Short Communication

The Anionic Polymers in the Cell Wall of *Bacillus subtilis* var. *niger* Grown in Phosphorus-Limiting Environments Supplemented with Increasing Concentrations of Sodium Chloride

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Tempest, Dicks & Meers (1967) showed that the affinities with which cell walls of *Bacillus subtilis* var. *niger* and *Aerobacter aerogenes* bound Mg^{2+} were different. This difference correlated with the ability of the Gram-negative organism to outgrow the bacilli in Mg^{2+} -limited mixed cultures in a chemostat. Meers & Tempest (1969) investigated this further and studied the cation-binding properties of *B. subtilis* var. *niger* cell walls produced from organisms grown in different environments. They found that cell walls of organisms grown in magnesium-limited environments bound Mg^{2+} more avidly than did cell walls of organisms grown in phosphorus-limiting conditions. This could be explained by the fact that cell walls of magnesium-limited bacteria contained a teichoic acid (i.e. a phosphorus-containing polymer) whereas phosphorus-limited organisms had little phosphorus in their cell walls but instead contained a teichuronic acid (an anionic polymer that does not contain phosphorus) (Ellwood & Tempest, 1967; Ellwood, 1970). These results, coupled with the fact that cell walls of *B. subtilis* var. *niger* grown under magnesium-limited conditions contained more teichoic acid than did cell walls of organisms grown in the other environments studied, suggested that teichoic acids could be involved in the assimilation of cations (perhaps specifically Mg^{2+}) on the bacterial surface (Ellwood, 1970).

Heptinstall, Archibald & Baddiley (1970) studied the binding of Mg^{2+} to cell walls of *Staphylococcus aureus* H. This organism contained a ribitol teichoic acid having ester-bound alanine in its cell wall when grown in a complex medium in batch culture, and the teichoic acid in the cell wall could be removed by chemical degradation procedures. They showed that when 75% of the cell-wall phosphorus was removed the Mg^{2+} -binding ability of the cell wall was decreased by 80%. When they grew the organism in their medium supplemented with 7.5% (w/v) of sodium chloride the cell walls contained less alanine ester residues and bound a substantially greater amount of Mg^{2+} . They concluded that it was the phosphodiester residues of the teichoic acid that were affecting the binding of

Mg^{2+} to the cell wall, again suggesting that this was the functional role of the teichoic acid.

Meers & Tempest (1970) showed that when they grew *B. subtilis* var. *niger* in phosphate environments, but with the osmolarity of the medium increased by the addition of 4% (w/v) of sodium chloride, cell walls were synthesized that had an increased binding capacity for Mg^{2+} .

These cell walls also had a pH-buffering capacity in the pH 2.0-2.5 region, indicating that there were ionized phosphate residues in the cell walls. This finding could explain the increase in the ability of these cell walls to bind Mg^{2+} , but was totally unexpected as it had been previously shown that phosphorus limitation generally caused deletion of teichoic acid from the walls (Tempest, Dicks & Ellwood, 1968).

This communication reports the results of an investigation of the ionic polymer composition of cell walls of *B. subtilis* var. *niger* grown in phosphate-limited environments with increasing amounts of sodium chloride in the feed medium.

B. subtilis var. *niger* (A.T.C.C. 9372) was grown in a 0.5-litre Porton-type chemostat (Herbert, Phipps & Tempest, 1965) at 35°C with the pH controlled at 7.0 ± 0.1 . The phosphate-limited medium (Tempest *et al.* 1968) was supplemented to give final concentrations of 2%, 4% and 6% (w/v) sodium chloride. Organisms were grown at a dilution rate of $0.3 h^{-1}$ and collected in a receiving vessel surrounded by melting ice. Bacteria were separated by centrifugation, washed once with deionized water and broken in a Braun M.S.K. homogenizer. The cell walls were isolated quantitatively and analysed for phosphorus, total carbohydrate, uronic acid, D-glucose and D-galactosamine as described by Ellwood (1970) (Table 1).

