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ISOLATION AND CHARACTERIZATION OF SEVERAL SOIL PHAGES THAT LYSE *BACILLUS BREVIS*

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PHAGES of *Bacillus brevis* have been isolated and investigated for a variety of reasons, including the problems they cause in the food industry and their use as taxonomic tools in genetic studies[1–3]. Several workers have demonstrated the use of these phages in recombinant DNA technology[1–5]. Mainly two factors are vital for a phage to become a useful vector. First, the phage should have a wide host range, and second, it must also be possible to transfect the phage DNA or its recombinant molecules. In the present investigation several soil samples were screened for phages that could lyse *B. brevis*. I have described the molecular characterization of some of these phages.

For the isolation of phages soil samples (about 50 g) were incubated for 4–6 days in phage medium[6] containing the phage-sensitive *B. brevis* strain (10^8 cfu/ml) at 30°C for 24 h under shaking conditions. After the incubation the culture filtrate was plated[6] to check for the presence of phage. In all, five phages, viz. BS12, BS25, BS65, BS138 and BS158, were isolated. Purification of the phages was done as described and lysozyme activity was measured according to Verma and Siddiqui[7]. Temperate phages could be distinguished from virulent phages by the appearance of plaques on the lysogenic strain. These lysogenic bacteria were immune to superinfection by the same phage. Moreover, in a culture of a lysogenic strain, free phage particles would be detected, since lysogenic strains would spontaneously go into lytic cycle at a low level. Exposure to ultraviolet light increased the lytic response. Serological properties, host range, melting curves of phage DNAs and restriction analysis were done by following methods described elsewhere[8–10]. For transfection small amounts (5 to 10 μg) of phage DNA were mixed with 10^8 cells of *B. brevis* and 0.5 ml of 25% (w/v) polyethylene glycol in 10 mM calcium chloride at pH 8 was added. After 10 min of incubation at 30°C the contents were centrifuged briefly and plated on a lawn of the sensitive strain of *B. brevis*.

Ten soil samples were tested and five phages, viz. BS12, BS25, BS65, BS138 and BS158, were isolated. No real differences were observed in isolation efficiency between shaking cultures or standing cultures at 30°C. High phage titres (in the range of 10^{10} pfu/ml) were obtained from confluent plate lysates.

All the phage heads were of an icosahedral form, although phage head size and tail length varied considerably (table 1). Short tail fibrils (6 to 10) were attached to the base plate of each phage. Continuous cesium chloride gradients showed that the bouyant density of these phages varied between 1.5 and

Table 1 *Morphological characterization of B. brevis phages*

	Phage dimensions (nm)						
	Head		Tail		Plaque morphology		
Phage	Length	Width	Length	Width	Size (nm)	Transparency	Edge
BS12	140.5	130.0	350.5	15.0	1.5	Turbid	Diffuse
BS25	155.0	146.5	405.0	15.0	1.6	Clear	Sharp
BS65	132.5	130.5	350.5	14.5	1.4	Clear	Sharp
BS138	154.0	140.0	300.0	13.5	1.5	Clear	Sharp
BS158	140 0	150 0	410 0	14 0	1.5	Turbid	Diffuse

Tobacco mosaic virus was mixed with phage samples to serve as internal standard for phage dimensions.

1.7 g cm^{-3}. Five strains of *B. brevis*, 10 strains of *B. subtilis* and three strains of *Escherichia coli* were tested for infection susceptibility to the five phages investigated in this work. Plaques were detected in all strains of *B. brevis*; the other bacteria were unaffected by these phages. Phage BS138 could grow on *Salmonella typhimurium* as well. Serologically all the phages were alike (neutralized by antisera of phage isolated in the present investigation), and they could not be neutralized by antisera to *B. subtilis*, *S. typhimurium* or *E. coli* phages.

Thermal denaturation experiments provided evidence for double-helical secondary structure of the phage DNAs. The melting profile of all phage DNAs displayed a narrow helix–coil transition typical of native double-stranded DNA. The melting temperature for BS12, BS65 and BS158 was 64°C (G+C = 29.3 mol%), and for BS25 and BS138 it was 77°C (G+C = 56.5 mol%). The low melting temperature for BS12, BS65 and BS158 suggests the presence of unusual bases in the genome of these phages[11].

