Short Communications

The Influence of Growth Conditions on the Presence of Bacteriophage-Receptor Sites in Walls of *Bacillus subtilis* W 23

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Recent studies have shown that the composition of walls of several Gram-positive bacteria is influenced by the conditions under which the organisms are grown. Thus the composition and degree of cross-linking in the peptidoglycan components of walls of *Staphylococcus aureus* and *Bacillus subtilis* vary according to the composition of the medium (Schleifer, 1969; Rogers, McConnell & Hughes, 1971). Dramatic alterations in wall composition have been observed also during growth of various Gram-positive bacteria under conditions of phosphate limitation, when teichoic acid is no longer synthesized and the walls contain instead the acidic polysaccharide teichuronic acid (Tempest, Dicks & Ellwood, 1968; Ellwood & Tempest, 1969; Ellwood, 1971).

The bacteriophage-receptor sites in the walls of certain Gram-positive bacteria appear to be organized structures involving both the teichoic acid and peptidoglycan components of the wall (Glaser, Ionesco & Schaeffer, 1966; Wolin, Archibald & Baddiley, 1966; Young, 1967; Murayama, Kotani & Kato, 1968; Coyette & Ghuysen, 1968; Chatterjee, 1969). The ability of walls to bind bacteriophage would therefore be expected to depend on the conditions under which the cells were grown, since changes in the nature of the anionic polymers present or in the composition and degree of cross-linking of the peptidoglycan could affect the structure of the receptor sites, so that determination of the ability of cells to bind or to be infected by bacteriophage could provide information on the structural organization of the wall that would be of value in studies of the factors controlling the synthesis of organized wall material. We have therefore examined the binding of bacteriophage SP50 to walls and cells of *B. subtilis* W 23 grown under conditions of excess of phosphate and limiting phosphate to determine whether such cells differ in their capacity to bind the bacteriophage.

B. subtilis W 23 was grown at 37°C in a 600 ml chemostat at pH 7.2 in the K⁺-limited medium described by Ellwood & Tempest (1969). Cells were also grown in the phosphate-limited medium

described by the same authors; after collection of an adequate number of phosphate-limited cells the medium in the reservoir bottle was changed to that containing excess of phosphate and limiting K⁺. Chemostat cultures were grown at a dilution rate of 0.2 h⁻¹ and the cultures were allowed to equilibrate for 48 h. Small samples (approx. 15 ml) of culture were drawn directly from the culture vessel; larger samples were collected continuously into a chilled receiver. Cells were also grown in batch culture for 8 h at 37°C in a medium containing nutrient broth (Oxoid) (12.5 g), yeast extract (Difco) (2.5 g), dipotassium hydrogen orthophosphate (2.5 g), glucose (A.R.) (10 g) and demineralized water (1 litre).

Cells were harvested by centrifugation at 17000g for 30 min, washed with cold water and freeze-dried. Walls were prepared as described by Davison (1968) and further purified, after thermal inactivation of the autolytic enzymes, by incubation with trypsin. *B. subtilis* bacteriophage SP50 was obtained from Dr T. A. Trautner and propagated on *B. subtilis* W 23 in a manner similar to that described by Foldes & Trautner (1964), giving stock suspensions containing approx. 10¹⁰ plaque-forming units/ml. Bacteriophage assays were performed by using the soft-agar-overlay method (Adams, 1959) by dilution of the stock bacteriophage suspension in a solution containing disodium hydrogen orthophosphate (7 g), potassium dihydrogen orthophosphate (3 g), sodium chloride (5 g), magnesium sulphate (1.2 g), calcium chloride (12 mg) and water (1 litre).

Bacteriophage-binding studies were carried out with whole cells and isolated walls. Cells were suspended at a concentration of about 60 µg/ml in 2.5% nutrient broth (10 ml) containing 10 mM-magnesium sulphate. Bacteriophage suspension (0.3 ml containing 2.3 × 10⁴ plaque-forming units) was added and the mixture incubated at 37°C. Samples (2 ml) were removed after 1 h and filtered through a Millipore filter (pore size 0.45 µm), and portions (0.1 ml) of the filtrates were plated in triplicate on lawns of *B. subtilis* 168 as indicator. The inactivation of bacteriophage by cell walls was

Table 1. *Composition of walls of B. subtilis W 23 from cells grown under different conditions*

Walls were hydrolysed in 6M-HCl for 16 h at 100°C and analysed for amino compounds as described in the text. The results for muramic acid and glucosamine are low because they have not been corrected for destruction during hydrolysis.

