ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF PHOTOSYNTHETIC BACTERIA

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INTRODUCTION

In 1967, the term photosynthetic or phototrophic bacteria according to Pfennig (1977), was used to designate the green and purple bacteria performing photosynthesis without the evolution of oxygen (anoxygenic photosynthesis). With the advances in cytology and electron microscopy, it was shown that the blue-green algae have the same prokaryotic cellular organization as the bacteria. The blue-green algae, however, perform oxygenic photosynthesis in common with the eukaryotic phototrophs.

In view of their common cellular structure with bacteria, the blue-green algae (cyanobacteria) are now considered as members of the kingdom Prokaryotae (Stanier, 1974). Figure 1 shows the cyclic photophosphorylation in anoxygenic photosynthesis of green and purple bacteria while Figure 2 shows the non-cyclic photophosphorylation which occurs in cyanobacteria in addition to cyclic photophosphorylation.

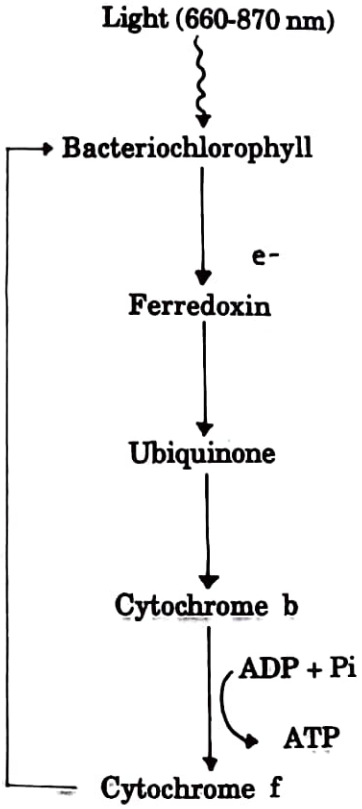

Figure 1. Cyclic photophosphorylation (Cohen et al., 1975; Pelczar et al., 1977).

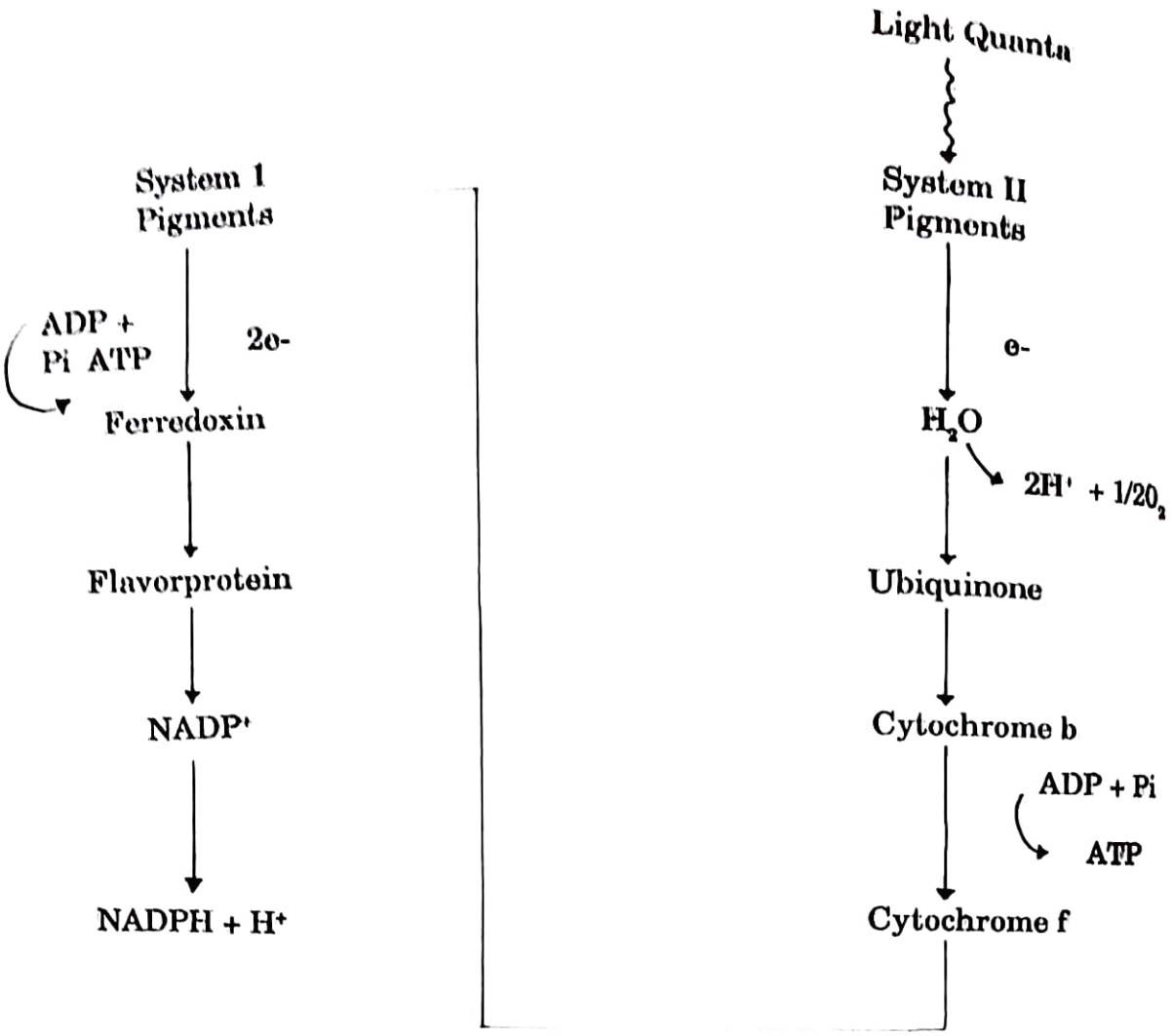

Figure 2. Non-cyclic photophosphorylation (Cohen et al., 1975; Pelczar et al., 1977).

Ecologically, phototrophic bacteria are important because they contribute to the productivity in the habitat where they thrive, in the cycle of matter as well as in the detoxification of sulfides to sulfates (Culver and Brunskill, 1969; Fenchel, 1969; Sorokin, 1970; Hayden, 1972). Figures 3a and 3b are diagrammatic representations of a meromictic lake showing the vertical distribution of oxygenic and anoxygenic phototrophs and the relative concentrations in the water profile of dissolved oxygen and H_2S.

The phototrophic green bacteria belong to two families: Chlorobiacase and Chloroflexaceae while the purple bacteria comprise the families Rhodospirillaceae (purple non-sulfur bacteria) and Chromatiaceae (purple sulfur bacteria) (Pfennig and Truper, 1974; Stanier, 1976).

The green and purple bacteria can be differentiated from one another by cell morphology and ultrastructures and physiological and biochemical characteristics.

The objective of the experiment conducted and of this report is to characterize and identify the isolated photosynthetic bacteria.

