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THE BACTERIAL FLORA OF DISTILLED AND STORED WATER. II. CAULOBACTER VIBRIOIDES HENRICI AND JOHNSON 1935 IN DISTILLED WATER

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SUMMARY. Caulobacter vibrioides is commonly found in tanks of distilled water. A detailed description is given of cultural, morphological and physiological characteristics. Attachment to other bacteria and to inanimate objects with rosette formation is described.

Caulobacter vibrioides was isolated from the Chicago, California, England and Washington samples of distilled water, but not from those from Japan, New Zealand and Vancouver. This does not necessarily mean that C. vibrioides was absent from the latter. The colonies of C. vibrioides are colorless and inconspicuous, and are readily overlooked on a plate with numerous colonies of other species. The distilled water supply in the author's laboratory has invariably contained the organism. From the four sources mentioned there were isolated 8 strains which were practically identical both morphologically and physiologically.

Cultural Characteristics.

<u>C. vibrioides</u> is heterotrophic and grows readily on simple peptone media but not on synthetic media unless certain accessory growth factors are present. The growth in broth is colorless and uniformly turbid without pellicle formation. On prolonged incubation in a casein-peptone medium melanins are produced and the culture turns brown. If a small amount of "tergitol" is added to a culture which is still colorless the brown melanin color may appear in a few minutes. The colonies on agar are colorless, smooth and opaque, and relatively small. It is a typical halophobe or osmophobe. Sodium chloride shows marked growth inhibition

INTERNATIONAL BULLETIN

PLATE I

Figures 1 - 7. Caulobacter vibrioides. Strain 127.

- 1. A typical single individual.
- An individual which has been attached, grown a stalk and is now about to divide. The daughter cell has developed its own flagellum. Note the flagellum at end of stalk of parent cell.
- 3 and 4. Small rosettes. In Figure 3 one parent cell still has its flagellum at base of stalk.
- 5. Two polar flagella. This was very rare.
- Stained directly from distilled water. The tremendously long stalk and greatly enlarged soma is only seen in water and never in media with a fair amount of nutrients.
- 7. An extremely rare type indicating that cellular division may occur in free-living cells. As a rule attachment (and perhaps some stalk formation) precedes growth and fission.

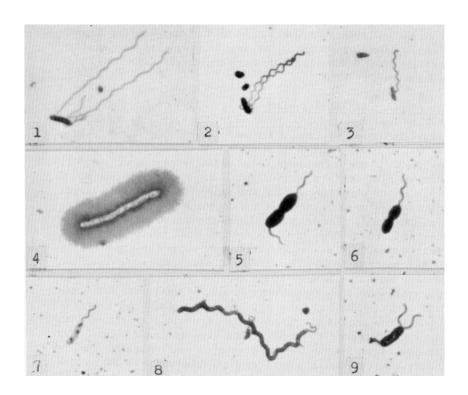


Plate I. Photomicrographs x 2,000. Leifson flagella stain.

INTERNATIONAL BULLETIN

at 0.5% concentration and more or less complete inhibition of growth at 1.0% concentration. Other salts have similar inhibitive effects. Glucose in 1.0% concentration reduces growth and 4.0% glucose is very inhibitive. The growth inhibiting effect appears to be a function of the osmotic pressure. The pH limits for fairly prompt growth are 5.5 to 7.5 with the optimum at 6.0-6.5. At pH 5.0 and 8.0 the growth is very slow, or absent if the medium and the temperature are not ideal. The optimum temperature is about 30°C with a maximum at 43°C.

Morphological Characteristics

The morphology of C. vibrioides (Figs.1-7) is subject to great variation depending on the cultural conditions. In a salt-free dilute peptone medium at pH 6.5 and temperature of 20-30°C the organism is a small straight or slightly curved rod with a predominantly single polar flagellum of wavelength averaging 0.9 μ. The individual organisms attach themselves to each other at the flagellated ends and stalks are produced. The length of the stalks varies with the medium. In a favorable medium such as 0.3% peptone and 0.1% yeast extract the stalks are relatively short but grow longer if this medium is diluted from 10 to 100 times. In distilled water, stalks as long as 100 \mu may be seen (see Fig. 6). As the stalk grows the flagellum remains at the end of the stalk and at the point of attachment. Thus are produced increasingly large groups of organisms in the form of rosettes with the cells attached by their stalks at a common point. The stalked organisms in the rosettes grow and elongate. Eventually binary fission begins and at this stage a flagellum develops at the distal ends. When fission is complete the "daughter" cell breaks away. The number of daughter cells which may be produced from a stalked cell has not been determined. Free-living individuals with a flagellum at each pole (Fig. 7) are extremely rare and evidence of division in the free-living individuals is inconclusive. This may indicate that growth and cell division usually does not take place in the free-living cells but only after attachment and stalk formation.

In slightly acid media of pH 6.0, <u>C</u>. <u>vibrioides</u> is a small straight rod, at pH 7.0 it is longer and has a slight curvature. At pH 7.6 and above the curvature becomes more pronounced and actual coils may form. At pH 7.5 growth is

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

poor and flagella are few. Sublimiting salt concentrations have a striking effect on morphology. At sodium chloride concentrations of 0.5 to 1.0% growth is slow and long filaments are produced. Flagella may occasionally be found at the end of such filaments but flagellation tends to be poor.

Physiological Characteristics

The 8 strains of <u>C. vibrioides</u> isolated were practically identical physiologically. All strains oxidized glucose, sucrose, maltose and xylose with slight acid formation. All were negative on d-sorbitol, d-mannose, raffinose, lactose, starch and cellulose. Also negative were gelatin, nitrate and catalase. The oxidase test was indefinite.

Attachment to other Bacteria

Cells of C. vibrioides not only attach themselves to each other but also to other microorganisms. The attachment takes place at the flagellated end, after which a stalk is formed. In a mixed culture or suspension of live caulobacters and other bacteria, live or dead, a small proportion of the other bacteria will be found with caulobacters attached. The fact that caulobacters become attached to only a few individuals of the other bacteria may indicate individual differences in these bacteria. When one caulobacter has once become attached to a foreign organism other caulobacters usually follow suit and large clusters result. The relative attack rate is somewhat higher when caulobacters are mixed with a suspension of other dead bacteria compared with live bacteria. Experiments to determine if the caulobacter attaches itself only to dead bacteria have been inconclusive. In a limited study caulobacters were found to attach themselves to all the types of bacteria tested but not to yeast cells. (For additional illustrations of caulobacter morphology and illustrations of attachment to other bacteria see Atlas of Bacterial Flagellation, by Leifson.)