A study of the sulphur bacteria of the hot springs of Yellowstone National Park
by Raymond H Howard

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Bacteriology
Montana State University

Abstract:
A chronological review of the literature was made concerning sulphur bacteria. These bacteria are defined by Ellis ('32) as a group of organisms which have sulphur globules in their cells, oxidize hydrogen sulphide to sulphur, store it temporarily in their cell, and then oxidize it to sulphates.

A differential staining technic was developed for sulphur organisms utilizing a mordanted malachite green or methylene blue stain and counterstaining with sodium nitroprusside. With this technic, the cell outline retains the primary stain, and the sulphur granules assume a contrasting red color.

Representative species of gram positive and negative organisms were studied in order to show that this stain did not indicate sulphur complexes present in small concentrations in bacterial protoplasm.

Thiobacilus thiooxidans was stained with the above technic and polar red granules were observed. These red granules indicated that the sulphur complexes in Thiobacillus thiooxidans are similar in nature to the granules found in the true sulphur bacteria studied in the present work.

To test this technic, organisms collected from thermal waters of Yellowstone National Park and immediate vicinity were studied

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INTRODUCTION

Since so few studies have been made on the methods of differentiating and staining of sulphur bacteria, it is the purpose of the writers to determine a differential staining technic which will facilitate further observations on these organisms found in thermal waters in Yellowstone National Park and immediate vicinity.

REVIEW OF LITERATURE

The study of sulphur bacteria is a comparatively recent study of microorganisms. Although casual observations of composite sulphur bacteria in hot springs were made as early as 1860, studies as to their isolation and pure culture, morphology and physiology were not made until several years later. Cramer ('70) was the first to suggest that granules in Beggiatoa, a genus of the sulphur bacteria, consisted of sulphur. From investigations carried out in 1869-71 on the vegetation of the Yellowstone Hot Springs, J. W. Harshbarger ('97) reported the presence of bacteria able to deposit sulphur as granules within their cells. Cohn ('75) then postulated the theory that the Beggiatoa and the purple bacteria produce hydrgen sulfide by reduction of sulfates. W. H. Weed ('89) in his article entitled "The Vegetation of Hot Springs" shows that "travertine" is the result of sulphur deposition by the

Beggiatoa. This work was substantiated by B. M. Davis ('97). His observations were that "travertine" and "felt", a closely woven mass of filamentous bacteria in which crystals of calcium carbonate were imbeded, were responsible for many of the colored deposits in the park. Controversy as to the origin of organisms in sulphur springs was the result of investigations carried out by W. A. Setchell, ('03). His contention was that no organisms were found in strictly thermal waters nor in springs which were reputed to have a decided acid reaction.

Engelmann ('87) first postulated the theory that purple and green sulphur bacteria belonged to the photosynthetic group of organisms. This theory was strongly opposed by Winogradsky ('88) who proposed the theory of chemosynthetic metabolism. In these processes, the energy supply of the organism is not furnished by decomposition of organic matter, but by the oxidation of inorganic substances. In these processes, also, hydrogen sulfide is oxidized by the organism to sulphuric acid. Molisch ('07) published his monograph of the purple bacteria in which he concluded that purple bacteria assimilate organic compounds in the light. This was his attempt to defeat the theory of an autotrophic mode of life for these organisms, as outlined by previous investigators. Such a view was in direct support of the work of Nadson ('03) who stated, also, that hydrogen sulfide is not required for nutrition, and sulphur is not accumulated. Buder ('19) in discussing the value of the

various theories presented up until this time, was inclined to believe that the metabolism of the purple bacteria should be considered as a combination of photosynthetic and chemosynthetic modes of life, independent of each other, but providing the organisms with the faculty to live and thrive under divergent conditions. This idea is called "only a well founded assumption" but even at the present time, we have come no further in our knowledge of this function.

Warming ('75) and Lankester ('76) in their early investigations, drew the conclusion that all the various forms and shapes of colored organisms with droplets inside the cells represented different developmental stages or "phases of growth" of one species. This idea was attacked by Cohn ('75) who held to the monomorphistic viewpoint, as did Winogradsky ('87). Such a viewpoint stressed the fact that distinct variations were characteristic of different species. On this basis, Winogradsky established an elaborate system of classification of the sulphur bacteria based upon the shape and size of the cells, as well as upon their mode of colony formation. The excellence of this system is shown by the fact that it has been perpetuated--with only minor modifications--to the present day. Van Niel ('30), through extensive investigation, concluded that variations as to size, shape, and growth are often encountered and are the result of environmental effects such as hydrogen sulfide concentration, pH of the medium, age of