aeruginosa) as well as actinomycetes (*Nocardia*, *Streptomyces*, *Micromonospora*) and fungi (*Trichoderma*, *Penicillium*, *Aspergillus*).

Sterilized soil as culture medium thus seems to keep vegetative cells of *R. meliloti* alive and effective in a desiccated condition for periods approaching half a century; moreover, it preserves for more than ten years the viability of several other micro-organisms that have never been claimed to form endospores.

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Inhibition of Lysozyme by 'Suramin'

IT has been shown that a number of non-chaining bacteria form long chains when grown in the presence of 'Suramin'; the chains are rapidly split by filtrates of ordinary 'short form' cultures of the organisms. It was assumed as a working hypothesis that separation of bacterial cells is an enzymic process and that 'Suramin' acts by inhibiting the cell-separating enzyme[1].

Detailed experiments showed that while *Streptococcus faecalis*, *B. megaterium*, *Cl. welchii* and *Cl. sporogenes* grow well in the presence of 'Suramin' at a concentration of 1 gm. in 40 ml. (w/v), some chaining still occurs at concentrations up to 1 gm. in 5,000 ml. (w/v). The hypothesis of enzyme inhibition was confirmed when it was found that the chain-splitting activity of the filtrates was indeed inhibited by 'Suramin' at concentrations ranging from 1 to 15 μgm./ml. (w/v).

It is also known that chains of various aerobic and anaerobic rods (obtained by growth in magnesium-deficient medium) and chains of *Streptococcus pyogenes* can be split by lysozyme[2,3]. Preliminary experiments confirmed these findings and extended them to *Cl. sporogenes* and *Streptococcus faecalis*. Relatively high concentrations of lysozyme were required to split the chains in the standard time of 30 min. (20–100 μgm./ml. w/v); the splitting was inhibited by 'Suramin'.

Evidence was afterwards obtained (to be published in detail elsewhere) that despite the similarity of effect between lysozyme and filtrates, splitting of chains is not in fact caused by lysozyme but is due to an enzyme of bacterial origin—apparently released by lysozyme from the cells. Since, however, 'Suramin' is a powerful inhibitor of lysozyme it was thought interesting to give an account of it.

Crystalline lysozyme (Sigma Co.) and 'Suramin' (Imperial Chemical Industries) were sterilized by filtration through sintered glass. Lysozyme was assayed by estimating its ability to lyse suspensions of *Micrococcus lysodeikticus* (N.C.T.C. 2665). The organism was grown at 28° C. on meat-extract broth agar for 48 hr. and the cells washed three times with saline. Suspensions of 10^9 cells/ml. were made in saline-phosphate buffer *p*H 5·5, and falling amounts of lysozyme added. Results were read after 30 min. at 20° C. taking 50 per cent clearing of the suspension on an EEL nephelometer as the end point.

The results can be summarized as follows: lysozyme caused clearing of the suspensions of *Micrococcus lysodeikticus* up to a concentration of 0·1 μgm./ml. (w/v). 'Suramin' gave 50 per cent inhibition of lysozyme when present at equal weight. Accepting the mass of lysozyme as approximately ten times that of 'Suramin', inhibition occurred when 'Suramin' was present at a ten-fold molar excess.

In some experiments 'Suramin' was first left in contact with the bacterial cells; provided that 'Suramin' was afterwards removed by washing the cells with saline no inhibition was observed. In other experiments 'Suramin' was allowed to interact with lysozyme before the cell suspension was added; maximum inhibition was reached rapidly in 5–15 min.

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Dihydroxyacetone Production in *Acetomonas*

IT has already been shown[1] that a useful primary criterion for differentiating *Acetomonas* strains from those of *Pseudomonas* is the ketogenic action of the former on glycerol which results in the production and accumulation of dihydroxyacetone. Thus the 165 strains of *Pseudomonas* studied all failed to show production of dihydroxyacetone, while 49 out of the 50 *Acetomonas* strains produced it abundantly.

As regards the single anomalous *Acetomonas* strain (*A. suboxydans* N.C.I.B. 3734) it was tentatively suggested that this might not be an *Acetomonas* strain at all, but a mutated *Acetobacter* strain which had possibly lost the typical ability of acetobacters further to oxidize the acetic acid produced to carbon dioxide and water, for production of dihydroxyacetone is much less common in *Acetobacter* than in *Acetomonas*. There was, however, no way of knowing whether it was originally dihydroxyacetone-negative when first isolated or whether it has lost dihydroxyacetone positivity in the course of long sub-cultivation.

However, we now have definite evidence that the ability to produce dihydroxyacetone can be lost by 'mutation' (in the broadest sense) in the course of laboratory sub-cultivation. A strain of *Acetomonas*, isolated from cider, was strongly dihydroxyacetone-positive when first isolated. Now, after sub-cultivation on apple juice agar for some two years, this strain is completely dihydroxyacetone-negative, although it has retained its original ability to produce deep brown to black water-soluble pigment on yeast extract/glucose/calcium carbonate agar.

It thus appears that production of dihydroxyacetone from glycerol, if negative, is not so conclusive as formerly in suggesting that a strain is a pseudomonad, but merely functions as a first presumptive indication to that effect. However, even if dihydroxyacetone-production is negative, the other three primary criteria[1] found always to be conclusive for differentiating *Acetomonas* from *Pseudomonas* strains