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# Immunodiffusion Analysis of Membranes of *Thermoplasma acidophilum*

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Isolates of *Thermoplasma acidophilum* were divided into serological groups based on immunodiffusion studies of solubilized-membrane preparations.

*Thermoplasma acidophilum* is a thermophilic, acidophilic mycoplasma first isolated from a coal refuse pile which had undergone selfheating (4). A considerable amount of information has accumulated concerning the serology of mycoplasmas and their membranes (1, 7), and it was thus of interest to examine the antigenic properties of *Thermoplasma*. The unusual stability of the *Thermoplasma* membrane to low pH and its sensitivity to neutral pH have been reported by Belly and Brock (3) and Smith et al. (8). Also, Langworthy et al. (6) have reported on the unusual ether-linked lipids of this organism. The immunofluorescence characteristics of *Thermoplasma* were studied by Belly et al. (2), and, based on immunofluorescence and immunofluorescence adsorption studies of 38 isolates derived from different geographical areas, five antigenic-affinity groups were proposed. The present study was initiated to investigate the antigenic composition of the *Thermoplasma* membranes by immunodiffusion. Since the membranes of this organism are rapidly solubilized at neutral pH, the preparation of soluble antigens suitable for immunodiffusion studies was quite simple.

The isolates used for this study were chosen as representative members of the five antigenic-affinity groups of Belly et al. (2). The antisera against the membrane fractions were prepared as described previously (2). Immunodiffusion and immunodiffusion-adsorption were performed by the method of Dudman (5). The *Thermoplasma* cells to be tested as antigen were grown for 10 days in a basal salts-yeast extract medium (4), harvested by centrifugation, and solubilized by suspension in phosphate buffer (pH 8.0) at one-tenth of the original volume. These antigen preparations were used in the peripheral wells of a hexagonal arrangement, with the central well receiving the undiluted antiserum. Preimmunization sera were used in all cases as negative controls. The plates were incubated in a moist chamber at 4 C and observed periodically over a 10-day period.

The three antisera tested, 122-1B3, 124-1, and R8D55, all exhibited three precipitin bands against their homologous antigens. These included one diffuse band, closer to the antigen well and specific for the homologous system. The other two bands were more distinct and less specific, since they were shared by more of the isolates tested. All three isolates showed a certain degree of cross-reaction when tested against each other. One precipitin band (designated 3) was shared by all the isolates tested and formed lines of identity with the homologous system. This indicates that at least one component of the membrane is identical in all the isolates tested. Another precipitin band (designated 2) was also present in the three test isolates (122-1B3, 124-1, and R8D55), but absent in several of the other isolates (Table 1). This band formed lines of identity between isolates 122-1B3 and 124-1, but partial identity between R8D55 and 122-1B3 and R8D55 and 124-1. This indicates that component 2 is identical in 122-1B3 and 124-1, but slightly different in R8D55.

Immunodiffusion-adsorption was used to further discern the antigenic differences of several isolates. Representative isolates from the five antigenic-affinity groups of Belly et al. (2) were tested for their cross-reaction with untreated antisera and antisera adsorbed with the test isolates. The results (Table 1) illustrate that there is at least one component of the membrane which is shared by all the isolates tested. The data also indicate that there is one component specific and unique to the homologous cells (band 1). This band is absent in all the other isolates, except in the case of isolate 96-2 when tested against 122-1B3 and 124-1 antisera, and isolate 97-2 when tested against 124-1 antiserum.

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TABLE 1. *Immunodiffusion and immunodiffusion-adsorption pattern of membrane antigens of Thermplasma isolates*

| Antiserum | Adsorbing antigen | Presence[a] of bands of test antigen | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Group I | | | | | | Group II | | | | | | Group III | | | | | | Group IV | | | Group V | | |
| | | 122-1B3 | | | 135-1 | | | 124-1 | | | 97-2 | | | R8D55 | | | 110-1 | | | 22-7 | | | 96-2 | | |
| | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 122-1B3 (group I) | 0 | + | + | + | 0 | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | + | + | + |
| | 124-1 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | R8D55 | + | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 |
| 124-1 (group II) | 0 | 0 | + | + | 0 | 0 | + | + | + | + | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | + | + | + |
| | 122-1B3 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 |
| | R8D55 | 0 | + | 0 | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | + | 0 |
| R8D55 (group III) | 0 | 0 | + | + | 0 | 0 | + | 0 | + | + | 0 | 0 | + | + | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | + |
| | 122-1B3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 124-1 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Presence or absence of precipitin bands 1, 2, or 3. Symbols: +, band present; 0, band absent. Groups I to V, from Belly et al. (3), defined by immunofluorescence.

Immunodiffusion-adsorption provided an antigenic pattern much more comprehensive than that developed by immunofluorescence, and it provided a quantitative means of assessing antigenic differences. The isolates may now be regrouped on the basis of the three precipitin bands within any of the three antiserum systems tested. For example, antiserum R8D55 forms three precipitin bands with its homologous antigen and two bands, both of identity, with 122-1B3 and 124-1. Both of these cross-reacting components are removable by adsorption with either 122-1B3 or 124-1. Likewise, isolate 124-1 forms two bands when tested with 122-1B3 antiserum, but none when the antiserum was preadsorbed with 124-1, and one (band 2) when adsorbed with R8D55. Furthermore, isolate 124-1 forms three bands with its homologous antiserum, loses bands 2 and 3 when adsorbed with 122-1B3, but retains band 1, which is specific for the homologous system. After adsorption with R8D55 cells, the antiserum against 124-1 still retains band 2, indicating some differences in this component for the two isolates.

From these data the following conclusions can be drawn. (i) Isolates 122-1B3, 124-1, and 96-2 are all closely related, but each possesses some unique and individual characteristics. (ii) Isolate 97-2 is more related to 124-1 than 122-1B3. (iii) Isolate R8D55 is antigenically more distinct than 122-1B3 or 124-1. (iv) The other isolates in Table 1 are related to the three isolates only by component 3. (v) The results obtained by immunodiffusion adsorption are quite different from those by immunofluorescence-adsorption (2). It must, however, be pointed out that in immunofluorescence it is the particulate and fixed components of the membrane which are the targets of the fluorescent antibody, whereas in immunodiffusion it is those portions of the membrane antigens that are solubilized at pH 8.0 and still retain their characteristic antigenicity which are the targets.

The present results emphasize further the considerable antigenic diversity of the genus *Thermoplasma* shown earlier (2) by immunofluorescence techniques.

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