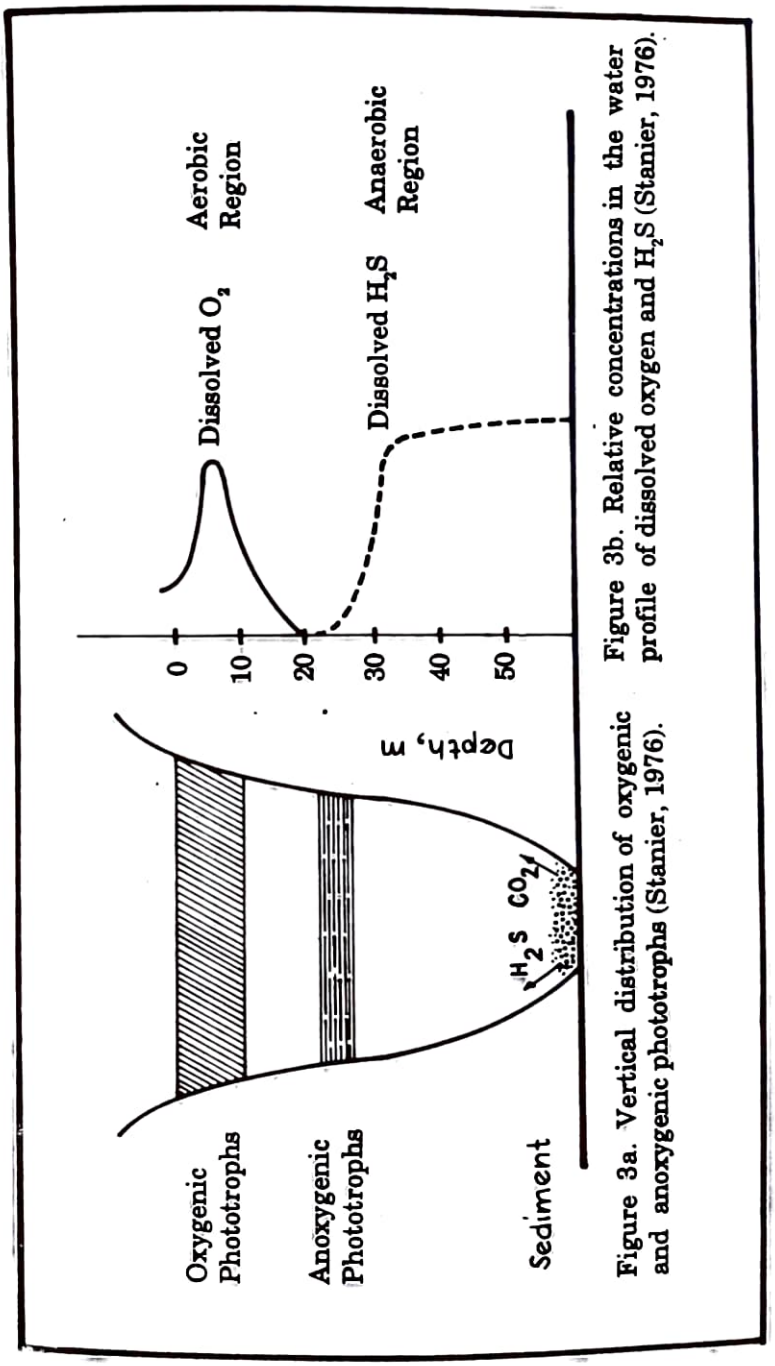

Figure 3a. Vertical distribution of oxygenic and anoxygenic phototrophs (Stanier, 1976).

Figure 3b. Relative concentrations in the water profile of dissolved oxygen and H_2S (Stanier, 1976).

Review of Literature

Molisch (1907), van Niel (1957), and Stanier (1976) described the purple sulfur bacteria as being photoautotrophic, with a very narrow range of photoassimilable organic substances, strictly phototrophic, able to oxidize H_2S and accumulate elemental sulfur as intermediate product in the oxidation of H_2S to SO_4^{-2}. The purple non-sulfur bacteria on the other hand, as described by the same authors, are photoheterotrophic with a broad range of photoassimilable organic substances, able to grow aerobically or anaerobically in the dark, do not oxidize H_2S or accumulate elemental sulfur. Only under special conditions does the second physiological group utilize sulfide as an electron donor for growth (Hansen and van Gemerden, 1972).

Flagellar motility together with the ability to respond photo- and chemotactically, positively or negatively, to environmental conditions allow each individual cell to adjust to optimum growth conditions in a particular habitat (Clayton, 1957; Pfennig 1967; Sorokin, 1970).

Stanier (1976) and Pfennig and Truper (1974) enumerated ten genera of purple sulfur bacteria and three genera of purple non-sulfur bacteria. One prominent difference in the metabolism of these two groups is that the key enzyme of the dissimilatory sulfur metabolism of purple (and green) sulfur bacteria, adenylylsulfate (APS) reductase, has been proven to be absent from all Rhodospirillacease studied so far (Truper and Peck, 1970; Hansen and Voldkamp, 1972; Kirchhof and Truper, 1974; Pfennig and Truper (1974) described five species of *Rhodospirillum,* six of *Rhodopseudomona* and only one species of *Rhodomicrobium.*

With the exception of the ability of *Rhodopseudomonas gelatinosa* to liquify gelatin, the known purple non-sulfur bacteria charateristically lack the capacity to break down organic macromolecules such as starch, cellulose, pectin, chitin, neutral lipids, and proteins (Pfennig, 1978). In natural habitats, they depend on the preceding activity of chemoorganotrophic bacteria capable of degrading such macromolecules. This dependence has been cited as one reason why Rhodospirillaceae are never seen in blooms comparable to those of the green and purple sulfur bacteria (van Niel, 1971). They are, however, found in relatively high proportions together with chemoorganotrophic bacteria (Kaiser, 1966; Biebl, 1973). The most commonly encountered species in nature are *Rhodopseudomonas gelatinosa* and *Rhodopseudomonas palustris* (Biebl and Drew, 1969)

Materials and Methods

Samples were obtained from nine shallow portions (3-5 meters deep) of Laguna Lake waters behind the Public Market of Los Baños, Laguna, using a modified water sampler. Samples were immediately brought to the Microbiology Laboratory of the Institute of Biological Science, UPLB, for isolation, characterization and identification. The samples were first inoculated to screw-capped tubes two-thirds filled with PBM I liquid medium, each separately enriched with 0.1% sodium succinate, 0.1% sodium acetate and 0.1% ethanol.

The tubes were then incubated 60 cm from a 25w bulb as a source of photic energy observed daily until a characteristic red or brown color was noted. Wet mounts were then made to check the morphology of the predominant bacteria.

Eight serial dilutions were made from each original tube which showed color change , into tubes with PBM II agar while still melted, sufficiently mixed, allowed to solidify and then sealed with vaspar. These tubes were incubated in the same conditions as before until well- isolated colonies were seen. A colony was then picked up using a sterile needle and a second set of serial dilution was made for further purification. When isolated colonies appeared, one was picked up using a sterile needle and transferred to another PBM II agar tube and sealed with vaspar. Nine stock cultures were produced using the abovementioned procedure which were used as sources for the different experiments conducted.

Obligate phototrophy, cell morphology and pigmentation were used as main bases to separate families. Among genera, cell morphology was used as differentiating characteristic whereas among species, methods of reproduction, flaggellations, cell morphology and size, pigmentation and unique cell groupings and cell structures were used for identification. These are indicated in Figures 4 and 5 and Tables 1 and 2

