

## Ecological and Physiological Studies on Purple Sulphur Bacteria (Chromatiaceae) at Aswan High Dam Lake

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**Abstract:** 322 isolates of purple sulphur bacteria were isolated from different localities of study in Aswan High Dam Lake. These comprise five genera of purple sulphur bacteria (*Chromatiaceae*). *Allochromatium* was the most common genus 161 (50%) followed by *Thiocystis* 50 (15.53%), *Thiocapsa* 40 (12.42%), *Thiodictyon* 38 (11.8%) and *Lamprocystis* 33 (10.25%) from the total number of isolates during the whole period of study. These genera represented by six species *Allochromatium vinosum* and *A. warmingii*; *Thiocystis violacea*; *Thiocapsa roseopersicina*; *Thiodictyon elegans* and *Lamprocystis roseopersicina*. Physico-chemical parameters of water samples were determined.

**Key words:** Purple sulphur bacteria • *Allochromatium vinosum* • *A. warmingii* • *Thiocystis violacea* • *Thiocapsa roseopersicina* • *Thiodictyon elegans* • *Lamprocystis roseopersicina*

### INTRODUCTION

The water areas in Egypt include the River Nile and Aswan High Dam Lake (AHDL) previously referred to as Lake Nasser. The purple sulfur bacteria included in the gamma subdivision of proteobacteria capable of photosynthesis [1]. They are Gram negative found in illuminated anoxic zones of lakes and other aquatic habitats where hydrogen sulfide accumulates and not use water as their reducing agent and so do not produce oxygen. Instead they use hydrogen sulfide, which is oxidized to produce granules of elemental sulphur S inside the cells. The combination of the yellow and red carotenoids with the bacteriochlorophylls *a* or *b* form colors of red, brown, purple and sometimes orange.

In the traditional classification, morphological properties such as cell form and size, motility, presence of gas vesicles and formation of cell aggregates were properties of major relevance. Because this classification was not in congruence with the phylogenetic relationships within the Chromatiaceae species, a reclassification was proposed on the basis of 16S rDNA sequence similarities and with support of selected phenotypic properties [2, 3].

A number of more recent reviews consider ecological aspects and the distribution of purple sulfur bacteria in nature [4-15]. The most important properties that

determine the development and distribution of phototrophic bacteria in nature are the availability of light, the light intensity and the concentrations of sulfide and oxygen, which form countercurrent gradients in most of the natural habitats of purple sulfur bacteria.

The objective of the present work is to isolate for the first time the photosynthetic purple sulphur bacteria (*Chromatiaceae*) from fresh water of two sites Khor-El Ramla (site I) and Khor Manam (site II) in Aswan High Dam Lake based on phenotypic properties.

### MATERIALS AND METHODS

**Sampling:** Fresh water samples were collected from Aswan High Dam Lake at two successive sites (khor-El-Ramla (I) to the west and khor-Manam (II) to the east).

Physico-chemical characteristics: temperature, pH values, dissolved oxygen, sulphate, Chlorophyll-a and bacteriochlorophyll-a of water samples were determined according to Pfennig [5], Jackson [16] and APHA [17].

**Enrichment Medium:** Samples from different depths and localities were inoculated into white screw-capped bottles containing an enrichment medium [18, 19]. Liquid medium was sterilized in 30 ml white screw-capped bottles. They were filled with a proper volume and autoclaved for about 15 minutes at 120°C. The enrichment media were

inoculated with water and sediment samples and then exposed to tungsten light source (light intensity, 100-300 lux) at 25-35°C. After 7 to 10 days, the colours which indicate the presence of purple sulphur bacteria were observed. Pure cultures were obtained by repeated subculturing in agar shake tubes [20]. Isolates were identified to species level microscopy, pigmentation and physiological characters according to the scheme of J. Imhoff [21].

## RESULTS AND DISCUSSION

**Physico-chemical Parameters:** During summer and autumn seasons water temperature decreased with depth from the surface to the bottom 32.1-15.5°C. This may be due to thermal stratification [22-26]. The pH values ranged from a minimum of 7.2 at the bottom of site II to maximum of 8.80 at the surface of site I in spring 2002. This may be due to the photosynthetic activity especially macrophytes [25-28].

Dissolved oxygen (mg/l) ranged from 8.08 at the surface of site II in winter to 0.25 at the bottom of site I in spring 2002. This may be attributed to photosynthetic production of oxygen by phytoplankton blooms [29-32]. The relative decrease in sulfate contents recorded at the bottom of the lake may be due to the reduction of sulfate ( $\text{SO}_4^{2-}$ ) to sulfides ( $\text{S}^2$ ) by the action of sulfate reducing bacteria. Similar, the decomposition of organic matter usually produces anaerobic conditions leading to the formation of hydrogen sulfide [27,33].

