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# IDENTIFICATION OF ATYPICAL MYCOBACTERIA ISOLATED FROM SOIL AND EXPERIMENTAL SENSITIZATION OF GUINEA PIGS TO MAMMALIAN AND AVIAN TUBERCULINS

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## INTRODUCTION

Atypical mycobacteria were isolated from soil (Tsukamura, 1966; Beerwerth, 1971) and water (Joynson, 1979). Brandes (1960) stated that atypical mycobacteria were the cause of tubercloid changes in the mesenteric lymph nodes of animals. Paterson (1965) found that Mycobacterium fortuitum was the cause of an outbreak of mastitis in a dairy herd. On the other hand , Krebs and Kappler (1971) incriminated atypical mycobacteria as the cause of 1 % of all infective cases of pulmonary tuberculosis. The principal importance of atypical mycobacteria, however, is the finding that they may sensitize man and animals to tuberculin and thus giving rise to false positive reactions.

In the present work trials were made for isolation and identification of atypical mycobacteria from soil. Also experimental sensitization of guinea pigs with the isolates was studied.

## MATERIAL AND METHODS

40 soil samples collected from animal dwellings were examined for the presence of atypical mycobacteria by three methods: Trisodium phosphate after Jones and Jenkins (1965), mice inoculation (Tsukamura, 1967) and combined treatment with sodium hydroxide and oxallic acid (Beerwerth, 1971). Lowenstein-Jensen slants were used for isolation. The

identification of the isolates was based on pigmentation, rate of growth, growth at temperatures of 37, 45 and 52°C and survival at 60°C as well as biochemical reactions recommended by Bojalil et al (1962) and Bönicke (1962), Cerbon& Trujillo (1963), Tskamura (1967) and Stanford and Gunthorpe (1971). These biochemical reactions were : Niacin, nitrate reduction, catalase, peroxidase, tween 80 hydrolysis aryl sulphatase, neutral red, acid production from glucose, mannose, fructose, rhamnose, lactose, sucrose, maltose, raffinose and mannitol, utlization of citrate and amidase activity using acetamide, benzamide, urea, isonicotinamide, nicotinamide, pyrazinamide, salicylamide, allantoin, succinamide and malonamide.

For the sensitization of guinea pigs, the various species were injected each in 4 guinea pigs intramuscularly (1 ml containing 1 mg of live bacilli) and after one month the animals were tested intradermally with 0.4 mcg (20 tuberculin units) of both mammalian and avian PPD tuberculins. All reactions were measured after 24 hours.

## RESULTS

As seen in Table (1): 39 isolates of atypical mycobacteria were recovered from 19 out of 40 soil samples examined. All of which grew rapidly (2-5 days) on Lowenstein-Jensen medium. Only 7 isolates produced pigments (scotochromogenic) while 32 isolates were non-chromogenic.

Table (1): Atypeical mycobacteria isolated from soil

| Species | N. of isolates |
|---|---|
| M. runyonii | 12 |
| M. fortuitum | 9 |
| M. phlei | 5 |
| M. parafortuitum | 3 |
| M. flavescens | 2 |
| Unidentified species | 8 |
| Total | 39 |

On the base of the various biochemical reactions and with the help of identification tables of Bojalil et al. (1962) and Bönicke (1962). Cerbon and Trujillo (1963), Tsukamura (1967) and Stanford and Gunthorpe (1971) the isolates could be identified as M. runyonii, M. fortuitum, M. phlei, M. parafortuitum and M. flavescens. 8 isolates could not be identified with the help of these tables. The results of the biochemical behaviours of the isolates are seen in Table 2.

Of the 8 unidentified isolates; 5 isolates could grow at 45 and 50°C, they were negative in aryl sulphatase and positive in nitrate reduction and Tween 80 hydrolysis tests, produced peroxidase and catalase activity, produced acid from fructose but not from mannose, galactose, lactose, sucrose, maltose and raffinose. Three strains were positive in sorbitol and only one strain was positive for rhamnose and mannitol and could utilize sodium citrate. The other, 3 strains grew at 45 but not at 52°C, they were negative in Tween 80 and arylsulphatase. They were negative in all sugars, but could utilize citrate. The amide reactions were variable in all 8 isolates.