Lysozyme is a significant enzyme produced by infected cells in the later stages of infection. The levels of lysozyme production were measured (table 2) and it was observed that the highest amount of lysozyme was produced by BS138-infected cells. This is expected because BS138 is a virulent phage.

Transfection could be observed by plaque formation in the regeneration plates after 24–30 h of regeneration or by soaking regeneration plates with 4 ml phage buffer and testing the latter suspension for the presence of phage DNA[12]. For transfection experiments 5 μg DNA was used and the number of infective centres was determined by counting the number of plaques on the transfectant plate. In order to investigate whether the phage DNA was integrated in the chromosome or excised as an extrachromosomal element, I attempted to isolate

Table 2 *Lysozyme activity in B. brevis infected with different phages*

Time after infection (min)	Lysozyme activity (units/ml) in cells infected with				
	BS12	BS25	BS65	BS138	BS158
0	0	0	0	0	0
5	0	0	0	0.5	0
10	0	0.3	0.4	2.0	0
15	0.1	0.5	0.6	3.5	0.4
20	0.2	0.8	0 8	5.0	0 8
25	0.8	3.0	2.0	12.0	2.0
30	2.0	5.0	5.0	16.0	5.0
40	5.0	8.0	7.2	18.0	5.0
50	8.7	8.0	7.5	18.5	5.0
60	8.8	8.0	7.5	18.5	5.5
70	8 8	8.0	7.5	18.5	5.5
80	8.8	8 0	7.5	18.5	5.5

The procedure for the assay of the enzyme has been described earlier[13].

the extrachromosomal element, if any, but the results were negative. This suggests that these phages probably integrate in the chromosome (the experiment was performed by cesium chloride gradient centrifugation as suggested by Joshi *et al.*[10])

Of the five phages isolated, four (BS12, BS25, BS65, BS158) were temperate and BS138 was a virulent phage. The temperate phages could switch to lytic cycle at a rate of about 2% of the number of viable cells. The transition from temperate to lytic can be enhanced by exposure of the lysogenic strain to ultraviolet radiation (240–280 nm), although long exposure decreases the number of viable cells (table 3).

The molecular weights of the phage DNAs, as determined by cesium sulphate gradient analysis, were as follows: BS12, 14.8 kb; BS25, 18.8 kb; BS65, 15.3 kb; BS138, 18.3 kb; BS158, 16.5 kb. The restriction maps of the phage DNAs are shown in figure 1. ^{32}P-

Table 3 *Effect of ultraviolet exposure on the viability of phages*

Time of exposure (min)	Per cent survivors remaining after exposure				
	BS12	BS25	BS65	BS138	BS158
No exposure	100	100	100	100	100
5	98	95	98	97	98
10	95	93	96	92	93
15	81	83	83	84	82
20	72	74	72	71	72
30	51	49	51	48	47
40	31	32	30	30	31
50	13	15	13	12	11
60	0.1	0.4	1	0.5	0.1

