TRANSFORMATION IN BACILLUS CEREUS 569: A CORRECTION OF STRAIN DESIGNATION

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Recently Goldberg and Gwinn (1968) reported that the strain designated Bacillus cereus 569 reported by Felkner and Wyss (1964) was in fact Bacillus subtilis. We would like to substantiate the findings of this critique with observations made in our laboratory. First, it should be said that this culture was secured at Fort Detrick and was labelled B. cereus strain 569, Pollock. This strain had at least 2 characteristics which identified it as an atypical B. cereus. Goldschmidt and Felkner (unpublished data) made the observation that this strain was easily disrupted with egg white lysozyme at a concentration of 0.4 mg/ml and/or 2% sodium lauryl sulfate. Since all other B. cereus strains were insensitive to this treatment, McDonald, et al. (1963) had to use an alternate procedure to obtain DNA. In this report, it was shown that the Tm for B. cereus 569 was 85.45 C (which corresponds to 40.1% guanine + cytosine) whereas the mean value for all other B. cereus strains studied was 82.25 C (which corresponds to 32.2% guanine + cytosine). These values are in excellent agreement with those reported by Goldberg and Gwinn for "B. cereus" 569-S^r and B. cereus NRRL B-569 ie, 85.5 C and 82.4 C respectively.

Methods and Results: Type strains of B. cereus and B. subtilis were compared to "B. cereus strain 569" with regard to diagnostic sugar fermentations, enzyme reactions and morphological characteristics. These results

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TABLE 1

COMPARISON OF CERTAIN DIAGNOSTIC CHARACTERISTICS OF B. SUBTILIS STRAIN TEXAS, B. CEREUS STRAIN TEXAS AND "B. CEREUS" STRAIN 569

Test Strain	Sugar Fermentations						Size (u)		B. Hydroxy* Butyric Acid	Lethicinase ‡ Production
	Mann.		Ara.		Xyl.		Length	Width		
B. subtilis strain Texas	A^+/G^-	24h	A^+/G^-	24h	A^+/G^-	24h	586	0.55	Few, small and bipolar globules	None
	A^+/G^-	36h	A^+/G^-	36h	A^+/G^-	36h				
	A^+/G^-	48h	A^+/G^-	48h	A^+/G^-	48h				
B. cereus strain Texas	A^-/G^-	24h	A^-/G^-	24h	A^-/G^-	24h	5.25	1.35	Many, large globules	Strongly positive at 24 hr and increasing 48 hr
	A^-/G^-	36h	A^-/G^-	24h	A^-/G^-	36h				
	A^-/G^-	48h	A^-/G^-	48h	A-/G^-	48h				
"B. cereus" strain 569	A^+/G^-	24h	A^+/G^-	24h	A^+/G^-	24h	3.17	0.94	Few, small and bipolar globules	None or very little
	A^+/G^-	36h	A^+/G^-	36h	A^+/G^-	36h				
	A^+/G^-	48h	A^+/G^-	48h	A^+/G^-	48h				

Symbols: A = acid; G = gas; *, using a fat straining procedure employing Sudan Black B;
‡ Detected by growth on Tryptone-egg yolk agar.

are summarized in TABLE 1. The mannitol and arabinose reactions of "B. cereus strain 569" are like those of B. subtilis and differ from B. cereus strain Texas. The same is true with xylose although a slightly positive reaction could be shown for B. cereus strain Texas. When the 3 strains were grown for 24 hr in nutrient broth and stained with Sudan Black B, followed by a counterstain with aqueous safranin, the number and size of fat globules (poly-B-hydroxybutyric acid) were few and small with bipolar orientations in B. subtilis strain Texas and "B. cereus strain 569" whereas there were many large ones in B. cereus strain Texas.

On Tryptone-egg-yolk agar, colonies which display an opalescent area (halo) around the periphery are considered to be lethicinase positive (Colmer, 1948). B. cereus strain Texas was lethicinase positive by this test whereas B. subtilis strain Texas was negative. "B. cereus strain 569" appeared to have a slight halo at 24 hr but it failed to increase up to 72 hr later. It was thus judged to produce little or no lethicinase.

A filar micrometer was used to measure the length and width of individual bacilli. As is seen from Table 1, "B. cereus strain 569" is shorter than either of the type strains and is intermediate in width. Both B. subtilis strain Texas and B. cereus strain Texas formed long chains in nutrient broth whereas "B. cereus strain 569" was usually in pairs, short chains or single. It is significant that B. subtilis 168 M ind (received from Dr. W. R. Romig) is the same relative size, and also exists in pairs, short chains or singularly. Recent reports (Singh and Pitale, 1968; Cahn and Fox, 1968; Hadden and Nester, 1968) show that uninucleate cells of B. subtilis 168 M ind which are lighter in a Renografin-76 (methylglucamine diatrizoate) density gradient are most likely to be the competent ones. Thus chaining and other properties which might be related (eg. length, width and density) might be important properties which prevent non-transformable strains of B. cereus and B. subtilis from becoming competent.

A personal communication (Dr. Curtis Thorne) indicated that "B. cereus

strain 569" shares several genetic properties with B. subtilis 168 M ind. These included sensitivity to bacteriophages known to attack B. subtilis and the ability for "B. cereus strain 569" and its mutants to transform and be transformed by B. subtilis strain 168 M and its derivatives. We have verified these findings in our laboratory and have suggested that this strain be classified as B. subtilis (as was suggested also by Goldberg and Gwinn, 1968). This reclassification suggestion was reported to the Federation Meeting in 1965 although it does not appear formally in the abstract (Felkner and Wyss, 1965). This correction does, however, appear elsewhere (Felkner, PhD thesis, 1966) but unfortunately was not made earlier to this journal. Although we feel that "B. cereus strain 569" and its derivatives reported earlier (Felkner and Wyss, 1964) are probably derivatives of B. subtilis 168 M ind, we cannot be certain and have therefore referred to it as B. subtilis strain 569.

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