The Journal of Medical Microbiology

Vol. 11 No. 1

LYSOGENY ASSOCIATED WITH MUCOID VARIATION IN *MYCOBACTERIUM KANSASII*

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PLATES I AND II

STUDIES on the experimental establishment of lysogeny in mycobacteria have shown that lysogenic variants are frequently of smoother colonial morphology than their non-lysogenic parent strains (Russell, Jann and Froman, 1960; Bönicke and Saito, 1970). Similarly, naturally-occurring lysogenic mycobacteria, although infrequently reported in the literature, may also be smoother than closely-related non-lysogenic strains (Buraczewska, Manowska and Rdultowska, 1971; Grange and Bird, 1975). Colonial variation due to simple mutation is common in the mycobacteria (Grange, 1975*a*); thus colonial modification due to lysogeny must be considered in relation to the normal variation within a species.

Mycobacterium kansasii may produce a number of different colonial forms (Kubica and Jones, 1965) and on Löwenstein-Jensen medium appears as either a smooth or rough growth. In a study of 200 isolates of *M. kansasii*, most of the smooth forms were of a butter-like consistency, but 10 strains were found to be of a thick, tenacious, mucoid consistency. This paper reports the association of this mucoid form with lysogeny.

MATERIALS AND METHODS

The bacteria. Two hundred strains of *M. kansasii* were obtained, 189 from clinical specimens and 11 from a study of hospital water supplies (McSwiggan and Collins, 1974). All strains were maintained on Löwenstein-Jensen medium.

Cultural tests. All strains were examined for their ability to grow on Löwenstein-Jensen and Sauton medium. Strains cultured on Löwenstein-Jensen medium were examined for pigment production in the dark and after exposure to a 100-watt lamp at a distance of 1m for

Received 9 July 1976; accepted 27 Sept. 1976.

4 h. The macroscopical appearance and texture of the strains were observed after 4 weeks' incubation on Löwenstein-Jensen medium.

Serological and biochemical tests. The identity of all strains was confirmed by immunodiffusion serology (Stanford and Grange, 1974). Selected strains were tested for nitratase by a modification (Bönicke, 1962) of the method of Virtanen (1960), for amidase (Bönicke, 1962), arylsulphatase (Whitehead, Wildy and Engbaek, 1953), deoxyribonuclease (Mankiewicz and Tamari, 1972) and Tween-80 hydrolysis (Wayne, Doubek and Russell, 1964).

Electronmicroscopy. The strains selected for electronmicroscopy were cultured at 35°C for 10 days in 25-ml amounts of Nutrient Broth No. 2 (Oxoid) containing 3% (v/v) glycerol.

The cultures were centrifuged at 1500 *g* for 15 min. to remove most of the bacteria. The supernates were then spun for 1 h at 25 000 *g* in a MSE Superspeed 50 ultracentrifuge. After the supernant fluids had been poured off, the pellets were kept at 4°C. Within 1 h of centrifugation they were resuspended in 0.05 ml of 2% (w/v) ammonium molybdate solution adjusted to pH 6.8 with 0.1N ammonia solution. A single drop of this suspension was pipetted on to Formvar carbon-coated grids (Smethurst New 200), and after 30 s excess negative stain was removed with filter-paper. The preparations were then allowed to dry before being examined in a GEC/AEI EM 801 electron microscope.

Detection of sensitive indicator strains. Sensitive indicator strains for the phages were sought by spotting culture filtrates on to 72 strains of *M. kansasii* and one strain (ATCC607) of *M. smegmatis*, incorporated in soft agar overlays (Grange and Bird, 1975). The filtrates were also spotted on to their homologous strains as a number of lysogenic mycobacteria showing susceptibility to their own phages have been described (Grange, 1975*a* and *b*).

RESULTS

Cultural characteristics

All strains grew within 3 weeks on Löwenstein-Jensen medium and 197 strains grew on Sauton medium; 174 of the strains were smooth but not mucoid, 16 were rough and 10 were mucoid. The mucoid strains were very tenacious and not easily harvested from the medium and their mucoid nature became more pronounced on prolonged incubation. Eight of the 10 mucoid strains were photochromogenic, one (no. K109) was scotochromogenic and one (no. K20) was non-chromogenic.

Biochemical properties

The amidase, arylsulphatase, Tween-80 hydrolase and nitratase activities of the 10 mucoid strains are shown in the table, together with the usual biochemical properties of the species. A strain that did not hydrolyse Tween 80 failed to grow on Sauton medium and two strains with weak or negative amidase activities showed a lack of nitratase activity. None of the mucoid strains produced detectable amounts of deoxyribonuclease.

Electronmicroscopy

The 10 mucoid strains all liberated large numbers of whole bacteriophages, whereas no phages or phage sub-units were observed in 30 randomly-selected non-mucoid strains. One lysogenic strain (K109) liberated a phage with a non-contractile tail 0.2 μm long and a cylindrical head 0.1 μm long and 0.05 μm in diameter (fig. 1). All other lysogenic strains liberated phages with heads hexagonal in outline and 0.7 μm in diameter (fig. 2). The tails of these phages

TABLE
Biochemical and cultural properties of the 10 lysogenic strains of M. kansasii

Strain number	Results of tests for															Mucoid growth	Growth† in Sauton medium	Pigment production
	the amidases*			arylsulphatase in culture aged (days)		hydrolysis of Tween 80 in cultures aged (days)			nitratase* at (h)									
	urease	nicotinamidase		3	10	3	10	3	10	0.5	1	2	24					
K18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P	
K20	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	
K25	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P	
K27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P	
K51	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P	
K55	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P	
K109	—	—	—	+	+	+	+	+	+	—	—	—	—	+	+	+	S	
K116	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P	
K119	—	—	+	+	+	+	+	+	+	—	—	—	—	+	+	+	P	
K135	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P	
Non-lysogenic strains	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	—	P	

* = Reactions graded + to +++ according to colour intensity.
† = Positive results indicate that growth occurred within 4 weeks.
W = Weak reaction; P = photochromogenic; N = non-chromogenic; S = scotochromogenic.

were long and non-contractile, with a small swelling at the distal end and a narrowing at the point of connection with the head (fig. 3). Although most of these phages had tails 0.2 μm long, a small number of phages in each culture filtrate had tails ranging in length from 0.4 to 0.8 μm . Short-tailed phages were also occasionally seen. The variation of phage-tail length is shown in fig. 4.