**Chlorophyll-a and Bacteriochlorophyll-a:** chlorophyll-a ( $\mu\text{g l}^{-1}$ ) content ranged from 4.3-12 at the bottom of site II in autumn 2001 to a maximum value at the surface of site I in summer 2001, while bacteriochlorophyll-a ( $\mu\text{g/l}$ ) increased from bottom to surface 2.5-9.9. In General, chlorophyll-a decreased with increasing depth, while bacteriochlorophyll-a ( $\mu\text{g/l}$ ) increased. The changes in chlorophyll-a were found to be correlated to the phytoplankton density [28, 29, 34, 35]; while bacteriochlorophyll-a due to the presence of photosynthetic bacteria [5, 28, 29, 36, 37].

**Characterization and Identification of the Purified Isolates:** 322 isolates of purple sulfur bacteria were obtained from different localities of study. These comprise five genera of purple sulfur bacteria (*Chromatiaceae*) with six different species. The characteristics of *Chromatiaceae* were shown in Table 1 and Fig. 1.

***Allochromatium vinosum*:** Cells are rod shaped 2 and 2.5-6  $\mu\text{m}$ , occasionally longer. Globules of S evenly distributed within the cell color of growing cultures, first yellowish to orange-brown, later brownish red. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin group.

Grow phototrophically under anoxic conditions in the light. Photosynthetic electron donors: sulfide, sulfur, thiosulfate, sulfite, molecular hydrogen, formate, acetate, propionate, pyruvate, fumarate, malate and succinate. Some strains utilize butyrate. Not utilized: sugars, sugar alcohols, alcohols, benzoate, citrate and amino acids. Nitrogen sources: ammonium salts and  $\text{N}_2$ . Mesophilic freshwater bacterium with optimum growth at 25-35°C and pH range 6.5-7.6. Habitat: ponds and lakes with stagnant freshwater, sewage lagoons, brackish waters, estuaries, salt marshes and marine habitats containing hydrogen sulfide and exposed to light. Among the most widely occurring species of the family *Chromatiaceae*. *The mol% G \_ C of the DNA is:* 61.3-66.3 (Bd).

***Allochromatium warmingii*:** Cells ovoid to rod shaped, 3.5-4.0 and 5-11  $\mu\text{m}$ , sometimes longer, motile by a flagellar tuft which is usually 1.5-2 times the cell length and visible by bright field or phase contrast microscopy. In the presence of sulfide and light, globules of S are predominantly located at the two poles of the cell. Color of individual cells is grayish to slightly pink, color of cell suspensions pinkish to purple-violet. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the rhodopinal group. Strictly anaerobic and obligately phototrophic. Sulfide reduced media required. Sulfide and S used as photosynthetic electron donors. In the presence of sulfide and bicarbonate, acetate and pyruvate are photoassimilated. Not utilized: thiosulfate, sugars, alcohols, higher fatty acids, amino acids, benzoate, formate and most intermediates of the tricarboxylic acid cycle. Assimilatory sulfate reduction is absent. Nitrogen sources: ammonium salts, urea and  $\text{N}_2$ . Vitamin B12 required. Freshwater bacterium with optimum growth at 25-30°C and pH range 6.5-7.3. Habitat: ditches, ponds and lakes with stagnant freshwater containing hydrogen sulfide and exposed to light. *The mol% G \_ C of the DNA is:* 55.1-60.2

***Lamprocystis Schroeter 1886, Brilliant Bag:*** *Lamprocystis roseopersicina* is distantly related to *Thiocapsa* and *Thiodictyon* species [38]. Sequence analyses of 16S rDNA place this species into the group of

Table 1: Characterization of *Chromatiaceae* isolated from two sites (Khor E-Ramla and Khor Manam) from Aswan High Dam Lake