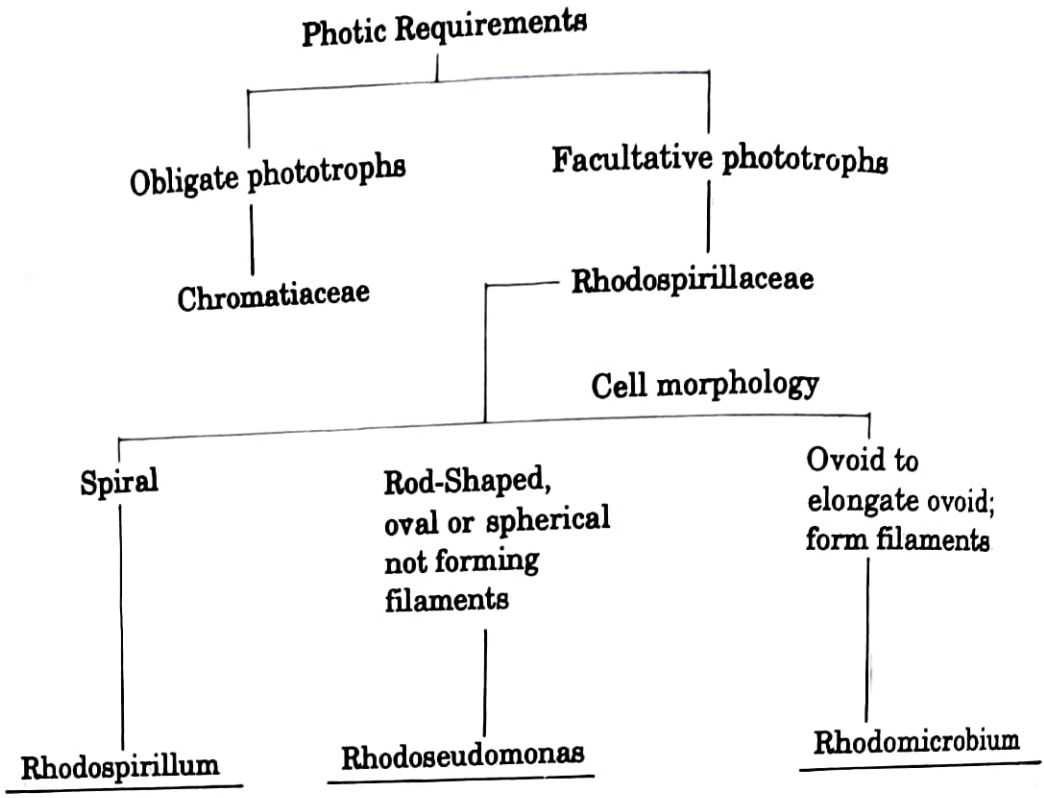

Figure 4. Key to the families of purple bacteria and genera of Rhodospirillaceae. (Modified from Pfennig and Truper, 1974.)

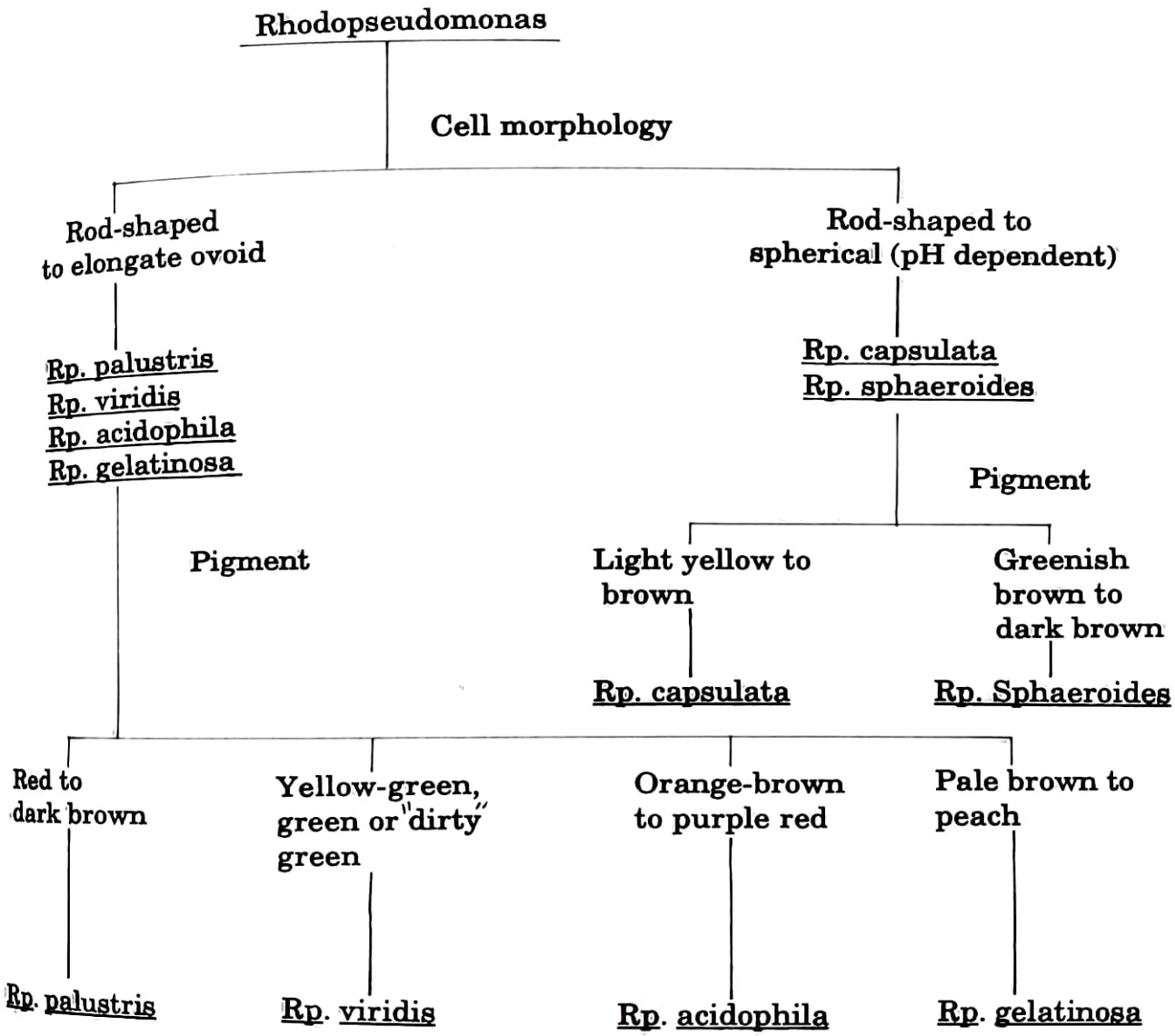

Figure 5. Key to the species of *Rhodopseudomonas*. (Modified from Pfennig and Truper, 1974).

Table 1. Some differentiating characteristics of *Rhodopseudomonas spp.**

	Rp. palustris	Rp. viridis	Rp. acidaphila	Rp. gelatinosa	Rp. cansulata	Rp. sphaeroides
Morphology affected by pH	-	-	-	-	-	-
Mode or reproduction	budding	budding	budding	fission	fission	fission
Color of pigment in anaerobic medium	red to dark brown	yellow - green, green or "dirty" green	orange - brown to purple red	pale brown to light pink	light yellow to brown	greenish brown to dark brown
Pigment molecules	Bchl a, carotenoids of the spirilloxanthin series	Bchl b, carotenoids of the neurosporene & lycopene series/ derivatives	Bchl a, carotenoids of the spirilloxanthin series	Bchl a, group 2 carotenoids	Bchl a, group 2 carotenoids	Bchl a, group 2 carotenoids
Flagella	polar	polar	polar	polar	polar	polar
Slender tube produced during budding	+	+	-	NA	NA	NA

NA ---- not applicable

* Modified from Pfennig and Truper (1974).

Table 2. Some properties of *Rhodomicrobium vannielii*.*

Mature cells ovoid to lemon-shaped.
Young cells spherical, originating as buds at the end of filaments which are approximately 0.3 um and one to several times as long as the mother cell.
Mature birds may remain attached to the filament and form another filament at the opposite pole.
Mature cells may produce as many as three daughter cells; one, by formation of a primary filament from the pole of the cell and one or two or more by lateral outgrowth of new filaments from the primary filament upon which the first daughter cell is borne.
Facultative phototroph.
Form from pinkish to reddish brown pigment.

Both wet mounts from agar stabs with a little amount of crystal violet and flagellar staining using Gray's flagellar stains were made. The photomicroscope at the Central Laboratory was used to take pictures of the isolates.

All taxonomic keys and schemes were based on references listed under Literature Cited in this report. Minor modifications, however, were made for better use of such keys.

* Modified from Pfennig and Truper (1974).

