Phage-induced Alteration of Enzymic Activity in Lysogenic
*Mycobacterium smegmatis* Strains

By S. E. JUHASZ, S. GELBART AND MONIQUE HARIZE

Department of Microbiology, Loyola University of Chicago, Stritch School of Medicine and Research Service, Veterans Administration Hospital, Hines, Illinois 60141, U.S.A.

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SUMMARY

Changes in the amidase, nitrate reductase and phenolphthalein sulphatase activity of *Mycobacterium smegmatis* were observed following B1-lysogeny. These changes were more consistent in lysogenic derivatives of strains SN2 and SN14 than in those of strain SN10. Strains SN2 and SN14 appeared to differ from SN10 only in that the former possessed a functional salicylamidase locus while the latter did not. Emergence of various phenotypes in the same strain and during the same lysogenic event were observed. Multiple sites of phage integration may have accounted for the variation of SN10 (B1) phenotypes.

INTRODUCTION

Soon after its discovery d'Herelle suggested that the bacteriophage was not merely a lethal agent but also an important factor of bacterial variability (d'Herelle, 1930). In 1951 Freeman found that toxigenicity in *Corynebacterium diphtheriae* was determined by phage β carried by toxic diphtheria strains (Freeman, 1951). Changes in colonial morphology (Ionesco, 1953) and antigenic composition (Uetake, Nagakawa & Akiba, 1955) due to the interaction of the host and phage genome have been demonstrated subsequently.

Lysogenic conversion resulting in altered enzymic activity has been reported previously in *Mycobacterium smegmatis* (Bönicke & Juhasz, 1965; Bönicke, 1967). In addition to altered α-nicotinic acid oxidase and malachite green reductase activity of *M. smegmatis* SN2, it has been observed recently that phage infection affected the ability of *M. smegmatis* ATCC607 to reduce nitrates to nitrites and to hydrolyse Tween 80 (Jones & White, 1968). The present paper describes changes in the amidase, nitrate reductase and phenolphthalein sulphatase activities of *M. smegmatis* strains following lysogenization by phage smegmatis B1.

METHODS

Bacterial strains. *Mycobacterium smegmatis* strains SN2, SN10 and SN14 of the Borstel Collection served as hosts in this study. The original designation of strain SN10 was ATCC607.

Phage smegmatis B1 (Juhasz & Bönicke, 1965) was used to infect and lysogenize *Mycobacterium smegmatis* strains.

Media. Bacterial cultures were maintained on Loewenstein-Jensen slopes, phage strains in heart infusion broth (Difco). Lysogenization experiments were performed on
nutrient agar plates (Difco); the agar overlay was prepared from nutrient broth (Difco) by the addition of 0.7% Bacto agar. For the enzymic experiments all the strains were grown on Loewenstein-Jensen plates.

Procedures for the recovery and purification of lysogens through single colony transfers were the same as described for B2-lysogenic Mycobacterium phlei (Juhasz & Bonicke, 1966).

**Amidase test.** Bönicke's amide series (Bönicke, 1960; Juhlin, 1967) was employed to detect amidase activity. Bacteria grown on Loewenstein-Jensen plates for 12 to 15 days were harvested and suspended in physiological NaCl. They were washed by centrifugation for 20 min at 3000 rev./min. and resuspended in pH 7.2 phosphate buffer to give equiv. 10 mg. dry weight/ml. One ml. samples were mixed with 1 ml. each of a 0.00164 M-amide solution. The ten amides used in these experiments were: acetamide, urea, isonicotinamide (Sigma Chemical Company), pyrazinamide, allantoin (courtesy of Dr R. Bönicke), benzamide, nicotinamide, salicylamide, succinamide, malonamide (J. T. Baker Chemical Company). Each amide + bacteria mixture was incubated for 22 hr at 37°C. Liberation of ammonia resulting from the hydrolysis of the given amide was detected by the phenol-hypochlorite method (Russell, 1944, Bönicke, 1960). Eau de Javelle obtained from Dr R. Bönicke was used as a source of hypochlorite. The amount of liberated ammonia was determined by comparison of the experimental tubes with standards containing measured amounts of ammonia. The two controls used were bacterial suspensions without amides and amide solutions without bacteria.

The nitrate reductase test of Virtanen (Virtanen, 1960) was used to measure reduction of nitrate to nitrite. Suspensions containing equiv. 0.4 mg. dry weight bacteria were inoculated into nitrate broth (Difco) and incubated at 37°C for 2 hr. The colour reaction obtained by Shinn's naphthylethylenediamine + sulphanilamide method (Shinn, 1941) was compared with that of standards containing known concentrations of NaNO₃. The latter was obtained from Merck, Sharpe and Dohme, Inc. N-1-naphthylethylenediamine from Mann Research Laboratories, sulphanilamide from Eastman Organic Chemicals.

Phenolphthalein sulphatase activity was determined as suggested by Wayne (Wayne 1961). Dubos oleic acid agar (Difco) containing 650 mg. phenolphthalein disulphate tripotassium salt (Eastman Organic Chemicals) per litre was inoculated with a suspension containing equiv. 0.4 mg. dry weight bacteria. Readings were made after 14 days of incubation at 37°C. The quantity of phenolphthalein produced was measured by comparison with standards containing known amounts of phenolphthalein (J. T. Baker Chemical Company).