Detection of sensitive indicator strains

No susceptibility to lysis by any of the 10 phages was detectable on 72 strains of *M. kansasii* or on *M. smegmatis*, strain ATCC607. The lysogenic strains were not susceptible to their own phages.

Origin of the lysogenic strains

The nine strains liberating phages with heads of hexagonal outline were isolated from sputum and the strain liberating the phage with a cylindrical head (K109) was isolated from a water tap in a hospital.

DISCUSSION

Lysogeny has been reported previously in only two species of slow-growing mycobacteria, *M. marinum* (Bönicke, 1969) and *M. scrofulaceum* (Mankiewicz and Tamari, 1972). Although phages able to lyse strains of *M. kansasii* have been isolated from the environment (Engel, 1975), naturally-occurring lysogeny in this species has not been described previously.

Apart from the use of the electron microscope, there is no simple way of detecting lysogeny in mycobacteria. It is not always possible to find sensitive indicator strains, as phages liberated from lysogenic mycobacteria usually have much narrower host ranges than those isolated from the environment (Grange, 1975*b*). In the present study, none of the phages showed any lytic activity on a large number of strains. Some lysogenic mycobacteria produce deoxyribonuclease (Mankiewicz and Tamari, 1972) or show other biochemical anomalies (Grange and Bird, 1975), but in this study no anomaly, other than mucoid growth, was constantly associated with lysogeny. The strains in this study have been included in unpublished phage-typing studies in which the 10 mucoid strains were indistinguishable as a group from other strains of *M. kansasii* on the basis of susceptibility to lysis by the typing phages (H. W. B. Engel, personal communication).

Colonial variation due to lysogeny is not limited to the genus *Mycobacterium*. Other examples are cited by Martin (1973), who described phage-induced mucoid variation in *Pseudomonas aeruginosa*. Although in this study there was an exact correlation between lysogeny and mucoid variation in the strains examined by electronmicroscopy, the possibility remains that non-mucoid lysogenic variants of *M. kansasii* exist. The biochemical basis of the mucoid variation has not been fully investigated, but preliminary observations suggest that it may be due, at least in part, to extracellular DNA.

In their morphology, the phages described in this study resemble those observed in other species of mycobacteria. Thus, most mycobacteriophages

MUCOID LYSOGENIC VARIANT OF *M. KANSASII*

FIG. 1.—Bacteriophages liberated by strain K109. Electronmicrograph (EM). $\times 120\,000$.

FIG. 2.—Bacteriophages liberated by strain K135. EM. $\times 126\,000$.

MUCOID LYSOGENIC VARIANT OF *M. KANSASII*

FIG. 3.—Bacteriophages liberated by strain K119. The tails show a constriction at the proximal end (large arrows) and a swelling at the distal end (small arrows). EM. $\times 120\,000$.

FIG. 4.—Bacteriophages with varying tail lengths in a culture filtrate of strain K119. (N = normal length.) EM. $\times 80\,000$.

has to have heads of hexagonal outline and non-contractile tails; less frequently encountered are phages with cylindrical heads, such as those liberated by strain K109. Buraczewska *et al.* (1972) described four such phages isolated from unidentified rapidly-growing mycobacteria, but unlike the phage from strain K109 they all lysed *M. smegmatis*, strain ATCC607. The variation in tail lengths of the phages may have resulted from multiple infection of the bacterial strains, but was more likely due to errors in the assembly of the tail sub-units. Long-tailed phages have been observed in small numbers in preparations of phage *Lambda*, but they had empty heads (Eiserling and Boy de la Tour, 1965).

This study adds to the evidence that, contrary to earlier opinion, lysogeny in the genus *Mycobacterium* is not a rare occurrence and may contribute significantly to variation within species. The limited clinical data available in respect of the strains did not suggest that the lysogenic variants were either more or less pathogenic than other strains of the same species. It is, however, often difficult to determine whether a strain of *M. kansasii* isolated from sputum is a primary cause of disease, a secondary invader or merely a contaminant. Nevertheless, as both mycobacteria and phages are common in the environment, the emergence of a highly-pathogenic host-phage combination at some time in the future is a definite possibility.

SUMMARY

Ten of 200 strains of *Mycobacterium kansasii* were found to produce very mucoid growth on Löwenstein-Jensen medium. By electronmicroscopy these 10 strains were found to be lysogenic, whereas no phage was observed in cultures of 30 non-mucoid strains. The cultural and biochemical properties of the lysogenic strains are compared with those of non-lysogenic strains, and the morphology of the phages is described.

We thank Drs A. Beck, H. Engel, E. Nassau and J. Stanford and Mr C. H. Collins for supplying the bacteria. Dr J. L. Stanford performed the immunodiffusion serology and allowed some of the studies to be made in his department. We also thank Mrs Linda Fathalla, Miss Roberta Hanley and Miss Pam Nye for technical assistance.

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