

**Research Article****ISOLATION OF PHOTOSYNTHETIC SULFUR BACTERIA FROM THE SOIL SAMPLES OF ELEVATED CO<sub>2</sub> CHAMBERS****R. SUNIL KUMAR<sup>1</sup>, B. RAJANI<sup>1</sup>, R. UDAY CHANDRA KUMAR<sup>\*2</sup>, MAHMOOD SHAIK<sup>2</sup>, M. VANAJA<sup>1</sup>, M. UMA DEVI<sup>1</sup>**

<sup>1</sup>Microbial Physiology Laboratory, Department of Botany, Postgraduate College of Science, Osmania University, Hyderabad 500 004, A.P., India <sup>2</sup>Bioinformatics Division, Environmental Microbiology Lab, Department of Botany, Osmania University, Hyderabad 500 007, A.P., India

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**ABSTRACT**

Soils are the largest carbon reservoir of the terrestrial carbon cycle. As the atmospheric CO<sub>2</sub> concentration rises, it is likely that microbial populations will change, as some bacterial strains may be favored over others. Soil samples were obtained from OTCs (open top chambers) and inoculated into selective enrichment media for isolation of anoxygenic photosynthetic bacteria. OTCs specially erected for climate change studies and are maintained at two elevated levels of carbon dioxide (550ppm and 700ppm). The chambers without any external CO<sub>2</sub> supply served as ambient (370ppm) control. Several photosynthetic bacteria have been isolated at 700ppm of CO<sub>2</sub> concentration whereas these photosynthetic bacteria were absent in ambient condition. Naturally adapted microorganisms may prove to be more suitable for climate change studies and the aim of the present work is to record the role of these bacteria in agriculture with reference to elevated atmospheric CO<sub>2</sub> conditions.

**Keywords:** Photosynthetic sulfur bacteria, Open top chambers, Elevated carbon dioxide concentration.

**INTRODUCTION**

An increase in atmospheric CO<sub>2</sub> content alters functioning of soil ecosystems. Nevertheless, the major influence of doubling of CO<sub>2</sub> atmospheric concentration on soil microbial communities is indirect because CO<sub>2</sub> concentration in soil is greater than 0.1 kPa<sup>1</sup>, whereas the current atmospheric content is 0.035 kPa. Soil bacterial communities probably play a key role in the response to the elevated atmospheric CO<sub>2</sub>. Under normal conditions, 12 to 54% of the carbon fixed by photosynthesis is released into the soil by the roots<sup>2,5</sup>.

Studies on bacterial numbers and biomass in the rhizosphere and under CO<sub>2</sub> enrichment are not well documented. Zak et al.<sup>6</sup> showed an increased bacterial biomass in the soil and the rhizosphere of *Populus grandidentata*. Schortemeyer et al.<sup>7</sup> found in samples taken in the spring a positive effect of the elevated CO<sub>2</sub> on the bacterial numbers in the rhizosphere of ryegrass, but a negative effect in the rhizosphere of *T. repens*. On the other hand, Runion et al.<sup>8</sup> detected no changes in bacterial numbers in the rhizosphere of cotton, and inconsistent results were found by Whipp<sup>9</sup> in *Zea mays* and by O'Neill et al.<sup>10</sup> in *Liriodendron tulipifera* rhizosphere. Studies are still needed to better understand the influence of the increased atmospheric CO<sub>2</sub> on soil bacterial processes, and therefore on plant growth. According to Diaz et al.<sup>11</sup>, the elevated CO<sub>2</sub> atmospheric content leads to an increase in substrate release into the rhizosphere. This induces a mineral nutrient sequestration by an expanding microbiota and a consequent limitation of plant growth. According to Cardon<sup>12</sup>, the influence is also linked to the nutrient status of the soil. On the other hand, Clarholm et al.<sup>13</sup> and Zak et al.<sup>14</sup> suggested an increased protozoan grazing due to enhanced carbon availability and therefore an increased mineralization and plant nutrient availability.

