OCCURRENCE OF *HYPHOMICROBIUM* AND *CAULOBACTER* SPP. IN BORE-WELL WATER

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INTRODUCTION

PAUCITY in the supply of protected water has necessitated examination in this laboratory of other water sources and one such source recently examined was the supply from a bore-well on this campus. While routine laboratory media have been found satisfactory for obtaining a general picture of microbial ecology of waters, their inadequacy for deriving a wider picture of the water microflora was ably demonstrated by Henrici several years ago.[1] Using the submerged slide technique he observed that quite a few bacteria that were not isolatable on routine laboratory media could be observed attached to the slides. He also drew attention to the various types of stalked bacteria occurring in water and provided valuable information on these species.[2]

With a view to study in great details such unusual forms of bacteria slides were suspended in the bore-well water stored in the laboratory. The slides were suspended by using a thin "iron" wire instead of rubber-covered copper wire ("Radio hook-up" wire) used by Henrici.[1] Incidental rusting of the wire, and consequential brown encrustation thereon and onto the slides thereafter, led to a thorough examination of the slides and the encrustations as well. A brief description of the various stalked bacteria observed on the slides and in cultures is presented in this communication.

METHODS AND RESULTS

The water sample examined was drawn from a bore-well nearly 130 feet deep. The pH of the water was 7·9. Clean sterile slides tied with alcohol sterilised "iron" wires were kept immersed in approximately 4-litre volume of the bore-well water stored in a 5-litre flask by inserting the wires between the plug and the rim of the flask. The flask was placed in the dark at room temperature (25–30° C.).

On chemical analysis the composition of the "iron" wire turned out to be: Fe—91·56%, Mn—8·18%, Zn—trace.

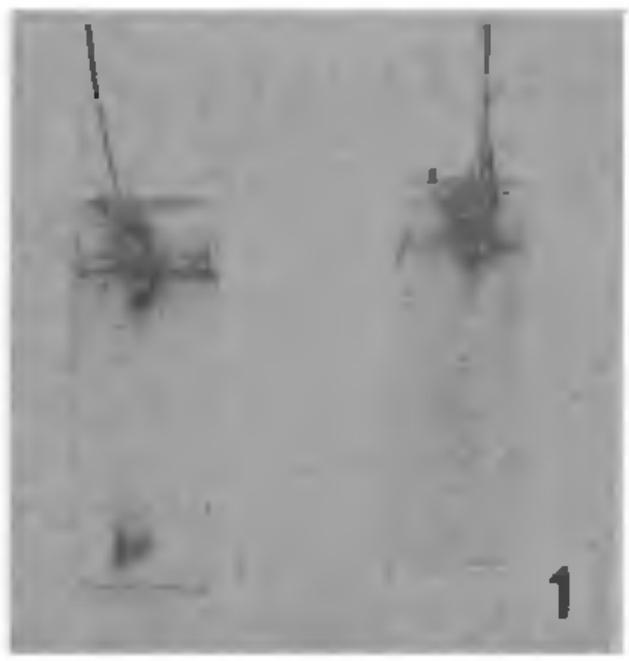
In about 10 days, a portion of the wire under water gave clear indications of rust at different points. Rusting was conspicuous at the water surface and where the wire was in contact with the slide. On prolonged incubation (about 15 to 20 days), the rust gradually spread all over the slide (Fig. 1). On simple staining with crystal violet (after either heat fixing or chemical fixing with Bouin's fluid), the slides revealed invariably the presence of stalked, budding—*Hyphomicrobium* cells in the phase contrast microscope. Sometimes the cells were found in clusters with repeated branching of stalks forming a network (Fig. 2). Occasionally some curved, stalked cells of *Caulobacter* were also observed. For photomicrographic work, the slides were treated with 5 to 10% tannic acid solution for 10 to 15 min. prior to staining with Hucker's crystal violet. The tannic acid treatment facilitated better resolution of the bacterial stalks.

