The Lipid Content of *Bacillus stearothermophilus* at 37° and at 55°

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The membrane composition of an obligate thermophile, *Bacillus stearothermophilus* NCA 1503-4R was studied by Bodman & Welker (1969). We have examined the membrane composition of a facultatively thermophilic strain of *B. stearothermophilus* strain B65, grown at a mesophilic (37°) and a thermophilic (55°) temperature. Isolated walls of this organism grown at 55° were reported to have an unusually high (8 %) lipid content (Forrester & Wicken, 1966). Sutow & Welker (1967), however, claimed that this lipid was due to contaminating membrane lipoprotein.

METHODS

Bacillus stearothermophilus B65 was obtained from Dr A. J. Wicken and grown in the medium of Forrester & Wicken (1966) in a New Brunswick Bench Top Fermentor with agitation (500 rev./min.) and aeration (1 l. sterile air/l. medium/min.). Protoplast membranes were prepared from freshly harvested late log-phase bacteria by the procedure of Perkins & Nieto (1970). Three different batches of membranes were analysed at each growth temperature. Freeze-dried membranes were solubilized by sonic treatment (Bertsch, Bonsen & Kornberg, 1969) before estimating protein (Lowry, Rosebrough, Farr & Randall, 1951), carbohydrate (Seifter, Dayton, Novic & Muntwyler, 1950) and RNA (Ceriotti, 1955). Lipids were extracted from membranes and freeze-dried sonically treated bacteria by 0.05 M-sodium citrate buffer pH 6.5 in a one-phase solvent system (Houtsmuller & Van Deenen, 1965). Phospholipids were precipitated from a methanol+chloroform (1+2, v/v) solution of total lipids with 6 vol. acetone at -20°. The solvents from the lipid extracts were removed in a stream of nitrogen gas and the lipid residues dried over P₂O₅ in a vacuum desiccator for 24 h. before weighing.

RESULTS AND DISCUSSION

The lipid composition of *Bacillus stearothermophilus* B65 grown at 37° and 55° to the mid-log and late-log stages of growth is shown in Table 1. The total lipid content decreased between mid-log and late-log stages of growth and also decreased with increased temperature. A similar decrease in total lipids was reported for *B. stearothermophilus* NCA 1518 grown at 37° and at 55° (Long & Williams, 1960). The decrease in total lipids with increased growth temperature was due entirely to a decrease in the acetone-soluble lipids since the phospholipid content increased slightly at 55°.

Isolated protoplast membranes from late log-phase culture of *Bacillus stearothermophilus* B65 accounted for 20.5 % (37°) or 16.4 % (55°) of the dry weight. The low yield of membranes isolated from bacteria grown at 55° may reflect the loss of certain membrane components during isolation rather than a true decrease in membrane content. Membrane yields of *B. stearothermophilus* have been reported as 24 % for strain 2184 at 60° (Card, Georgi &

Militzer, 1969), 16.5 % and 17.8 % for mid log-phase bacteria of strain NCA 1503-4R at 55° and 65°, respectively (Bodman & Welker, 1969). Protein and lipid were the major components of the membranes; RNA (8.7 % at 37°, 9.0 % at 55°) and carbohydrate (4.3 % at 37°, 2.8 % at 55°) constituted the remainder. The membrane protein content increased from 64.60 % at 37° to 80.26 % at 55° while the membrane lipid content decreased from 21.28 % (4.36 % bacterial dry weight) at 37° to 10.64 % (1.75 % bacterial dry weight) at 55°. Bodman & Welker (1969) reported similar but smaller changes in membranes of the obligate thermophile *B. stearothermophilus* NCA 1503-4R for a temperature increase from 55° to 65°.

Table 1. *Lipid composition of Bacillus stearothermophilus.*
The results show the mean and standard deviation for the different batches

No. of batches	Total lipids (% dry wt)	Phospholipids		Acetone-soluble lipids	
		(% dry wt)	(% total lipids)	(% dry wt)	(% total lipids)
37°, Mid-log					
4	5.54 ± 0.29	3.18 ± 0.18	57.48 ± 2.07	2.36 ± 0.17	42.52 ± 2.07
37°, Late-log					
3	4.46 ± 0.43	2.75 ± 0.24	61.90 ± 1.13	1.73 ± 0.20	38.10 ± 1.13
55°, Mid-log					
4	4.29 ± 0.14	3.26 ± 0.09	76.11 ± 1.80	1.02 ± 0.10	23.89 ± 1.80
55°, Late-log					
3	3.91 ± 0.08	2.96 ± 0.06	75.63 ± 2.32	0.95 ± 0.11	24.37 ± 2.32

Since at 37° late log-phase bacteria contained 4.46 % lipid, 4.36 % being the amount in the isolated membranes, it would appear that all the lipids were located on the membrane. At 55°, however, the late log-phase bacteria contained 3.91 % lipid, only 1.75 % in the membranes. It is unlikely that the difference, mostly comprising phospholipid, represented lipid which was not associated with the membrane since (i) at 37° almost all the lipids were isolated with the membrane and (ii) Card *et al.* (1969) showed that in *Bacillus stearothermophilus* 2184 grown at 60° the phospholipids were almost exclusively associated with the membrane, a condition widely recognized in Gram-positive bacteria. It is probable that when grown at 55° some *B. stearothermophilus* B65 membrane lipids became loosely associated and were more easily dissociated from the membrane during the isolation and washing of the membrane. This would explain the high lipid contamination of isolated walls of *B. stearothermophilus* B65 (Forrester & Wicken, 1966), as suggested by Sutow & Welker (1967).

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