Results and Discussion

All enrichment cultures turned reddish purple from an original brownish to grayish due to muddy sediments. It was also shown that all isolates (nine in all) were able to grow in the dark. However, growth in the dark was observed to be slower than in the presence of light, in anaerobic cultures. Based on these characteristics, it was established that all isolates belonged to the family Rhodospirillaceae (Figure 4).

This family is composed of only three genera which can be separated from one another by their cell morphology and ability to form filaments. These genera are *Rhodospirillum*, *Rhodopseudomonas* and *Rhodomicrobium* (Figure 4).

Wet mounts with a little amount of crystal violet for contrast and flagellar staining of the nine isolates showed that eight isolates were rods that didn't produce filaments and one isolate having an elongate ovoid or lemon-shaped form which produced filaments. It was evident that after three trials made showing the same results, eight isolates belonged to the genus *Rhodopseudomonas* and one isolate to the genus *Rhodomicrobium*.

Pfennig and Truper (1974) in the 8th edition of the Bergey's Manual of Determinative Bacteriology recognized only one species of *Rhodomicrobium*, that of *Rhodomicrobium vanielii*. Figures 6a-6b are photomicrographs of the *Rhodomicrobium vanielii* isolated. Table 2 and Figure 4 provide evidence for this identification.

The same authors recognized only six species of *Rhodopseudomonas* (Table 1 and Figure 5). Microscopic examinations revealed that out of eight *Rhodopseudomonas* isolates, seven showed rod-shaped cells, which produced slender tubes during budding, giving the appearance of dumbbells, form rosettes and cell clusters, each cell about 0.6 to 0.9 um x1.2 to 2.0 um (Figures 7-12). One out of the eight showed budding cells but without the formation of slender tubes between mother cells and daughter cells. Each cell was 1.0 to 1.3 um x 2.0 to 5.0 um. Based on Figure 5 and Table 1, seven *Rhodopseudomonas* isolates were *Rhodopseudomonas palustris* (Figures 7-10, 12-12a, 13-14) and one *Rhodopseudomonas acidophila* (Figures 11a-11d).

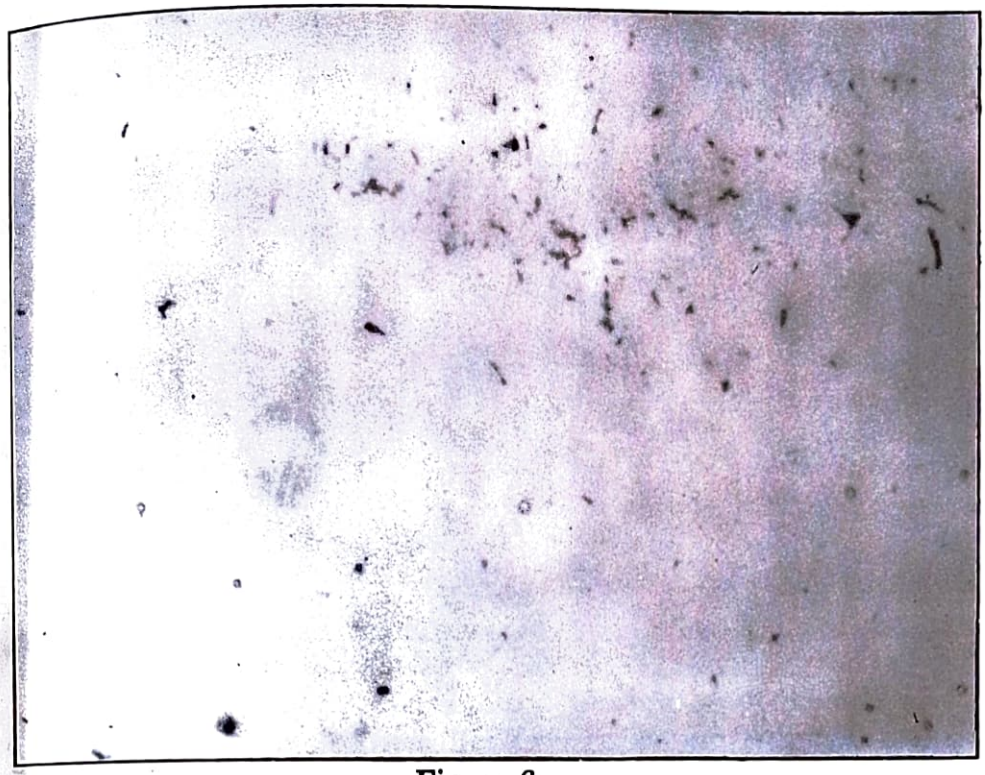

Figure 6a.

Rhodomicrobium vanielii (Isolate No. 2, 100x on flagellar staining).

Figure 6b.

Rhodomicrobium vanielii (Isolate No. 2, 200x on flagellar staining) showing lemon-shaped mother cell, prominent filaments and small buds (cells) at tip of filaments formed between the mother cells and daughter cells (buds).

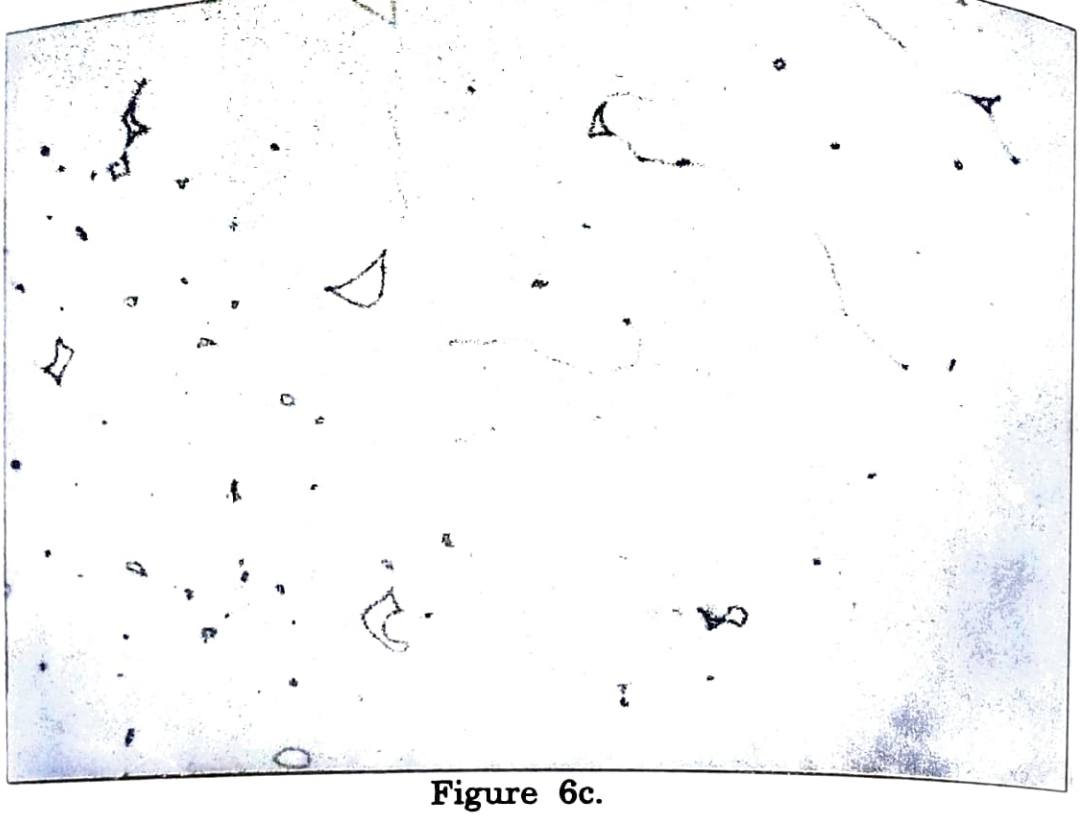

Figure 6c.