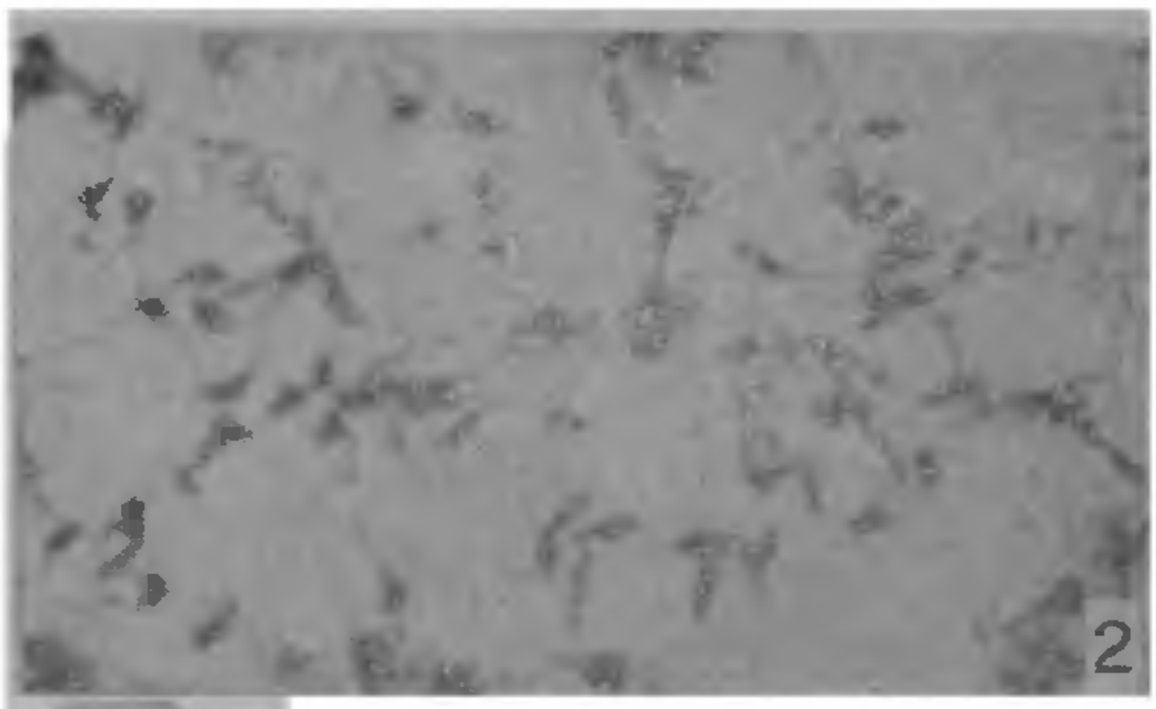
A month long incubation resulted in the appearance on the wire at different points, of rusty nodules, 1-2 mm. in diameter. The nodules were generally soft, easily detachable and were amenable to easy breakage by mere agitation. They gave characteristic Prussian blue reaction for ferric ions when treated with a few drops of 5 N H_2SO_4 and $K_4[Fe(CN)_6]$ solution. A drop of the broken nodular material was put on the copper grid for examination of the air-dried specimen in the 100 kV electron microscope and showed the presence of a few unusual types of "stalked cells" (Fig. 3). The arrow in Fig. 3 points presumably to a lump of the iron material enclosing a cell joining the bifurcated stalk. Some typical *Hyphomicrobium* cells were also observed. Such a cell having two bud-like structures attached to the stalk is shown in Fig. 4 in a reconstructed form. Since *Hyphomicrobium* is known to be pleomorphic and gives rise to many forms,[3,4] it would seem that the "stalked cells" depicted in Fig. 3 represent only the pleomorphic forms of *Hyphomicrobium*. This observation called for a further enquiry and prompted an enrichment of *Hyphomicrobium* spp. and was achieved in the following way:—