All guinea pigs sensitized by the various atypical mycobacteria reacted to both mammalian and avian PPD tuberculins. it is clear from Table (3) that the mean diameter of erythema in sensitized guinea pigs varied between 5.1 and 6.7 mm in case of mammalian tuberculin and between 5.6 and 7.3 mm in case of avian tuberculin. The highest reaction to both tuberculins was observed in animals sensitized with M. parafortuitum. The standard deviation was higher in case of reactions to avian PPD than to mammlian PPD in animals sensitized with M. parafortuitum, M. flavescens and unidentified species. Statistcal analysis of the results revealed that the increase in the average diameter of reactions to avian PPD was significant in guinea pigs sensitized with M. runyonii, M. phlei, M. flavescens and unidentified species.

Table (2): Identification of atypical mycobacteria isolated from soil

| | M.runyonii (12) | | M.fortuitum (9) | | M.parafort. (3) | | M.phlei (5) | | M.flavescens (2) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Pigmemtation | non-chrom. | | non-chrom. | | non-chrom. | | soctochromogenic | | | |
| Rate of growth | rapid | | rapid | | rapid | | rapid | | slow | |
| Growth at 37°C | + | (12) | + | (9) | + | (3) | + | (5) | + | (2) |
| at 45°C | - | (10) | - | (5) | - | (2) | + | (5) | + | (2) |
| at 52°C | - | (12) | - | (9) | - | (3) | + | (5) | - | (2) |
| Surviv.at 60°C | - | (12) | - | (9) | - | (3) | + | (5) | - | (2) |
| Niacin | - | (12) | - | (9) | - | (3) | - | (5) | - | (2) |
| Nitrate reduc. | + | (11) | + | (9) | + | (2) | + | (5) | + | (2) |
| Catalase | + | (12) | + | (9) | + | (3) | + | (5) | + | (2) |
| Peroxidase | + | ( 9) | + | (9) | + | (3) | + | (5) | + | (2) |
| Tween 80 hyd. | + | (12) | + | (9) | + | (3) | + | (5) | + | (2) |
| Arylsulphatase | | | | | | | | | | |
| 3 days | - | (12) | + | (9) | - | (3) | - | (5) | + | (2) |
| 2 weeks | - | (12) | + | (9) | + | (3) | - | (5) | + | (2) |
| Glucose | + | (12) | + | (9) | + | (3) | + | (5) | + | (2) |
| Mannose | + | ( 7) | - | (7) | - | (3) | - | (3) | + | (2) |
| Fructose | + | ( 8) | + | (7) | + | (2) | + | (4) | + | (1) |
| Galactose | - | (10) | - | (9) | - | (3) | - | (5) | - | (2) |
| Rhammose | - | (11) | - | (7) | - | (2) | - | (5) | - | (2) |
| Lactose | - | (12) | - | (8) | - | (3) | - | (4) | - | (2) |
| Sucrose | - | (12) | - | (9) | - | (3) | - | (5) | + | (1) |
| Maltose | - | (12) | - | (5) | - | (3) | - | (4) | - | (2) |
| Raffinose | - | (12) | - | (9) | - | (3) | - | (4) | - | (2) |
| Nannitol | - | (12) | - | (8) | - | (3) | - | (5) | + | (1) |
| Sorlitol | - | (12) | - | (7) | - | (3) | + | (3) | + | (2) |
| Sod. citrate | - | (11) | + | (9) | - | (3) | - | (4) | + | (1) |
| Acetamide | + | ( 6) | + | (6) | + | (3) | - | (5) | - | (2) |
| Benzamide | - | (12) | - | (9) | - | (3) | - | (5) | - | (2) |
| Urea | + | (11) | + | (9) | + | (3) | + | (5) | + | (2) |
| Isonicotinamide | - | (12) | - | (9) | - | (3) | - | (5) | - | (2) |
| Nicotinamide | + | ( 9) | + | (9) | + | (3) | + | (4) | + | (2) |
| Pyrazinamide | + | ( 9) | + | (6) | + | (3) | + | (4) | + | (2) |
| Salicylamide | - | (12) | - | (9) | - | (3) | - | (5) | - | (2) |
| Allantoin | + | ( 7) | + | (9) | + | (3) | - | (4) | - | (2) |
| Succinamide | - | (11) | - | (9) | - | (3) | - | (4) | - | (2) |
| Malonamide | - | (12) | - | (9) | - | (3) | - | (5) | - | (2) |

( ) = no. of isolates.