Photosynthetic sulfur bacteria constitute the purple and green bacteria belonging to the families Chromatiaceae and Chlorobiaceae. They are ubiquitous in nature. Phototrophic purple and green bacteria are found in nearly all aquatic environments. Purple or green sulfur bacteria can be easily recognized when they form water blooms. Purple nonsulfur bacteria, however, rarely appear in visible concentrations. Their distribution in nature, therefore, can only be evaluated from results obtained by enrichment techniques or the membrane filter method<sup>15</sup>. The presence of purple bacteria is particularly dependent upon the degree to which water is polluted by organic matter. Their growth contributes to the purification of heavily polluted water exposed to sunlight, as, for example, in

sewage lagoons<sup>16</sup>. In Japan, phototrophic bacteria are used in the main purification stage of organic wastewater treatment<sup>17-19</sup>.

The environments such as waste water stabilization ponds, polluted ponds, the hypolimnion of lakes etc. have the nutrients like H<sub>2</sub>S, H<sub>2</sub> and simple organic compounds make the photosynthetic bacteria predominant. However, there is no information available with respect to the photosynthetic sulfur bacteria from elevated atmospheric CO<sub>2</sub>. In an approach to understand the types of photosynthetic sulfur bacteria growing in the soils with different levels of CO<sub>2</sub>, samples were obtained from OTCs (Open Top Chambers at CRIDA) specially erected for climate change studies. They were analysed and enriched with specific media for the isolation of photosynthetic sulfur bacteria.

**MATERIALS AND METHODS****Sample collection**

Soil samples were collected from OTCs which are maintained at elevated atmospheric CO<sub>2</sub> of 550ppm, 700ppm. Those chambers without any external CO<sub>2</sub> supply served as ambient control. Parameters such as soil pH, organic carbon (OC), OTCs average temperature and relative humidity were recorded during sampling.

Cell suspensions with sterile distilled water were made out of these soil samples, later serial dilutions were carried out to 10-6 dilutions.

**Enrichment of photosynthetic sulfur bacteria****Preparation of PFENNIG'S medium**

The following recipe gives 3 liters of medium. Screw cap bottles of 125 ml and 65 ml volume were used. The medium was prepared as four different solutions:

Solution I: Distilled water: 2500 ml

CaCl<sub>2</sub> • 2H<sub>2</sub>O 1.3 g

NaCl 105.0 g (giving a final concentration of 3% NaCl)

Of solution I, 500 ml were autoclaved in an Erlenmeyer flask, and 2000 ml were distributed to the screw cap bottles, 80 ml for 125 ml-bottles, and 40 ml for 65 ml-bottles. Then the bottles were autoclaved with the caps not tightly closed. After autoclaving the bottles were allowed to cool to room temperature slowly, and then tightly closed.

Solution II: Distilled water: 67 ml

Trace element solution

(PFENNIG & LIPPERT 1966): 30 ml

Vitamin B<sub>12</sub> (2 mg/100 ml) solution: 3 ml

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

NH<sub>4</sub>Cl 1.0 g

MgCl<sub>2</sub> • 6H<sub>2</sub>O 1.0 g

KCl 1.0 g

Solution III: distilled water: 900 ml

NaHCO<sub>3</sub> 4.5 g

Solution III was enriched with CO<sub>2</sub> by bubbling until the pH was down to 6.2. Then solutions II and III were mixed and instantly sterilized by filtration through a sterile Seitz filter. (The sterilized filter was washed by filtration of 200 ml distilled water, before solutions II and III were sterilized.) After sterile-filtration, the combined solutions II + III were distributed to the bottles which already contained sterile solution I. The portions were 40 ml and 20 ml, respectively.

Solution IV: Distilled water: 200 ml

Na<sub>2</sub>S • 9H<sub>2</sub>O 3.0 g

A Teflon-covered stirring magnet was added, the solution autoclaved and set to cool. Then, under sterile conditions, about 1.5 ml of sterile (autoclaved) 2M H<sub>2</sub>SO<sub>4</sub> was added drop wise while the solution was stirred magnetically. This solution was distributed to the bottles in 6 ml and 3 ml amounts, respectively. Then the bottles were filled up with solution I; a pea-size air bubble was left to meet possible pressure changes. For the adjustment of the final pH, which should be 6.8 for the Chlorobacteriaceae and 7.2 for Thiorhodaceae, more or less H<sub>2</sub>SO<sub>4</sub> was added to solution IV.

