Conversion of Salicylate to Catechol by *Mycobacterium fortuitum*

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SUMMARY

It was reported in a previous paper that most strains of *Mycobacterium fortuitum* blackened a modified Sauton medium agar containing 0.1% (w/v) sodium salicylate. The mechanism of this blackening has been investigated. When a modified Sauton medium containing 0.1% salicylate was inoculated with any of three strains of *M. fortuitum*, the medium became black after incubation for four days at 37°. The brownish black formazan was isolated by paper chromatography and compared with formazans from the same medium containing 0.1% (w/v) catechol inoculated with the test organisms or without inoculation. These formazans showed similar R_F values in three solvent systems and the same absorption spectra (maximum absorption at below 220 and 275 m μ). It has been shown that *M. fortuitum* is capable of converting salicylate to catechol and that the formazan in the medium is an oxidation product of catechol.

INTRODUCTION

The author (Tsukamura, 1965) had found that *Mycobacterium fortuitum* decomposed salicylate and blackened a modified Sauton medium agar containing salicylate and that this reaction was specific for *M. fortuitum*. The present work is concerned with the mechanism of the blackening by *M. fortuitum* of medium containing salicylate.

METHODS

Strains. *Mycobacterium fortuitum*, nos. 606, 607 and 302, were used. As described previously, these strains caused a marked blackening of a modified Sauton medium agar containing 0.1% (w/v) sodium salicylate within 7 days when the medium was inoculated with one loopful of the stock cultures of the test strains (Tsukamura, 1965).

Medium. A modified liquid Sauton medium was used, with the following composition: sodium glutamate, 4.0 g.; glycerol, 50 ml.; K_2HPO_4 , 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; citric acid, 2.0 g.; ferric ammonium citrate, 0.05 g.; distilled water, 950 ml. The medium was adjusted to pH 7.0 by addition of 10% (w/v) KOH, poured in 50 ml. quantities into 200 ml. Erlenmeyer flasks, and sterilized by autoclaving at 115° for 30 min. Sodium salicylate solution was sterilized by heating at 100° for 5 min. and added to the medium aseptically. Catechol was sterilized by Seitz filtration and added to medium aseptically. All reagents used were

extra pure grade (Katayama Chemical Co., Osaka, Japan). Sauton medium with 0.1 % (w/v) sodium salicylate and with 0.1 % (w/v) catechol were prepared.

Paper chromatography. Paper chromatography was done by upward flow. Toyo filter paper no. 50 was used. Three solvent systems were used: (1) *n*-butanol saturated with ammonia water (28 % NH_4OH); (2) *n*-butanol saturated with distilled water; (3) *n*-butanol + glacial acetic acid (4 + 1).

Absorption spectra were measured with a Hitachi spectrophotometer type EPU-2A (Hitachi Co., Tokyo, Japan).

RESULTS

The following series of media were set up: (1) uninoculated Sauton medium containing 0.1 % sodium salicylate; (2) Sauton medium containing 0.1 % sodium salicylate inoculated with 300–400 mg. (moist weight) of test organism; (3) uninoculated Sauton medium containing 0.1 % catechol; (4) Sauton medium containing 0.1 % catechol inoculated with 300–400 mg. (moist weight) of test organism. All media were incubated at 37°.

Table 1. R_F values in paper chromatograms of coloured formazans produced by *Mycobacterium fortuitum* nos. 606, 607, 302 when incubated with salicylate or catechol in Sauton medium

Solvent	Strain no.	R_F values		
		Salicylate + organisms	Catechol + organisms	Catechol
A*	606	0.09	0.10	0.13
	607	0.10	0.13	0.13
	302	0.10	0.12	0.13
B	606	0.83	0.84	0.83
	607	0.83	0.82	0.83
	302	0.83	0.85	0.83
C	606	0.85	0.87	0.86
	607	0.84	0.86	0.86
	302	0.89	0.86	0.86

* Solvent A: *n*-butanol saturated with ammonia water. Solvent B: *n*-butanol saturated with distilled water. Solvent C: *n*-butanol + glacial acetic acid (4 + 1), by vol.

All samples showed another black spot at point 0 (R_F 0.00).

The two media with catechol showed an immediate violet colouring before inoculation. When each component of the medium was mixed with catechol, at the same final concentration, it was found that ferric ammonium citrate alone of the ingredients produced this colour. Catechol medium became dark brown or black after incubation for 4 days at 37°, even uninoculated medium in the absence of the test organisms. After incubation for 4 days medium (2), the inoculated Sauton media with salicylate, showed similar blackening. Only medium (1) uninoculated Sauton medium with 0.1 % salicylate remained colourless.

Media (2) and (4) were centrifuged at 3000 rev./min. to remove the bacteria, and the supernatant fluids obtained from these with medium (3) were concentrated to $\frac{1}{2}$ to $\frac{1}{3}$ volume under reduced pressure. Evaporation was done for 2 days, during

which the media were maintained cold in the dark, the cold state being produced by the reduced pressure. The three concentrates (salicylate + organisms, catechol + organisms and catechol) were used for paper chromatography.

R_F values of the brownish black formazans in the concentrates are shown in Table 1. The formazans showed one spot in different solvent systems and their R_F values were almost identical. A brownish black spot remained at the original point. Elution of this spot with distilled water showed no marked absorption between 230 and 550 $m\mu$, but showed an absorption in a region of less than 230 $m\mu$; the nature of this spot is still unknown.

Fig. 1. Absorption spectra of the colour formazans produced by 'salicylate + organisms', 'catechol + organisms' and 'catechol'. In this example *M. fortuitum* no. 607 was used. Coloured sections of the paper chromatograms with R_F values 0.82–0.85 (Solvent: *n*-butanol saturated with distilled water) were eluted with distilled water and the absorption spectra of the eluates measured.

Coloured sections in the paper chromatograms developed in *n*-butanol saturated with water were cut out and extracted with distilled water for 16 hr. The extracts were examined by spectrophotometry. An example of the absorption spectra of the extracts is shown in Fig. 1. Extracts from the samples 'salicylate + organisms', 'catechol + organisms', and 'catechol' showed the same type of absorption spectra. Maximum absorptions were observed at a region of wavelength less than 220 $m\mu$ and at 275 $m\mu$. Sodium salicylate showed maximum absorption at 230 and 295 $m\mu$ (data not shown).

In view of these results, the coloured formazan from salicylate was identified with the formazan from catechol since both formazans showed similar R_F values and similar absorption spectra. It was assumed that the salicylate was converted to catechol by *Mycobacterium fortuitum*, for when, instead of the living bacteria, heat-killed bacteria (100° for 5 min.) were added to salicylate medium, no blackening of the medium was observed.

It seems probable that the conversion of salicylate to catechol is enzymic. The reaction is described as follows:

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REFERENCE

TSUKAMURA, M. (1965). Salicylate degradation test for differentiation of *Mycobacterium fortuitum* from other mycobacteria. *J. gen. Microbiol.* **41**, 309.