Isolation of *Clostridium felsineum* from Samples of Indiana Mud

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Although the statement has been made by Baker (1) that anaerobic bacteria do not form pigments, a thorough search of the literature (particularly the reports of the Italian laboratory at Milan) reveals several pigmented species of more than passing interest. For a complete bibliography to these see: Section Ee3 of McCoy and McClung (10) and McClung and McCoy (6). As stated by Carbone and Venturelli (2) probably the first was *B. rubellus* of Okada (1892) and this was followed by the bacillus discovered by Ghon and Mucha in an abscess of a kidney (1906).

An organism of special interest is the pigmented species discovered in 1916 by Carbone and later named by him *Bacillus felsineus* [now *Clostridium felsineum* (Carbone and Tombolato) Bergery *et al.*] Donker (3) gives credit to Ruschmann and Bavendamm (12) for being the first to isolate the species in pure culture. This organism is able to ferment pectin and is used by Carbone in a patented process for the retting of flax in linen production. It has been proposed also as a fermentation agent for the disposal of garbage according to a patent by Jean (4). The literature records isolation of strains similar or identical to *Cl. felsineum* from Holland by van der Lek, (14), Argentina by Sordelli and Soriano (13), Russia by Muratova (11) and perhaps Germany by Ruschmann and Bavendamm (12).

The organism is a long, slender bacillus producing oval spores and, in addition to the production of a light orange pigment, it is characterized by the production of ethyl alcohol, butyl alcohol and acetone by fermentation of carbohydrate containing material. In this respect it is similar to the non-pigmented butyl species *Cl. acetobutylicum* described by McCoy et al. (8). That is a distinct species and different from a new orange pigmented form, *Cl. roseum*, first described by McCoy and McClung (9) was firmly established by serological studies by McClung and McCoy (5).

Perhaps difficulty of isolation and obscurity of the original literature explains the infrequency of isolation of the species. In a survey of the distribution of various types of carbohydrate fermenting clostridia it was somewhat surprising to find several positive enrichments of this organism. Included in the series were soil and mud samples from Brown, Monroe and adjoining counties. From these studies it would seem that the organism is distributed more widely than has been thought to be the case previously.

Pasteurized and also unheated dilutions of the samples have been incubated in a variety of media known to favor the carbohydrate fermenting types. Both room temperature and 37°C. series have been

included in our studies. Frequently pigmentation does not occur in the original tube until after 3 or more weeks of incubation. Perhaps peculiarly, media with pectin as the main source of carbon did not prove satisfactory in the studies made to date.

Successful isolation of pure cultures from the original enrichments is attained only with difficulty. Selective pasteurization and a variety of other techniques designed to decrease the aerobic contamination have been useful. Final purification has been accomplished by plates incubated in the vegetable tissue jar of McClung, McCoy and Fred (7). One needs to be constantly on guard for the loss of the power to produce pigment by the strains on serial passage incidental to purification procedures.

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