Table (3): Average diameter and standard deviation of reactions (erythema) in comparative tuberculin test (100 units) in sensitized guinea pigs (4 animals for each species)

| Mycobacterium | Mammalian PPD | | Avian PPD | |
|---|---|---|---|---|
| | Average diam. mm | Stan. dev. | Average diam. | Stan. dev. |
| M. runyonii | 5.500 | 0.35 | 6.500 | 0.35 |
| M. fortuitum | 5.375 | 0.41 | 5.625 | 0.41 |
| M. phlei | 5.167 | 0.41 | 6.000 | 0.41 |
| M. parafortuitum | 6.667 | 0.62 | 7.330 | 0.85 |
| M. flavescens | 5.500 | 0.41 | 7.167 | 0.62 |
| Unidentified species | 5.750 | 0.75 | 6.500 | 0.94 |

## DISCUSSION

The trisodium phosphate and animal inoculation methods failed to yield any atypical mycobacteria. In case of trisodium phosphate, the contamination rate was very high particularly with anthracoids and this may be the reason. In case of mice inoculation, the failure of isolation maybe explained by the fact that all isolates were rapid growers and according to Tsukamura (1967) the mouse body eliminates rapidly the rapid growers of atypical mycobacteria and only slow growers can be isolated by this method. The best method was that of Beerwerth (1971) by which atypical mycobacteria could be isolated from 47.5 % of soil samples examined. Such incidence is in agreement with the results of Kubica et al. (1963), but it is much lower than results obtained by other authors, such as Jones and Jenkins (1965), who reported the isolation of atypical mycobacteria from 83 % of soil samples examined and Beerwerth (1971) who reported an incidence of 84 %.

The identification of atypical mycobacteria is not an easy job because the tables of identification published by Bojalial et al (1962) and Bönicke (1962), Cerbon and Trujillo (1963), Tsukamura (1967) and Stanford and Gunthorpe (1971) are not fully in agreement. An example of such disagreement is the utilzation of sodium citrate and fermentation of sorbitol. Stanford and Gunthorpe(1971) found that sodium citrate was utilized and sorbitol was fermented by M. runyonii, while Bojalil et al. (1962), Cerbon and Trujillo (1963) and Tsukamura (1967) reported that M. runyonii was negative in both tests. The isolated strains in the present work were also negative in sorbitol and with the exception of one isolate were also negative in citrate test. Another example is the Tween 80 hydrolysis. The isolated strains of M. runyoji and M. fortuitum hydrolyzed Tween 80, while Stanford and Gunthorpe (1971) stated that the 2 species are negative in this test. Our results are however supported by the statement of Runyon and Dietz (1971) that most strains of atypical mycobacteria isolated from soil hydrolyze.Tween 80. Accordingly one should not depend on one single table of identification and several schemes are to be consulted in order to reach a

correct identification.

The results of the comparative tuberculin test substantiate the observation of Dekantor et al (1978) with regard to the sensitizing properties of atypical mycobacteria in guinea pigs. This point needs however further studies especially on large animals.

## SUMMARY

39 isolates of atypical mycobateria were recovered from 19 out of 40 soil samples collected from animal dwellings in Egypt. The isolates were identified on the base of pigmentation, rate of growth, sensitivity to heat and 29 biochemical tests as M. runyonii (12 isolates), M. fortuitum (9), M. phlei (5), M. parafortuitum (3), M. flavescens (2) and unidentified sp. (8). All species showed the ability to sensitize guinea pigs to mammalian and avian tuberculins (PPD).

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