Rhodomicrobium vanielii (Isolate No. 2, 400x on flagellar staining) showing the typical cell morphology of *Rhodomicrobium vanielii*.

Figure 6d.

Rhodomicrobium vanielii (Isolate No. 2, 1000x on flagellar staining). A distortion (bulging) on the left and of the filament was caused by agar that got in between a secondary filament and a lateral filament branching off from the former. The lower cell on the right end is the mother cell followed by the daughter cell in between filaments.

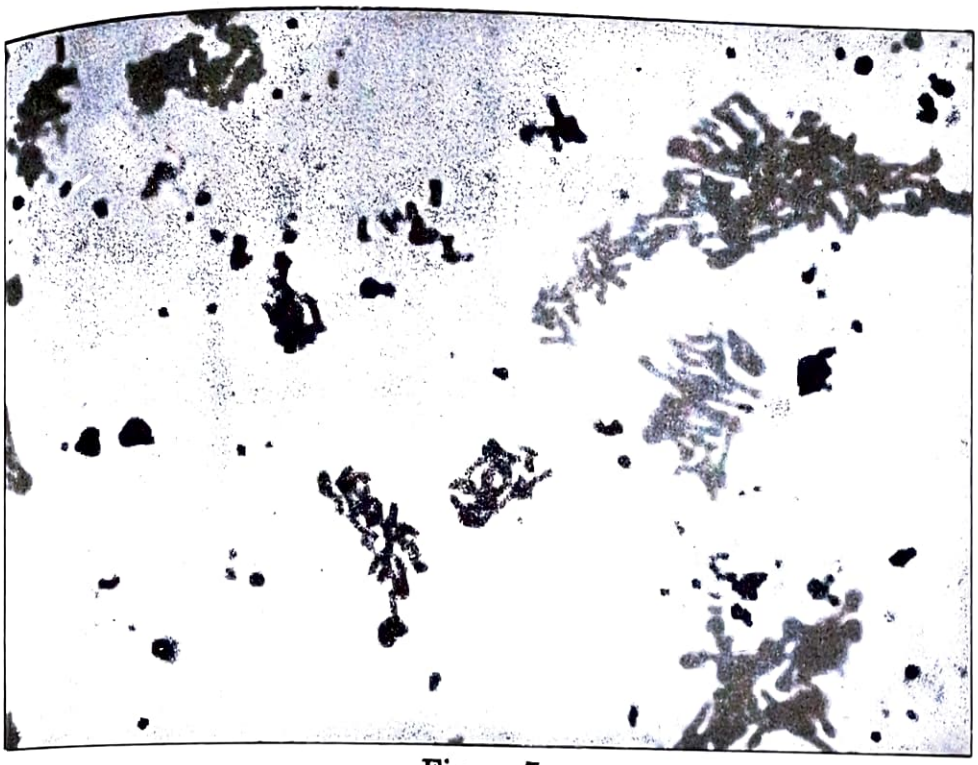

Figure 7.

Rhodopseudomonas palustris (Isolate No. 1, 1000x on flagellar staining). Budding cells formed dumbbells showing tubes in between dividing cells. Also shown are rosettes and clusters common to this species.

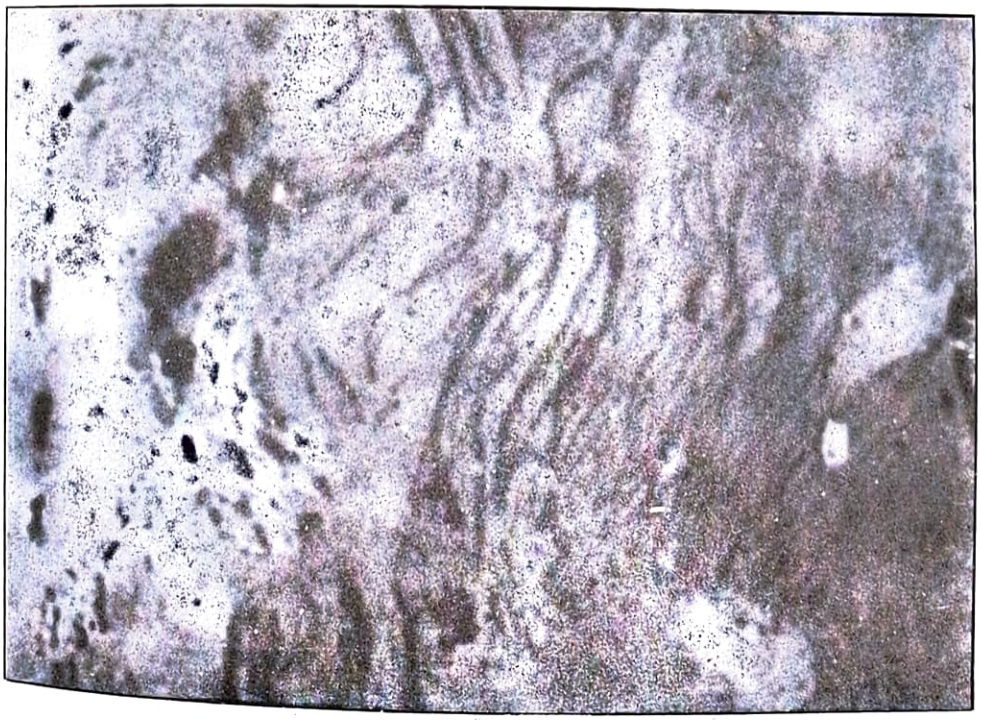

Figure 8.

Rhodopseudomonas palustris (Isolate No. 3, 1000x on flagellar staining)showing very visible flagella.
Wet mount of this isolate shows the characteristic cell morphology of the species.

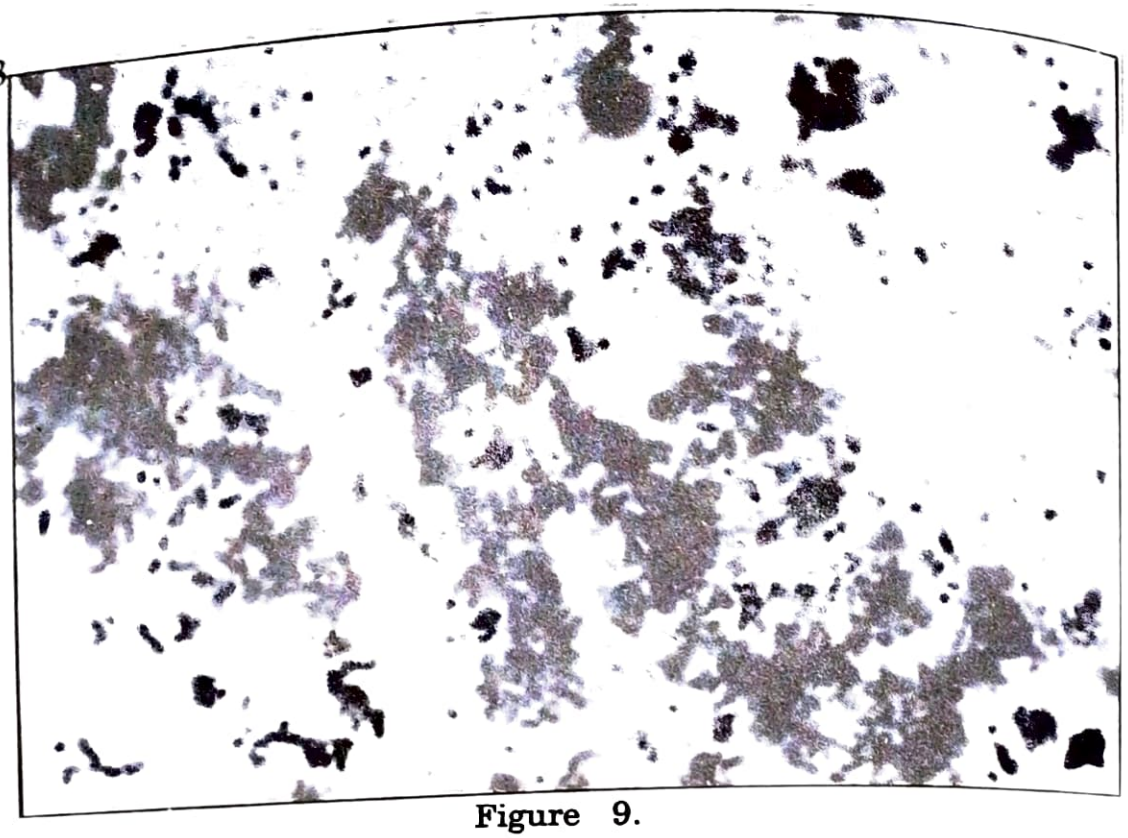

Figure 9.