**RESULTS**

The effect of phage B1 on various enzymic activities of Mycobacterium smegmatis strains SN2, SN10 and SN14 is shown in Table 1. While the four SN2 (B1) lysogens emerged in the same lysogenic event, SN10 (B1) and SN14 (B1), lysogens were obtained in two different lysogenic events (as indicated by arabic and roman numerals).

A definitive decrease in salicylamidase and nitrate reductase activity characteristic for SN2 and SN14 lysogens was accompanied by a decreased phenolphthalein sulphatase activity in strains SN2 (B1), nos 2, 3 and 4, and in SN14 (B1), nos. 1, 3 and — to a lesser degree—in SN14 (B1), no. 1. The decrease in these enzymic activities varied in degree. Nitrate reductase activity seemed to disappear completely in strains SN2 (B1), nos 2,
### Table 1 Amidase activity

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<tr>
<th>Strains</th>
<th>Acetamide</th>
<th>Benza-</th>
<th>Urea</th>
<th>Iso-</th>
<th>nicotinamide</th>
<th>Pyrazininamide</th>
<th>Salicylamide</th>
<th>Allan-</th>
<th>Succini-</th>
<th>Malona-</th>
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<th>Phenolphthalein sulphatase activity</th>
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All the cultures were grown on Loewenstein-Jensen plates for 12 to 15 days. Arabic and Roman numerals indicate different lysogenic events. Readings are expressed in pg. per ml. of the reaction product, i.e.: ammonia in the amidase test—0 to 2 pg. = 0, 2 to 3 pg. = (+), 3 to 5 pg. = +, 5 to 8 pg. = ++, 8 pg. = +++ (Juhlin, 1969); nitrate in the nitrate reduction test—0 to 0.1 pg. = 0, 0.1 to 1 pg. = (+), 1 to 2 pg. = +, 2 to 5 pg. = ++, 5 to 10 pg. = +++; phenolphthalein in the phenolphthalein sulphatase test—0 to 2 pg. = 0, 2 to 3 pg. = (+), 3 to 6 pg. = +, 6 to 16 pg. = ++, 16 to 40 pg. = ++++; nitrate reductase activity after 2 hr and phenolphthalein sulphatase activity after 14 days. All figures are based on at least two experiments.
3. 4 and in SN14 (B1), nos. 2 and I, but not in strains SN2 (B1) no. 1 and SN14 (B1), no. 3. On their part, strains SN2 (B1), no. 2 and SN14 (B1), nos. 1 and 3 revealed no detectable phenolphthalein sulphatase activity, a residual activity was found in strains SN2 (B1), nos. 3, 4, finally SN2 (B1), no. 1 and SN14 (B1), no. 2 showed a phenolphthalein sulphatase activity resembling that of the parental type. In addition to the enzymic changes already mentioned, SN2 (B1) lysogens displayed varying degrees of decrease in benzamidase and, with the exception of SN2 (B1), no. 2, in isonicotinamidase activity. Similar, but less significant changes, have also been observed in the amidase spectrum of SN14 (B1) lysogens.

In contrast to strains SN2 and SN14, B1-lysogeny did not appear to modify strain SN10 in a consistent manner. Although nitrate reductase activity decreased in strain SN10 (B1), no. I, it increased significantly in strain SN10 (B1), no. 1. Phenolphthalein sulphatase activity, undetectable in strain SN10 (B1), no. II and decreased in strain SN10 (B1), no. I, showed, on the other hand, definitive increase in strain SN10 (B1), no. I. The only obvious difference between strain SN10 and the former strains was the ability of parental type SN2 and SN14 to hydrolyse salicylamide.

**DISCUSSION**

The changes observed in B1-lysogenic strains of *Mycobacterium smegmatis* SN2 and SN14 resulted in a decrease of the salicylamidase, nitrate reductase and to somewhat lesser extent the phenolphthalein sulphatase activity of wild strains. No consistent changes were observed in SN10 (B1) lysogens. The different behaviour of *M. smegmatis* strains appeared to be related to the presence of a functional salicylamidase locus in wild SN2 and SN14 and to its absence in wild SN10. It would be premature to speculate whether mutation or repression of the salicylamidase locus at a possible site for phage integration or some other mechanism accounted for the differences.

Various degrees of change were observed, not only when different hosts were lysogenized, but among derivatives of the same strain emerging in the same lysogenic event. (Compare, for example, the nitrate reductase activity of SN2 (B1), no. I and SN2 (B1), no. 2 strains.) Since decrease of the salicylamidase activity appeared to be the primary and most consistent change, multiple sites for the integration of phage B1 was considered unlikely in SN2 and SN14 lysogens. It may have accounted, however, for the diversity of SN10 (B1) phenotypes, in which the salicylamidase locus was presumably not available for phage integration. Missing its preferential site the phage may have integrated at alternative sites. This would explain the apparent loss or decrease of phenolphthalein sulphatase activity compensated by an increased nitrate reductase activity in some strains and the converse in others.

Rapidly growing mycobacterial strains which cannot be identified and classified by the available methods are isolated from time to time in various laboratories. Such strains often show: (1) resistance to the known mycobacteriophages, and (2) enzymic activities which are different from that of the known rapidly growing mycobacterial species. Although attempts to isolate phages from them usually fail, the non-availability of susceptible indicator strains or the defectiveness of the prophage they carry may account for this failure. The reported in vitro experiments have shown that a considerably wide range of changes may follow lysogeny and alter potentially important taxonomic characters.
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REFERENCES