When sodium chloride was added to phosphate-limited media, the phosphorus content of the wall was increased and teichuronic acid synthesis diminished. With 6% (w/v) of sodium chloride in the medium the phosphorus content of the wall was considerable and glucuronic acid was virtually absent, indicating that under these conditions the

Table 1. *Composition of the cell wall of B. subtilis var. niger* A.T.C.C. 9372 when grown in phosphate-limited medium with increasing amounts of sodium chloride added to the medium at a dilution rate of 0.3 h^{-1} at 35°C and at pH 7.0

Total hexose, glucuronic acid and phosphorus determinations (after acid digestion) were carried out on an unhydrolysed suspension of bacterial walls (5 mg/ml of water). Galactosamine was determined enzymically on samples that had been hydrolysed with 4M-HCl (at 100°C for 4 h in a sealed tube) and neutralized with NaOH. The results of duplicate determinations are given for the amount of cell wall.

NaCl added to the medium (g/100 ml)	Amount of wall (g/100 g of dried organism)	Wall composition (g/100 g of wall)			
		Phosphorus	Hexose	Glucuronic acid	Galactosamine
None	17.0, 17.5	0.5	2.0	24	17.0
2.0	22.8, 21.8	0.7	10.4	19.7	17.0
4.0	19.4, 18.7	3.1	16.9	7.5	3.0
6.0	16.1, 16.0	4.3	18.8	<3.0	<3.0

organism no longer can conserve phosphorus by synthesizing a non-phosphorus-containing cell-wall anionic polymer.

Anionic polymers were extracted from the cell-wall preparations with 10% (w/v) trichloroacetic acid at 4°C and precipitated from solution with 1 vol. of acetone (Ellwood, 1970). Only 14% of the cell-wall material from organisms grown in the absence of sodium chloride was extracted by this treatment, whereas 32% of the cell-wall material was extracted from organisms grown in the presence of 6% (w/v) sodium chloride. Extracts were hydrolysed with 2M-hydrochloric acid for 4 h at 100°C and, after two evaporations under reduced pressure (to remove hydrochloric acid), examined by paper chromatography for the presence of teichoic acid and/or teichuronic acid components (Ellwood, 1970).

Acid-hydrolysed extracts of cell-walls of organisms grown in phosphate-limited environments in the absence of sodium chloride gave only galactosamine and glucuronic acid. This confirmed the results of Tempest *et al.* (1968) and shows that these cell walls contained no teichoic acid (only teichuronic acid). However, when sodium chloride was present in the growth medium, glycerol phosphates and glucose were detected in the cell-wall extracts. These hydrolysis products, characteristic of teichoic acid, were the only products found on analysis of trichloroacetic acid extracts from the cell walls of organisms grown in the presence of 6% (w/v) sodium chloride.

The teichoic acid extracted from the 6%-(w/v)-sodium chloride-grown organisms had a phosphorus/glucose ratio 1.07:1, and alkaline hydrolysis followed by enzymic dephosphorylation indicated that it has the structure of a glycerol phosphate polymer with the sugar moiety as part of the chain, i.e. it was similar to the type found in the cell wall of *Bacillus licheniformis* A.T.C.C. 9544 (Burger &

Glaser, 1966) and similar in structure to the teichoic acid found in the cell wall of *B. subtilis* var. *niger* grown in all phosphate-sufficient media so far examined (D. C. Ellwood, unpublished work). In agreement with previous observations on teichoic acids found in this organism, grown at pH 7.0 and at 35°C , no evidence of ester-bound alanine was found in any of the teichoic acid extracts.

The results in Table 1 also show that when 2% or 4% (w/v) sodium chloride was present in the growth media both teichoic acid and teichuronic acid coexisted in cell-wall preparations. This is in contrast with earlier results (Ellwood, 1970) where these polymers were found to be mutually exclusive. Previously it was shown that when *B. subtilis* var. *niger* was grown in phosphate-limited medium teichuronic acid was the only anionic polymer present in the cell wall. The present work shows that after growth of this organism in phosphate-limited medium containing sodium chloride the cell walls contained both teichoic acid and teichuronic acid. The amount of teichoic acid found in the cell wall increased as the sodium chloride concentration in the medium was increased; no teichuronic acid was found in the cell walls of organisms grown in the presence of 6% (w/v) sodium chloride.

Meers & Tempest (1970) showed that when *B. subtilis* var. *niger* was grown in phosphate-limited media in the presence of sodium chloride the isolated cell walls bound increasing amounts of Mg^{2+} as the sodium chloride concentration in the growth media was increased. The previous and present results indicate a correlation between Mg^{2+} -binding capacity and the teichoic acid content of cell walls, and suggest that one of the functional roles of teichoic acid is to bind Mg^{2+} to the bacterial surface as the initial step in the assimilation of this cation by this bacterium.

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