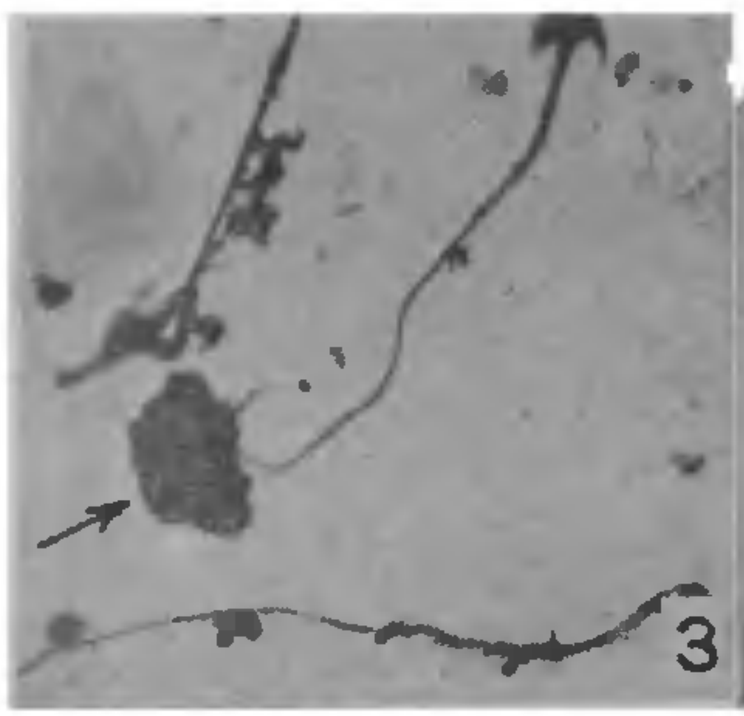
The rusted portion of the wire or a nodule was cut out and inoculated into (1) a 250 ml. flask containing 150 ml. of "337" basal salt

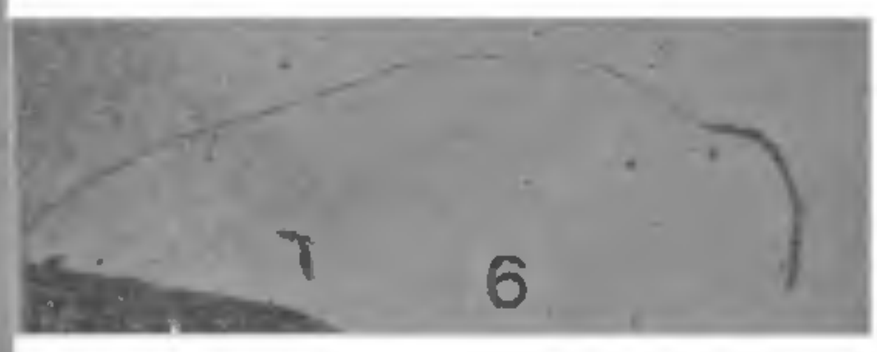
medium of Hirsch and Conti[5] with filter-sterilised methylamine-HCl, and (2) in a 250 ml. flask filled with 150 ml. autoclaved bore-well water. Both the flasks were incubated at 30° C. for two weeks.