| Feature                               | Genera and Species              |                                 |                                 |                                 |                                 |                                    |
|---------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|------------------------------------|
|                                       | <i>Allochromatium vinosum</i>   | <i>Allochromatium warmingii</i> | <i>Thiocystis violacea</i>      | <i>Thioapsa roseopersicina</i>  | <i>Thiodictyon elegans</i>      | <i>Lamprocystis roseopersicina</i> |
| Bacteriochlorophyll absorption maxima | 375<br>590<br>805<br>830<br>890 | 375<br>590<br>805<br>830<br>890 | 375<br>559<br>805<br>830<br>890 | 375<br>590<br>805<br>830<br>890 | 375<br>590<br>805<br>830<br>890 | 375<br>590<br>805<br>830<br>890    |
| Bacteriochlorophyll present           | a                               | a                               | a                               | a                               | a                               | a                                  |
| Carotenoid absorption maxima          | 425<br>460<br>490<br>525        | -<br>465<br>490<br>525          | -<br>470<br>490<br>523          | -<br>485<br>515<br>545          | -<br>470<br>-                   | -<br>464<br>490<br>-               |
| Carotenoid present                    | spirillo-xanthin                | rhodopinol and rhodopinol       | okenone and rhodopinol          | spirillo-xanthin                | rhodopinol and rhodopin         | lycopinol and lycopinol            |
| Culture pigmentation                  | orange-brown                    | purple-violet                   | purple-violet                   | pink                            | purple-violet                   | purple-violet                      |
| Morphology                            | ovoid                           | ovoid to rod                    | spherical                       | spherical                       | rod shaped                      | spherical to ovoid                 |
| Cell size (µm)                        |                                 |                                 |                                 |                                 |                                 |                                    |
| width                                 | 2                               | 3.5-4                           | 2.5-3.0                         | 1.5                             | 1.5 - 2                         | 2.0 - 3.5                          |
| length                                | 2.5-6                           | 5-11                            |                                 |                                 | 3.8                             | 6.5 -7.3                           |
| pH range                              | 6.5-7.5                         | 6.5 - 7.3                       | 6.5-7.6                         |                                 | 6.7-7.3                         | 25-30°C                            |
| Temperature range                     | 30-35°C                         | 30-35°C                         | 25-35°C                         |                                 | 25-30°C                         |                                    |
| Photo assimilation:                   |                                 |                                 |                                 |                                 |                                 |                                    |
| Acetate                               | -                               | +                               | +                               | +                               | +                               | +                                  |
| Pyruvate                              | +                               | +                               | +                               | +                               | +                               | -                                  |
| Fumarate                              | +                               | -                               | -                               | +                               | -                               | -                                  |
| Malate                                | +                               | -                               | -                               | +                               | -                               | -                                  |
| Succinate                             | +                               | -                               | -                               | +                               | -                               | -                                  |
| Formate                               | +                               | -                               | -                               | -                               | -                               | -                                  |
| propionate                            | +                               | -                               | -                               | -                               | -                               | -                                  |
| Glycerol                              | -                               | -                               | -                               | +                               | -                               | -                                  |
| Fructose                              | -                               | -                               | -                               | +                               | -                               | +                                  |
| Sulphide                              | +                               | +                               | +                               | +                               | +                               | -                                  |
| Thiosulphate                          | +                               | -                               | +                               | -                               | -                               | +                                  |
| Bicarbonate                           | -                               | +                               | +                               | +                               | +                               | -                                  |
| Nitrogen souece:                      |                                 |                                 |                                 |                                 |                                 |                                    |
| ammonium salts                        | +                               | +                               | +                               | +                               | +                               | +                                  |
| urea                                  | -                               | +                               | +                               | -                               | -                               | -                                  |

*Chromatiaceae* with motile, without gas vesicles typically found in freshwater habitats

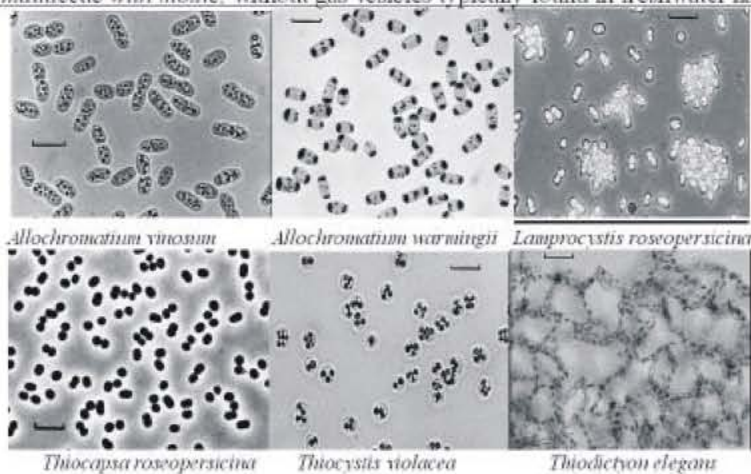


Fig. 1: *Chromatiaceae* isolated from two sites (Khor E-Ramla and Khor Manam) from Aswan High Dam Lake

spherical to ovoid, non motile freshwater *Chromatiaceae* species, many of which contain gas vesicles and in close proximity to the species originally described as *Amoebobacter purpureus* [39]. In accordance with the rules of the International Code of Nomenclature of Bacteria, the transfer of this species to the genus *Lamprocystis* as the new combination *Lamprocystis purpurea* comb.nov. was proposed [40].