Rhodopseudomonas palustris (Isolate No. 4, 1000x on flagellar staining) showing rod cells in cluster, budding not prominent, but cell morphology in wet mounts was typical of the species.

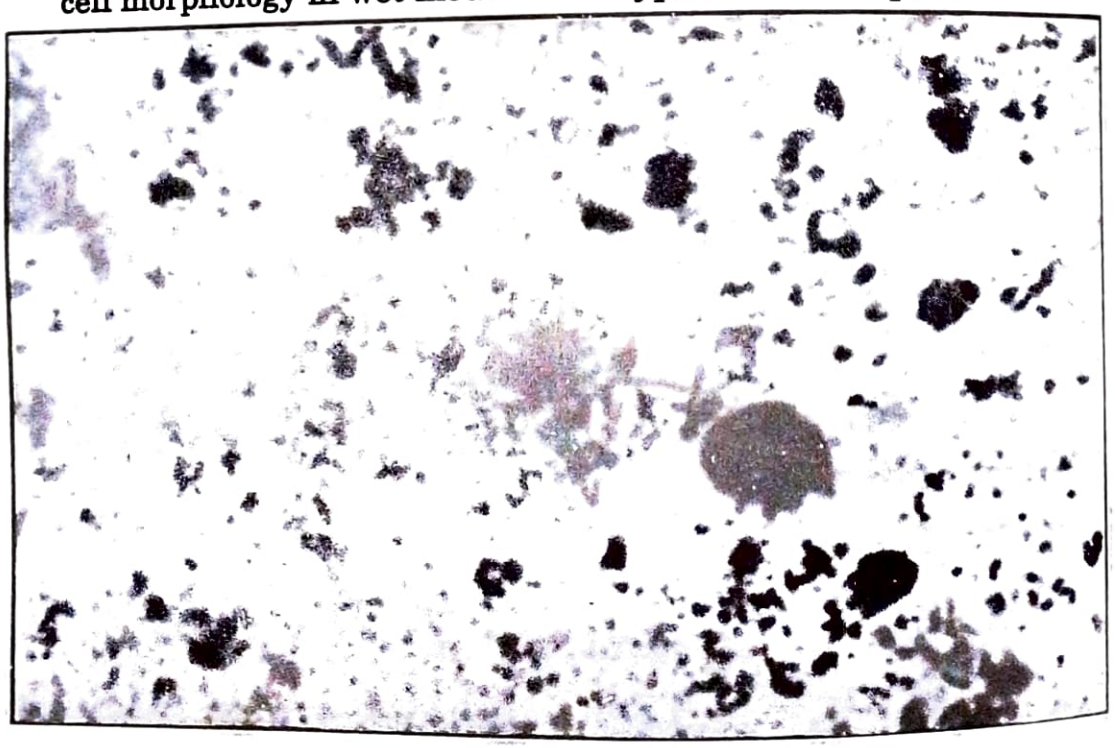

Figure. 10.

Rhodopseudomonas palustris (Isolate No. 5, 1000x on flagellar staining). Rod cells in rosette formation. Budding not prominent in this picture, but morphology in wet mount was typical of the species.

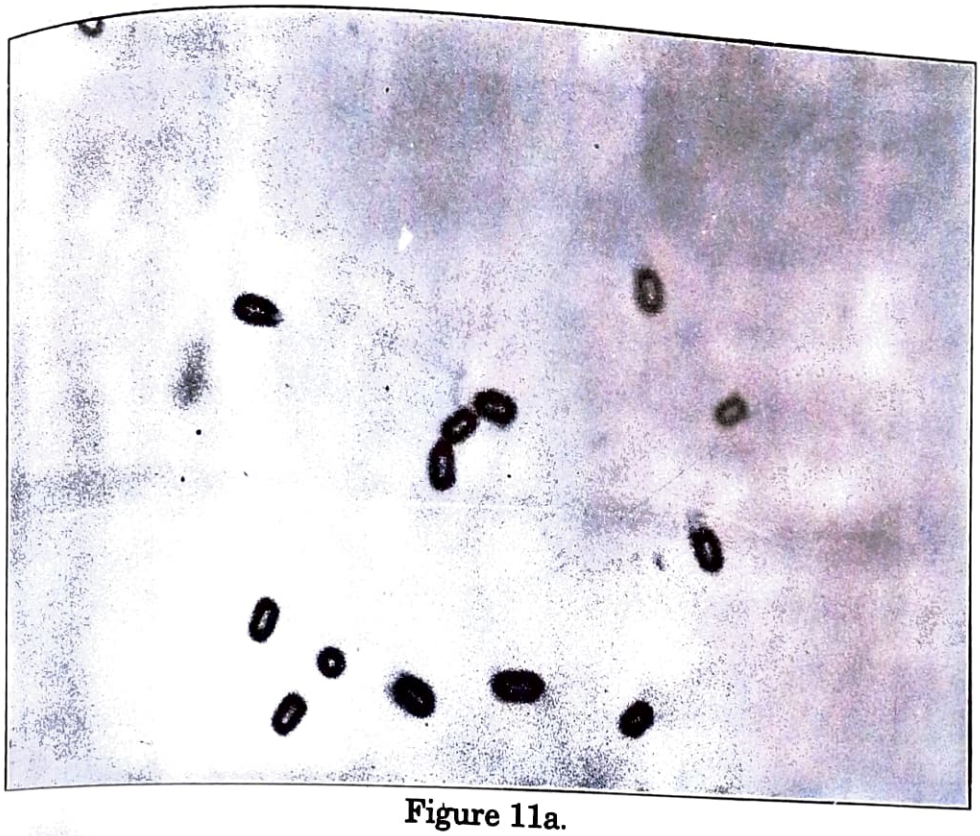

Figure 11a.

Rhodopseudomonas acidophila (Isolate No. 6, 1000x in wet mount with small amount of crystal violet). Cells were larger than *Rp. palustris*. No slender tube was formed. Budding was by constriction of the mother cell (sessile buds).

