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Bacteriophage SfII Mediated Serotype Conversion in *Shigella flexneri*

by

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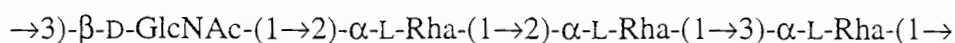


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Abstract

Shigella flexneri is the causative agent of bacillary dysentery and is responsible for approximately 10^5 deaths per year. Organisms that belong to this species can be classified according to the O-antigenic component of their lipopolysaccharide (LPS). Determination of the serotype is based on agglutination of the organism with polyclonal antisera. *S. flexneri* exhibits at least 12 different serotypes, all of which are variations of the basic tetrasaccharide repeat:



I

II

III

Variations resulting in different epitopes are due to the addition of either glucosyl or O-acetyl residues at specific positions along the repeat unit. Immunity to *S. flexneri* strains is serotype specific and therefore protection against each serotype is required.

The modifications have been found to be mediated by temperate bacteriophages. To date, two such phages have been identified and characterised; Sf6, responsible for the O-acetylation of rhamnose III and SfX which encodes genes which add a glucosyl residue to rhamnose I.

S. flexneri strains of serotype 2 have been associated with a highly virulent phenotype and are more prevalent in nature. Bacteriophage SfII is responsible for the glucosylation of rhamnose III of the repeat unit and has been isolated from a strain of serotype 2b. Phage SfII possesses an hexagonal shaped head, a tail with a contractile sheath and tail fibres and has a genome of 43.5 kb in size.

A 4 kb *Bam*HI fragment was isolated which was found to mediate serotype conversion. This fragment was found to contain 4 open reading frames, however, only two were required for serotype conversion. These genes were named *bgt*, which encodes a putative bactoprenol glucosyl transferase, and *gtrII* encoding the putative type II antigen determining glucosyl

transferase. These genes are adjacent to the integrase and excisionase genes and the attachment site (*attP*) which are highly homologous to those of *Salmonella* bacteriophage P22. In addition, a gene named ORF2 was identified of unknown function, which did not appear to be required for serotype conversion.

In vitro T7 polymerase /promoter analysis identified a protein of 34 kDa in size corresponding to Bgt. Subsequent subcellular fractionation localised this protein to the cytoplasmic and membrane fractions. Topology studies determined that the carboxy and amino terminal ends were cytoplasmically located. The active site was located to the cytoplasmic domain which is consistent with the role of Bgt. A protein was not observed for GtrII.

The ability of SfII phage and plasmid copies of serotype converting genes to modify LPS with only a single repeat unit was assessed. O-antigen polymerase mutants (*rfc*) of serotype X and Y, were transformed with various serotype converting plasmids and characterised by colony immunoblotting for reactivity to a variety of type and group antisera. In *S. flexneri*, it appears that a single O-antigen repeat unit is sufficient to be modified.

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