

# Revision of Serological Grouping of *Actinomyces*

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Recent work on the microaerophilic to anaerobic actinomycetes has established at least four species in the genus *Actinomyces*: *A. bovis*, *A. israelii*, *A. naeslundii*, and *A. eriksonii*. Conclusive separation of *A. bovis* and *A. israelii* has been reported (L. Pine, A. Howell, and S. J. Watson, *J. Gen. Microbiol.* **23**:403, 1960) following the description of *A. naeslundii* by A. Howell et al. (*J. Bacteriol.* **78**:82, 1959), and most recently the identification of *A. eriksonii* (L. K. Georg, G. W. Robertstad, S. A. Brinkman, and M. D. Hicklin, *J. Infect. Diseases* **115**:88, 1965). These authors used microcolony morphology, cell wall composition, and the catalase reaction as the primary basis for classification. The anaerobic diphtheroids, including *Corynebacterium acnes*, are not included in the genus.

J. M. Slack, A. Winger, and D. W. Moore, Jr. (*J. Bacteriol.* **82**:54, 1961) demonstrated that certain serological relationships exist between the anaerobic diphtheroids and *Actinomyces*, and proposed serological groups which included both organisms. However, when the additional knowledge of morphological and biochemical characteristics is taken into account, it seems that a re-evaluation of the serological groups proposed previously is appropriate.

One to four strains of each of the four *Actinomyces* species were adapted to growth in non-antigenic peptone dialysate medium, and immunizing antigens were prepared. Antiserum prepared against washed whole-cell antigens was used for grouping cultures by the fluorescent-antibody and gel-diffusion techniques.

Two to twelve cultures of each species were examined by the direct fluorescent-antibody technique with the use of fluorescein isothiocyanate-conjugated  $\gamma$  globulin from the above-described antiserum (Nairn, *Fluorescent Protein Tracing*, The Williams & Wilkins Co., Baltimore, 1964). The serological tests correlated quite well

with morphological and physiological characteristics. Therefore, the following serological groups, excluding the anaerobic diphtheroids, are proposed: group A, *A. naeslundii*; group B, *A. bovis*; group C, *A. eriksonii*; and group D, *A. israelii*. The serological relationships among the groups are shown in Table 1.

The cross-reaction between groups A and D can be removed by sorption with the heterologous antigen or by appropriate dilutions of the antiserum.

TABLE 1. Serological relationships among *Actinomyces* groups A, B, C, and D

Antiserum	Antigens			
	A	B	C	D
Group A	4+	—	—	2+
Group B	—	4+	—	—
Group C	—	—	4+	—
Group D	2+	—	—	4+

Whole cells of *Actinomyces* were disrupted by sonic treatment, and were centrifuged to remove particulate matter; the resulting supernatant fraction was concentrated by flash evaporation. Gel-diffusion tests were done by use of these antigens and a microslide method (S. Landfried, M.S. Thesis, West Virginia Univ., Morgantown, 1966.)

The results confirm the above-designated serological groups, although there were cross-reactions among groups A, B, and D that were not observed with the fluorescent-antibody technique. The fact that there is some serological relationship between *Actinomyces* and *C. acnes* was also shown by the gel-diffusion tests. Antigens from *A. naeslundii*, *A. bovis*, and *A. israelii* formed lines with antiserum to *C. acnes*.