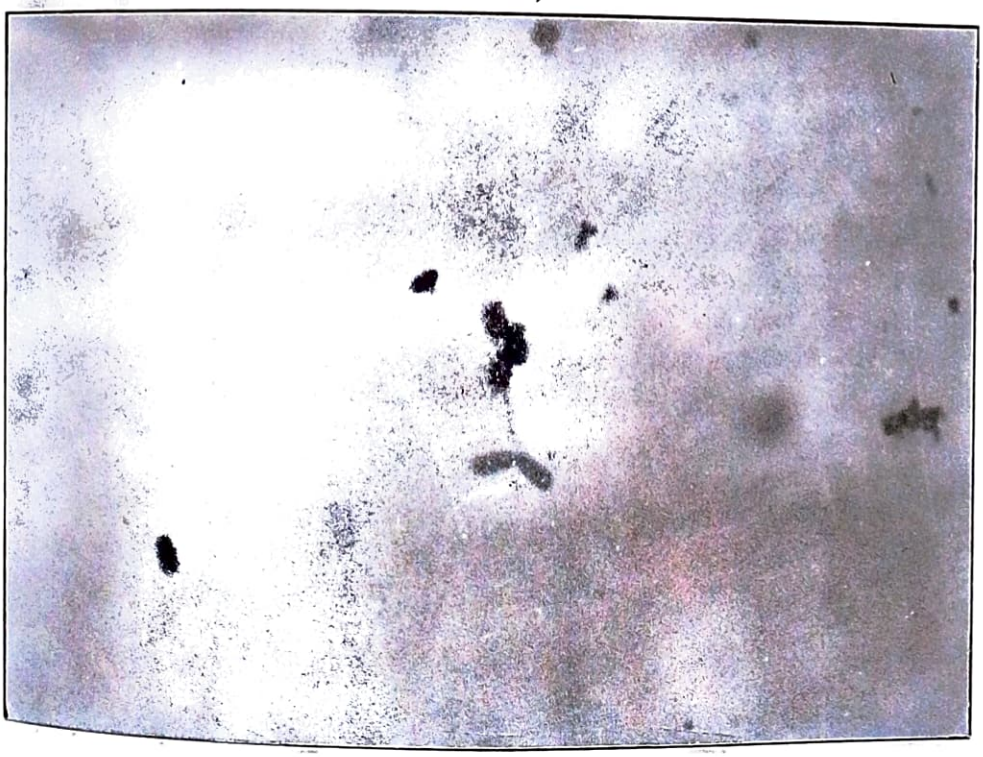

Figure 11b.

Rhodopseudomonas acidophila (Isolate No. 6, 1000x on flagellar staining) showing two cells about to separate from the point of constriction. A flagellum can be seen from one of the cells above.

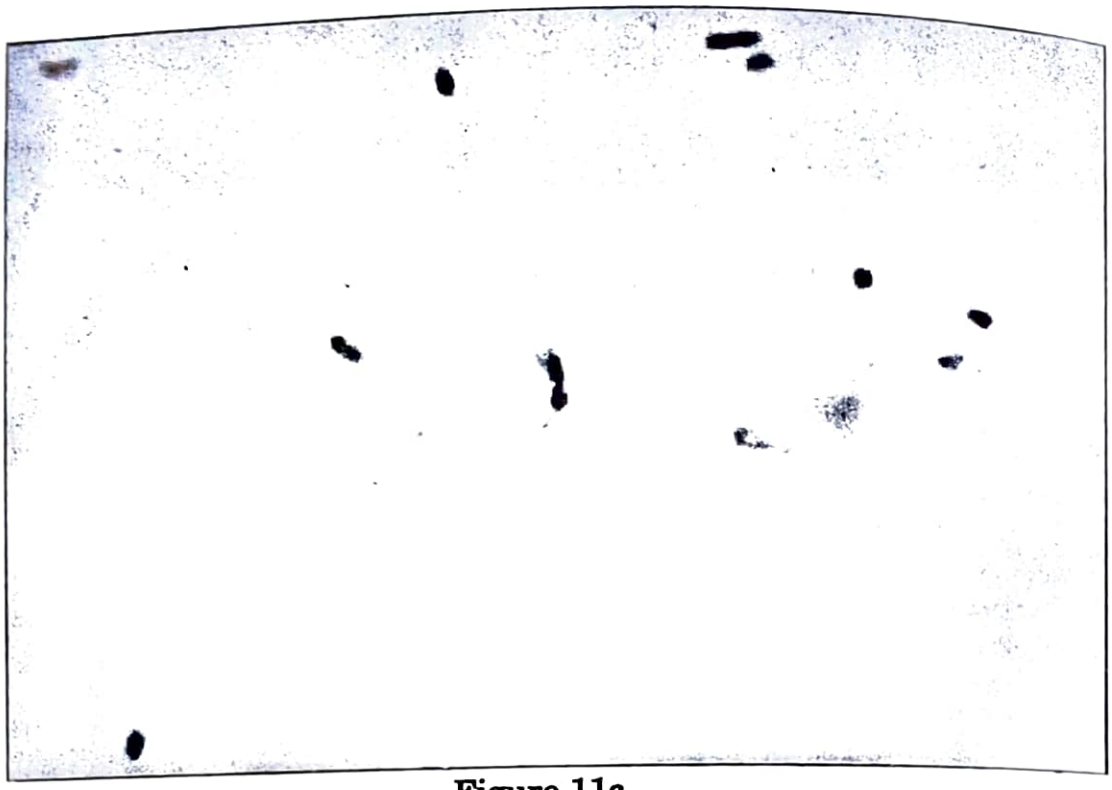

Figure 11c.

Rhodopseudomonas acidophila (Isolate No. 6, 1000x on flagellar staining). Polar flagellum visible.

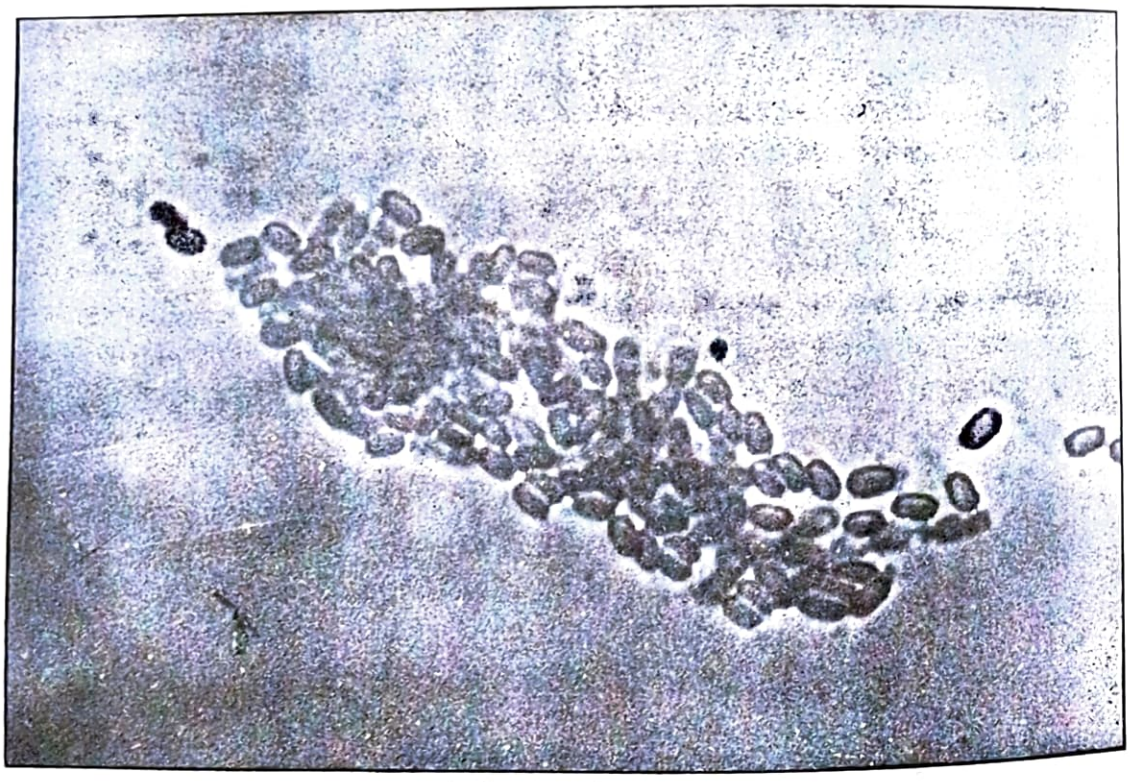

Figure 11d.