***Lamprocystis roseopersicina*:** Rosy peach-colored. Cells spherical to ovoid, 2.0-3.5 µm in diameter motile by flagella, contain gas vesicles. Color of cell suspensions pinkish violet to purple-violet. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the rhodopinal group, with lycopinal as main component [18]. Obligately phototrophic and strictly anaerobic. Photolithoautotrophic growth with sulfide and S as electron donors. In the presence of sulfide and bicarbonate, acetate and pyruvate are photoassimilated. Nitrogen sources: ammonium salts. Mesophilic freshwater bacterium with optimum growth at 20-30°C and pH 7.0-7.3. Habitat: mud and stagnant water of ponds and lakes containing hydrogen sulfide. *The mol% G \_ C of the DNA is: 63.8 (Bd).* [40], 1700VP (*Pfennigia purpurea* [38, 41], 1308; *Amoebobacter purpureus* [39], 399).

***Thiocapsa roseopersicina*:** Rosy peach-colored. Cells spherical, 1.2-3.0 µm, usually 1.5 µm in diameter and surrounded by a strong slime capsule. Aggregates of two, four, or more cells common; irregular clumps of cells usually surrounded by slime. Gas vesicles absent. Individual cells colorless, color of cell suspensions pink to rose-red. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin group [42, 43]. Photolithoautotrophic growth under anoxic conditions in the light with hydrogen, sulfide, thiosulfate and sulfur as electron donors. Acetate, pyruvate, fumarate, malate, succinate, glycerol and fructose are photoassimilated. Not utilized: lactate, propionate, butyrate, tartrate, citrate, benzoate and alcohols. Chemoautotrophic or chemoheterotrophic growth under microoxic to oxic conditions in the dark is possible. Most strains capable of assimilatory sulfate reduction. Nitrogen sources: ammonium salts. Nitrogenase activity was present. Growth factors not required. Mesophilic bacterium with optimum growth at 20-35°C and pH range 6.5-7.5. Habitat: stagnant water and mud of ponds, pools or wastewater lagoons containing degradable organic substances and hydrogen sulfide.

Also common in estuaries and salt marshes. *The mol% G \_ C of the DNA is: 63.3-66.3*

***Thiocystis violacea*:** Violet-colored. Cells spherical and about 2.5-3 µm in diameter. Depending on the culture conditions, larger individual cells may occur. Irregular cell aggregates surrounded by slime are formed under unfavorable conditions. Sulfur globules appear randomly distributed within the cell. Color of individual cells grayish, color of cell suspensions purple-violet. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the rhodopinal group [42, 43]. Photoautotrophic growth occurs under anoxic conditions in the light with sulfide, sulfur, thiosulfate, sulfite (used by some strains) and molecular hydrogen as electron donors. In the presence of sulfide and bicarbonate, acetate, pyruvate and fumarate are photoassimilated. In addition, some strains use propionate, succinate, oxoglutarate, glucose, fructose, propanol or glycerol. Chemoautotrophic or mixotrophic growth possible under microoxic to oxic conditions in the dark. Some strains capable of assimilatory sulfate reduction. Nitrogen sources: ammonium salts, urea and N<sub>2</sub>. Mesophilic bacterium with optimum growth at 25-35°C and pH range 6.5-7.6. Marine isolates may tolerate or require low concentrations of NaCl.

**Habitat:** ponds and lakes with stagnant freshwater, brackish water, or seawater containing hydrogen sulfide; sewage lagoons, estuaries, salt marshes and sulfur springs. *The mol% G \_ C of the DNA is: 62.8-67.9*

***Thiodictyon elegans*:** Cells are rod shaped 1.5-2.0 and 3-8 µm grow in the form of irregular netlike aggregates, dependent on the sulfide concentration and light intensity. In cultures of higher population densities, cell chains break apart and the cultures contain predominantly single cells. Color of single cells grayish, color of cell suspensions light violet to purple-violet. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the rhodopinal group, with rhodopinal and rhodopin as major components [18]. Obligately phototrophic and strictly anaerobic. Photolithoautotrophic growth under anoxic conditions in the light with sulfide and S as electron donors. During oxidation of sulfide, S is transiently stored in the peripheral part of the cell that is free of gas vesicles. In the presence of sulfide and bicarbonate, acetate and pyruvate are photoassimilated. Nitrogen sources: ammonium salts. Mesophilic freshwater bacterium with

optimum growth at 20-25°C, pH range 6.7-7.3. The mol% G \_ C of the DNA is: 65.3-66.3.

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