#### Isolation of photosynthetic sulfur bacteria

For isolation 10 ml screw cap test tubes were used. 1 ml of the filtered samples was inoculated into the test tubes containing the enrichment medium. The test tubes were filled completely with growth medium, tightly stoppered and incubated at room temperature (30 ± 2°C) under illumination of 1000 lux. A color change in the supernatant portion of the medium after one week incubation indicated the growth of photosynthetic sulfur bacteria. The supernatant portions containing the photosynthetic sulfur bacteria were used for further isolation of different strains by serial dilution in liquid media and by the routine plating procedures.

#### RESULTS

Data recorded at the time of sampling along with some parameters are given in the table below.

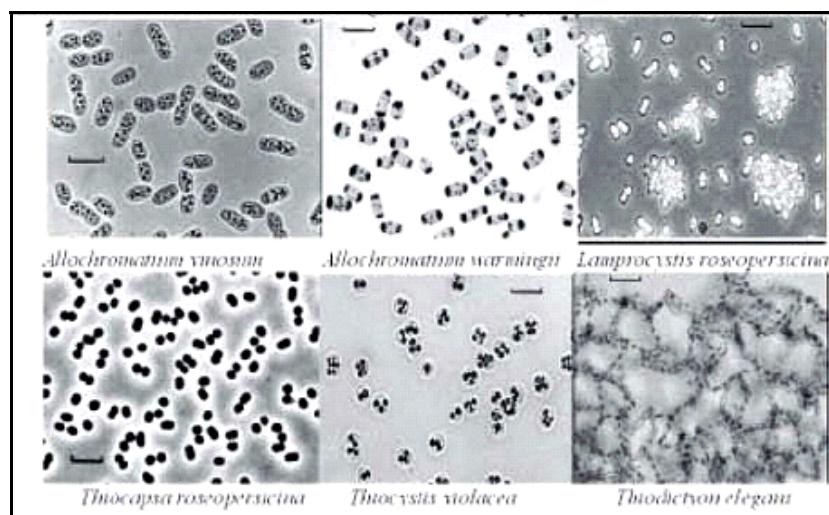
**Table 1: Physicochemical parameters of the site sampling**

S. No	Treatment	pH	Average Temperature	Relative Humidity (%)	OC (%)
1	Ambient control	6.9	28°C	50	0.13
2	550ppm	6.8	28.5 °C	42	0.18
3	700ppm	6.8	29.1 °C	38	0.22

At 700ppm CO<sub>2</sub> six strains of photosynthetic sulfur bacteria were isolated from the soil samples derived from OTCs, where as these strains were not detected at ambient and 550ppm CO<sub>2</sub> levels. This clearly indicates that the isolated strains are adapted to higher CO<sub>2</sub> levels. Further studies are in progress to identify and characterize these isolated strains of photosynthetic sulfur bacteria.

**Table 2: Isolated strains of photosynthetic sulfur bacteria from soil samples**

Feature	<i>Thiocystis violacea</i>	<i>Thiodictyon elegans</i>	<i>Lamprocystis</i>	<i>Allochromatium vinosum</i>	<i>Allochromatium warmingii</i>	<i>Thioapsa</i>
Morphology	Spherical	Rod shaped	Spherical to ovoid	Ovoid	Ovoid to rod	Spherical
Cell size (μM)	2.5-3.0	1.5-2	2.0-3.5	2	3.5-4	1.5
pH range	6.5-7.6	6.7-7.3	6.5-7.3	6.5-7.5	6.5-7.3	6.5-7.6
Temp range	25-35°C	25-35°C	25-35°C	30-35°C	30-35°C	25-35°C
Culture pigmentation	Purple-violet	Purple-violet	Purple-violet	Orange-brown	Purple-violet	Pink



**Fig. 1: Chromatiaceae isolated from the soil samples**

## CONCLUSION

Higher CO<sub>2</sub> condition appears to be more congenial for the growth of photosynthetic sulfur bacterial strains. These microorganisms can prove to be more suitable for climate change studies. The successful isolation of taxonomically distinct bacterial strains from conventional samples reinforces the hypothesis that some microorganisms remain unidentified since they are high-CO<sub>2</sub>-dependent. This in turn suggests that cultivation upon feeding CO<sub>2</sub> may become an effective method to isolate new microorganisms.

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