Rhodopseudomonas acidophila (Isolate No. 6, 1000x on flagellar staining). Cells in cluster reminiscent of clusters of *Rp. palustris*.

Figure 12.

Rhodopseudomonas palustris (Isolate No. 9, 1000x on flagellar staining). Rods were shown to form a rosette of three cells.

Figure 12a.

Rhodopseudomonas palustris (Isolate No. 9, 1000x on flagellar staining). Bands of flagella visible. Cells were not prominent.

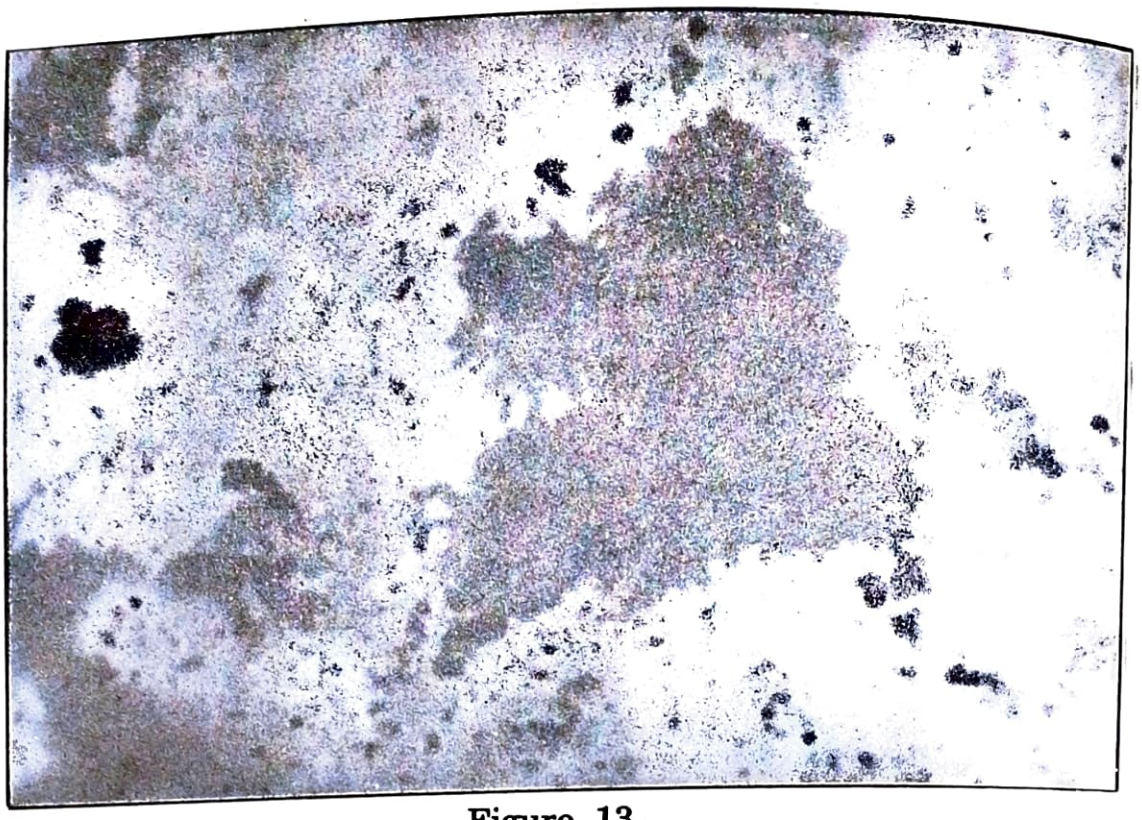

Figure 13.

Rhodopseudomonas palustris (Isolate No. 8, 1000x on flagellar staining). Rods in clusters.

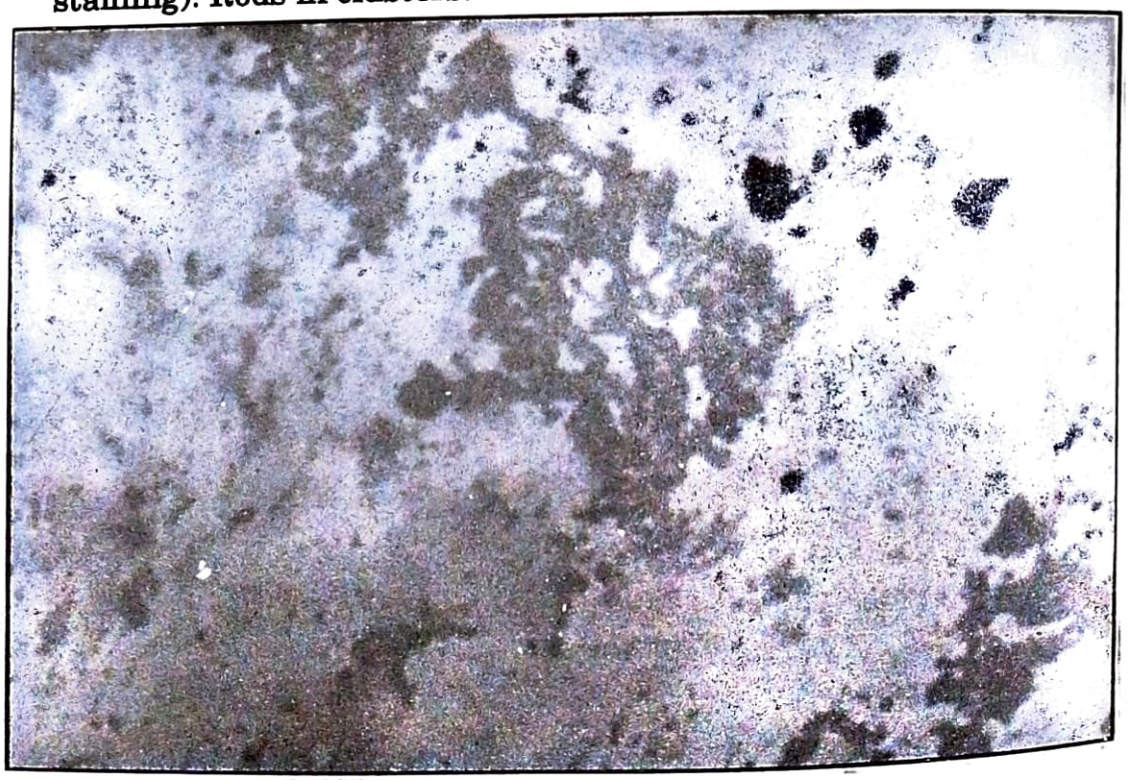

Figure 14.

Rhodopseudomonas palustris (Isolate No. 7, 1000x on flagellar staining). Rod cells in cluster.

Summary and Conclusion

Water samples were collected from various points in Laguna Lake behind the Public Market of Los Baños, Laguna. All species of isolated photosynthetic bacteria belong to the family Rhodospirillaceae. Morphological characteristics as shown in photomicrographs revealed that the isolates belong to two out of three recognized genera of Rhodospirillaceae, namely; *Rhodomicrobium* and *Rhodopseudomonas*.

It was shown further that seven isolates belong to the species *Rhodopseudomonas palustris*, one to *Rhodopseudomonas acidophila*, and one to *Rhodomicrobium vanielii*.

These results were insignificant because it was shown that members of Rhodospirillaceae can be speciated without resorting to electron microscopy. Moreover, one of the isolates, *Rhodomicrobium vanielii*, was reported to be a very rare species and the only member of the genus. Finally, the isolation technique described may prove useful in growing cultures of photosynthetic bacteria for physiological and biochemical studies and for biotechonological research as there has been recent interest in using photosynthetic bacteria for single cell protein (SCP) production.

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