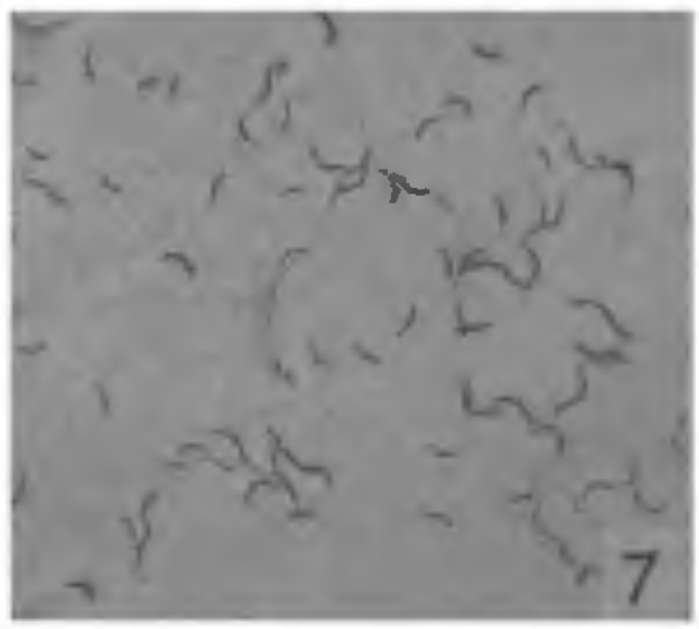
(1) *Enrichment in "337" Medium.*—This medium showed a pellicle formation on the surface which on examination revealed to be that of *Hyphomicrobium* cells mixed with many other types of bacteria. When sufficient











FIGS. 1–7. Fig. 1. Photograph of the slides tied to "iron" wires showing rust encrustation. Fig. 2. Phase contrast, crystal violet stain, × about 2,000. Fig. 3. Electron micrograph, × about 4 000 Fig. 4. Electron micrograph, × about 20,000. Fig. 5. Phase contrast, crystal violet stain × about 1,000. Fig. 6. Electron micrograph, × about 1,575. Fig. 7. Phase contrast, crystal violet stain, × about 1,100.

Hyphomicrobium cells got enriched in the methylamine-HCl medium, they were isolated on the plate containing the same medium solidified with agar after about a week's incubation at 30° C. In this way a few more strains of *Hyphomicrobium* were successfully isolated from the bore-well as well as two other water sources for a systematic study.

(2) *Enrichment in Autoclaved Bore-Well Water.*—This was attempted with the expectation that the wire would show progressive corrosion and might result in the continued stimulation of the growth of *Hyphomicrobium* spp. It was also hoped that the limiting nutritional conditions of the water medium might suppress the growth of other associated microorganisms and permit almost exclusive growth of the desired species.

As long as four months incubation, however, did not show any remarkable progress in corrosion, though a flocculent growth at the bottom and a thin film on the surface became clearly visible. Both the growths were found to be that of the stalked bacteria. The contents were stained after shaking well. The preponderance of the stalked bacteria in this can be witnessed from Fig. 5. Certain elongated, curved bacteria with long stalks were also observed. An electron micrograph of such a stalked bacterium is shown in Fig. 6. The organism appeared to be a *Caulobacter* species; an attempt was therefore made to isolate it by the method described by Stove.[6] Surprisingly several types of *Caulobacter* appeared on the plate from this single enrichment culture flask. Detailed study on them will be published elsewhere.

A typical vibrioid *Caulobacter* isolate possibly related to the one in Fig. 6 is shown in Fig. 7. The undisturbed storage conditions of the autoclaved bore-well water medium must have resulted in the enrichment of *Caulobacter* spp. associated with the rusted wire which perhaps serves as a support for their attachment. Likewise the flocculent material from the bore-well water medium gave rise to the growth of *Hyphomicrobium* on methylamine-HCl agar plate.

The deposition or accumulation of iron and manganese by *Hyphomicrobium* and related spp. has been reported by several workers.[7-9] Tyler and Marshall[3,10,11] described manganese depositing *Hyphomicrobium* spp. from water pipe-lines. Recently, Hirsch[12] reported epicellular deposition of iron by *Hyphomicrobium* spp. It is not surprising therefore that *Hyphomicrobium* was associated with the rust of the wire containing both iron and manganese and both the metals might have enriched these species. It must however be mentioned that the growth of *Hyphomicrobium* is probably not chemolithotrophic as it can occur in the absence of oxidizable iron as well. Its association with the iron or manganese environments is however more frequent and perhaps more significant though we have at present no unequivocal explanation for this phenomenon. It can be said that the pipe-line in the source might have facilitated a preliminary enrichment of these organisms in the bore-well water.

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