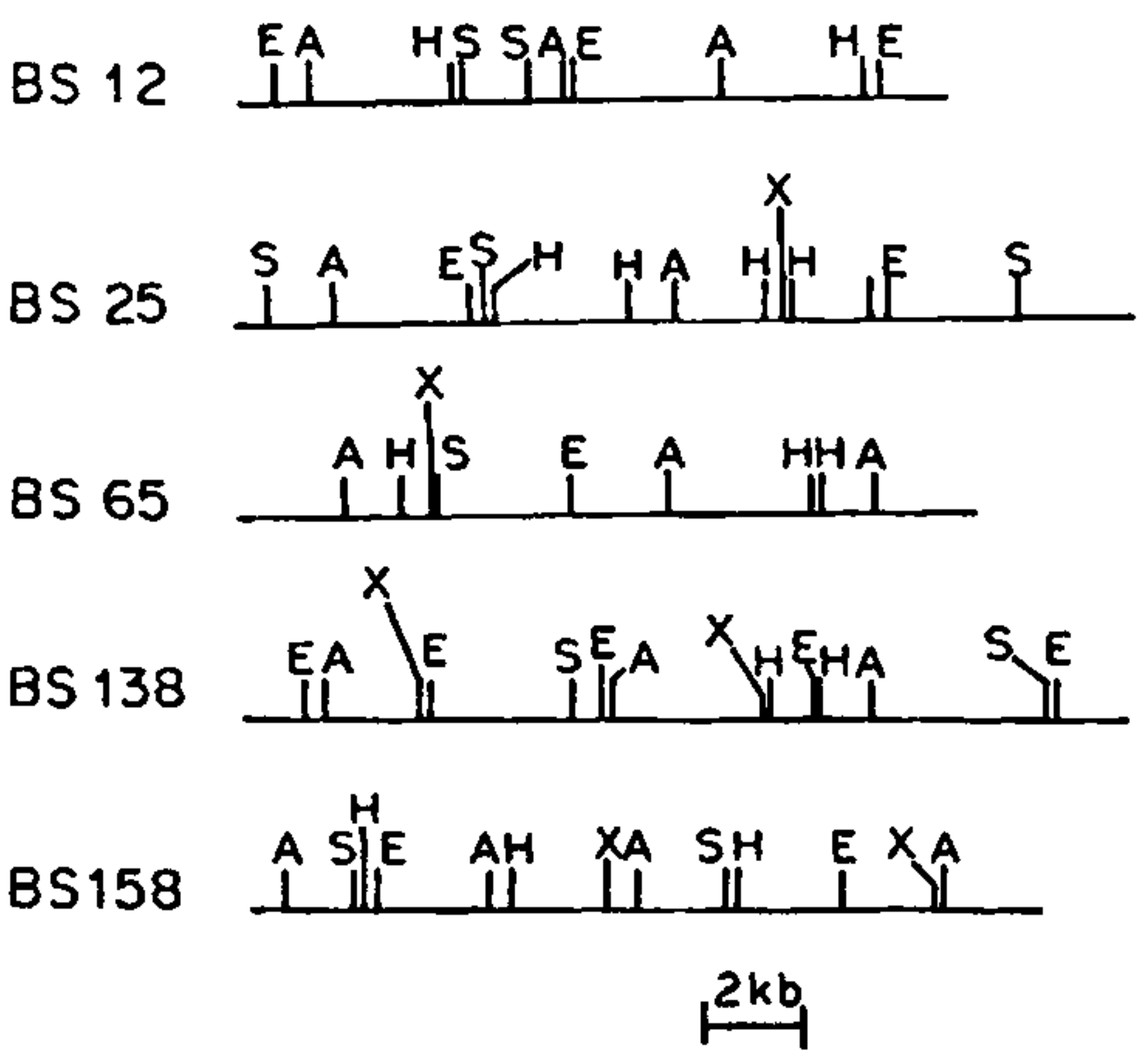

Figure 1. Restriction maps of *B. brevis* phages BS12, BS25, BS65, BS138 and BS158. E, *Eco*RI; A, *Ava*I; H, *Hind*III; S, *Sal*I; X, *Xho*I.

Labelling of phage DNAs using the Klenow fragment of DNA polymerase resulted in the labelling of only two *Eco*RII fragments (BS12 and BS138), two *Sal*I fragments (BS25), and two *Ava*I fragments (BS158 and BS65), all of which lie at the ends of the respective restriction maps (figure 1) indicating that the DNAs of these phages have 5′ protruding single-stranded ends. Further experimentation suggested that the single-stranded protrusions are complementary in base sequence but not palindromic.

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PLASMID-ENCODED PRODUCTION OF HYDROCARBON-SOLUBILIZING FACTOR BY *PSEUDOMONAS* PG–I

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ISOLATION of a polymeric hydrocarbon-solubilizing substance produced by *Pseudomonas* PG-I during growth on liquid and solid hydrocarbons has been reported previously[1,2]. Production of extracellular alkane-solubilizing factor by the yeast *Endomycopsis lipolytica* YM has also been reported[3]. It was shown that hydrocarbon solubilization by solubilizing factors could fully account for substrate uptake and growth of the organisms on hydrocarbons.

Many reports have appeared on the specific action