name of this species. *B. psychrophilus* is thus a later subjective synonym of *B. globisporus*.

Four new psychrophilic *Bacillus* species, *B. psychrosaccharolyticus*, *B. insolitus*, *B. globisporus*, and *B. psychrophilus*, were named and described in 1967 by Larkin and Stokes (4).

B. psychrosaccharolyticus and *B. insolitus* can be distinguished from each other as well as from the other two species by their different nutritional and enzymatic properties and by the shape and arrangement of their spores. On the other hand, *B. psychrophilus* and *B. globisporus* are very closely related (2, 4). Gyllenberg and Laine (3), in a numerical taxonomy study of psychrophilic bacilli, were unable to separate these two species on the basis of their biochemical characters.

In our studies on halophilic bacilli, the type strains of *B. psychrophilus* and *B. globisporus* were included, but we could not detect any differences between them (6). Therefore, we attempted to determine whether a comparison of the cell wall compositions of these two strains would help clarify their relationship. The results of these examinations are presented here.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study were *Bacillus globisporus* DSM 4 (= ATCC 23301 = Larkin and Stokes W 25) and *B. psychrophilus* DSM 3 (= ATCC 23304 = Larkin and Stokes W 16 A). Both of these strains were designated by Larkin and Stokes (4) as the type strains of their respective species.

Cultures of these strains were obtained from the Deutsche Sammlung von Mikroorganismen (DSM), Göttingen, Federal Republic of Germany.

Methods. The bacteria were cultivated in a modified ZoBell 2216 seawater broth (7), which consisted of: peptone, 5 g; yeast extract, 1 g; $\text{FePO}_4 \cdot 4\text{H}_2\text{O}$, 0.01 g; aged seawater, 750 ml; and distilled water, 250 ml (pH 7.6).

The cultivation method, in which successively increasing amounts of the inoculum and of the culture medium were used to prevent the formation of spores,

was as follows. Each of four test tubes containing 3 ml of seawater broth was inoculated with a loopful of a young culture. After a few hours of incubation, the contents of each tube were transferred to a bottle containing 30 ml of seawater broth and incubated overnight. From each culture, 30 ml of broth was then used to inoculate 300 ml of seawater broth in a 1-liter Erlenmeyer flask, and the flasks were shaken at 75 rpm for 48 h at 20°C. The cells were subsequently harvested by centrifugation. This procedure yielded 2 to 3 g (wet weight) of cells. For the purpose of determining cell wall composition, the cells were then washed first in distilled water and subsequently in 96% ethanol. After washing, the cells were stored at -20°C until use.

The cell wall analyses were performed as described by Richter (5), except that the cells of *B. globisporus* DSM 4 were extracted with 0.3 N KOH and those of *B. psychrophilus* DSM 3 were extracted with 0.25 N KOH.

RESULTS AND DISCUSSION

Larkin and Stokes (4) separated *B. psychrophilus* from *B. globisporus* on the basis of their distinctive behaviors with respect to nitrate reduction and their different abilities to ferment lactose, mannitol, and xylose. These species differed only slightly with respect to some other characters. Gordon et al. (2) subsequently examined the strains of Larkin and Stokes. The characters used by these two sets of authors to separate the two species are given in Table 1.

As shown by the data of Gordon et al. (Table 1), *B. psychrophilus* contains not only strains that ferment mannitol and xylose but also strains that do not. In this case, *B. psychrophilus* and *B. globisporus* overlap each other and thus cannot be differentiated solely on this basis.

According to Eiroma et al. (1), the deoxyribonucleic acid base compositions of the two type strains are different: the deoxyribonucleic acid of *B. globisporus* DSM 4 contained 36.8 mol% guanine plus cytosine, whereas that of *B. psychrophilus* DSM 3 contained only 32.7 mol% guanine plus cytosine. However, our examina-

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TABLE 1. Differentiation of *B. globisporus* and *B. psychrophilus* according to Larkin and Stokes (4) and Gordon et al. (2)^a

Character	<i>B. globisporus</i> , according to:		<i>B. psychrophilus</i> , according to:	
	Larkin and Stokes	Gordon et al.	Larkin and Stokes	Gordon et al.
Cell size (μm)	0.8–1.1 by 1.3–6.4	0.8–1.0 by 1.3–5.0	0.8–0.9 by 1.2–4.5	0.8–0.9 by 1.8–4.5
Spore size (μm)	1.0–1.1	n	0.9–1.3	n
Motility	+	±	+	±
Maximal growth temperature (°C)	25	25–30	30	25–30
Glucose agar slants	Growth as on nutrient agar	n	Growth poorer than on nutrient agar	n
Milk agar plates/casein hydrolysis	+	±	+	±
pH in VP broth	n	6.0–6.6	n	6.2–7.4
Starch hydrolysis	–	±	±	+
Methylene blue reduction	+	n	±	n
Nitrate reduction	–	±	+	+
Acid production				
Lactose	+	n	–	n
Mannitol	–	–	+	±
Xylose	–	–	+	±

^a +, All strains positive; –, all strains negative; ±, positive and negative strains are known; n, not reported.

TABLE 2. Cell wall composition of *B. globisporus* DSM 4 and *B. psychrophilus* DSM 3^a

Cell wall component	<i>B. globisporus</i> DSM 4	<i>B. psychrophilus</i> DSM 3
Alanine	+++	+++
Glutamic acid	+++	+++
Glycine	++	+++
Asparagine ^b	Tr	+
meso-DAP ^c	–	Tr
Lysine	+++	+++
Valine	Tr	+
Muramic acid	+	+
Glucosamine	+++	+++
Leucine/isoleucine	++	++

^a Symbols: +++, ++, +, Tr (traces), and – represent the relative amounts of substances present (or absent) on the chromatograms; in the case of the amino acids, + and Tr indicate negligible amounts.

^b As a result of acid hydrolysis, aspartic acid instead of asparagine appeared in the chromatograms.

^c meso-DAP, meso-Diaminopimelic acid.

tions (6) showed these values to be in the same range: 39.7 ± 0.3 mol% and 40.6 ± 0.6 mol% guanine plus cytosine for *B. psychrophilus* DSM 3 and *B. globisporus* DSM 4, respectively.

Because of the difficulty in differentiating between *B. globisporus* and *B. psychrophilus*, as previously discussed by Gyllenberg and Laine (3), we decided to analyze the cell walls of the type strains of these species to see if the information derived therefrom would help to clarify the relationship of these organisms. The results, presented in Table 2, showed that both strains have the same cell wall composition.

Because the type strains of *B. globisporus* and *B. psychrophilus* are very nearly identical (6),

we regard them as belonging to the same species, the correct name of which is *B. globisporus*. (Although the names *B. globisporus* and *B. psychrophilus* were proposed by the same authors in the same paper, the former was proposed first, and thus has priority [page priority]. Thus *B. psychrophilus* is a later subjective synonym of *B. globisporus*.)

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REPRINT REQUESTS

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