Component	Growth conditions	...	Composition (mol.prop. relative to glutamic acid)		
			Batch	K ⁺ -limited	Phosphate-limited
Muramic acid			0.61	0.52	0.48
Glutamic acid			(1.00)	(1.00)	(1.00)
Alanine			1.45	1.47	1.26
Diaminopimelic acid			0.75	0.76	0.56
Glucosamine			0.77	0.74	0.60
Galactosamine			0.05	0.16	1.90
Phosphorus			2.84	3.90	0.42

assayed as above with wall suspensions at a concentration of approx. 10 µg/ml. This assay gave reproducible results, and no inactivation of bacteriophage was observed in the absence of cells or walls. Under these conditions batch-grown cells and walls inactivated 71% and 87% of the added bacteriophage respectively. The K⁺-limited cells and walls inactivated 78% and 81% of the bacteriophage, whereas the phosphate-limited cells and walls did not cause any detectable inactivation.

Walls (3mg) were heated at 100°C in 1M-hydrochloric acid (2ml) for 4h and examined chromatographically by using methods described previously (Archibald & Coapes, 1971). Walls from cells grown in batch culture and under conditions of K⁺ limitation contained 3.7% and 4.6% phosphorus respectively (determined by the method of Chen, Toribara & Warner, 1956), and on acid hydrolysis gave anhydrosorbitol, glucose, P_i and ribitol phosphates. Walls from phosphate-limited cells contained only 0.57% phosphorus, and acid hydrolysis gave no anhydrosorbitol or any of the other products characteristic of teichoic acid; these walls did, however, contain large amounts of galactosamine and uronic acid. These results are consistent with the observations made by Ellwood & Tempest (1969) and show that teichoic acid is not incorporated into the wall when cells of *B. subtilis* W 23 are grown under conditions of phosphate limitation.

Further samples of wall (approx. 4mg) were heated at 100°C in 6M-hydrochloric acid (4ml) *in vacuo* for 16h and then analysed for amino compounds in a Jeol autoanalyser. The results (Table 1) show that, although there were some differences in the composition of the peptidoglycan, the major apparent difference between the walls that inactivated bacteriophage SP50 and those that did not was the replacement, in the latter, of teichoic acid by teichuronic acid. It is likely that the inability of phosphate-limited cells to bind bacteriophage SP50 is due to the absence of teichoic acid from

their walls. This conclusion is supported by the observation (A. R. Archibald, H. E. Coapes & D. J. Barnfield, unpublished work) that treatment of walls of the batch-grown cells with dilute sodium periodate destroys the bacteriophage-receptor sites; under these conditions the teichoic acid was removed from the wall, leaving intact peptidoglycan. On change-over from phosphate to K⁺ limitation teichoic acid synthesis resumes, and walls obtained from cells harvested between 24h and 48h after change-over had closely similar compositions and bacteriophage-binding capacities to those of cells grown directly under K⁺ limitation.

The above results show that the ability of cells of *B. subtilis* W 23 to bind bacteriophage SP50 is critically determined by the growth conditions. Phosphate-limited cells do not bind the bacteriophage, but on change-over from phosphate to K⁺ limitation synthesis of receptor sites is resumed. Measurement of the bacteriophage-binding capacity of cells undergoing transition from phosphate to K⁺ limitation (or to growth under other suitable conditions) should provide a sensitive procedure for the detection of the synthesis of receptor sites. The location of the newly synthesized receptor sites in the wall should be readily demonstrable by electron-microscopic examination of the bacteriophage-wall system. Since, as indicated above, receptor sites are organized structures, such procedures should be of considerable value in studies on the factors regulating the synthesis of organized wall material.

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- Adams, M. H. (1959). *Bacteriophages*, pp. 450–451. New York: Interscience Publishers Inc.
 Archibald, A. R. & Coapes, H. E. (1971). *Biochem. J.* **124**, 449.
 Chatterjee, A. N. (1969). *J. Bact.* **98**, 519.
 Chen, P. S., Toribara, J. Y. & Warner, H. (1956). *Analyt. Chem.* **28**, 1756.

- Coyette, J. & Ghuysen, J. M. (1968). *Biochemistry, Easton*, **7**, 2385.
- Davison, A. L. (1968). *Biochem. J.* **110**, 557.
- Ellwood, D. C. (1971). *Biochem. J.* **121**, 349.
- Ellwood, D. C. & Tempest, D. W. (1969). *Biochem. J.* **111**, 1.
- Foldes, J. & Trautner, T. A. (1964). *Z. VererbLehre*, **95**, 57.
- Glaser, L., Ionesco, H. & Schaeffer, P. (1966). *Biochim. biophys. Acta*, **124**, 415.
- Murayama, Y., Kotani, S. & Kato, K. (1968). *Biken's J.* **11**, 269.
- Rogers, H. J., McConnell, M. & Hughes, R. C. (1971). *J. gen. Microbiol.* **66**, 297.
- Schleifer, K. H. (1969). *J. gen. Microbiol.* **57**, xiv.
- Tempest, D. W., Dicks, J. W. & Ellwood, D. C. (1968). *Biochem. J.* **106**, 237.
- Wolin, M. J., Archibald, A. R. & Baddiley, J. (1966). *Nature, Lond.*, **209**, 484.
- Young, F. E. (1967). *Proc. natn. Acad. Sci. U.S.A.* **53**, 2377.