ABSTRACT

Microbial diversity constitutes the most extraordinary reservoir of life in the biosphere and it is the key to human survival and economic well being and provides a huge reservoir of resources which can be utilized for our benefit. The microbial diversity of the Indian subcontinent is one of the richest in the world owing to its vast geographic area, varied topography and climate, and the combination of several biogeographical regions. Though India is recognized as a one of the top 12 mega diversity regions of the world, prokaryotic diversity is very little studied in our country and the situation is even worse in studying the diversity of an important group of bacteria called Anoxygenic Phototrophic Bacteria (APB), which has a lot of biotechnological potentials (Sasikala and Ramana, 1995).

Based on sulfur metabolism, APB are broadly classified into four groups: Purple sulfur bacteria (PSB), Purple nonsulfur bacteria (PNSB), Green sulfur bacteria (GSB) and green nonsulfur bacteria (GNSB). These are physiologically and phylogenetically diverse group of bacteria with multiple colours, perform photosynthesis in the absence of air and without producing oxygen in the presence of light. They contain several types of bacteriochlorophylls and a variety of carotenoides as pigments, which function in the transformation of light into chemical energy. They are widely distributed in most of the habitats including extreme environments. Purple bacteria were extensively studied compared to green bacteria because green bacteria are obligate anaerobes, whose isolation is more complex than that of purple bacteria. These bacteria are
being commercially used since many years for several purposes, such as bioremediation, hydrogen production, enzymes, hormones, carotenoides, ubiquinones, waste water treatment, single cell protein, aqua feed and etc.

The study of bacterial diversity consists of classification, nomenclature and identification. However, the methods used in the bacterial taxonomy are being improved since their invention. The rapidly increasing number of potentially novel species, combined with the methodologically laborious polyphasic approach used in bacterial systematics, makes identification and, consequently, the description of novel taxa a highly demanding discipline.

**The thesis is organized into 5 chapters.**

**Chapter 1** includes the introduction and literature review that covered past to recent studies regarding biodiversity and its importance with a special emphasis on taxonomy, classification, occurrence, selective enrichments, isolation, and pigments of phototrophic *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*. This chapter also covered Indian scenario of the purple bacterial diversity. Broad objectives of the present work are also included in this chapter, which are as follows:

I. To study cultured diversity of phototrophic purple bacteria from diverse habitats of India.

II. To describe novel taxa, if any, based on polyphasic taxonomic analysis.
Chapter 2 provides insight into the materials and methods used for the sampling, enrichment, isolation, purification and polyphasic characterization which includes morphological, cultural, physiological, biochemical, chemotaxonomic, phylogenetic and genetic characterization of anoxygenic phototrophic purple bacteria. This chapter also included the processes of long time preservation and maintenance of bacterial stock cultures.

Chapter 3 elucidates the results of the present experimental work which is categorized into 7 sections as follows: 3.1 to 3.7

3.1 *Enrichment, isolation, purification and rapid typing of phototrophic purple bacteria from diverse habitats of India.*

A total of 75 samples were collected from diverse habitats (including aquatic and terrestrial) of India, including fresh water, marine, estuarine, extremes of temperature and saline, natural purple blooms and marine invertebrates. Among 75 samples, 65 samples showed positive results in which purple bacteria were enriched. From 65 positive enrichments, 59 pure cultures were obtained which include 35 purple non sulfur bacteria and 24 purple sulfur bacteria. Based on rapid typing of pure isolates, 11 strains were selected and subjected to polyphasic characterization after which, 9 are confirmed and described as novel species of respective genera among which, 5 were validly published and remaining 4 to be validated.

3.2 *Polyphasic characterization of Rhodobacter* sp. *JA276^T*

During investigations into the diversity of anoxygenic phototrophic bacteria in marine habitats, an ovoid to rod-shaped purple non-sulfur
bacterium, designated strain JA276\textsuperscript{T}, was isolated from enrichments under photoheterotrophic conditions from marine sediment sampled from the seashore of Cochin, India. Strain JA276\textsuperscript{T} is a Gram-negative, motile, chain-forming bacterium that shows optimum growth under photoheterotrophic conditions and is also able to grow chemoorganotrophically. Thiamine is required as a growth factor. Strain JA276\textsuperscript{T} contains vesicular intracytoplasmic membranes, bacteriochlorophyll \textit{a} and the carotenoids spheroidene and spheroidenone. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain JA276\textsuperscript{T} belongs to the genus \textit{Rhodobacter} and is closely related to the type strain of \textit{Rhodobacter capsulatus} (96.2\% sequence similarity). On the basis of the results of 16S rRNA gene sequence analysis and morphological and physiological data, strain JA276\textsuperscript{T} is significantly different from other species of the genus \textit{Rhodobacter} and represents a novel species of the genus, for which the name \textit{Rhodobacter maris} sp. nov. is proposed. The type strain is JA276\textsuperscript{T} (JCM 14794 = ATCC BAA-1549 = CCUG 55129).

### 3.3 Polyphasic characterization of \textit{Rhodobacter} spp. JA194\textsuperscript{T} and JA247

Two strains (JA194\textsuperscript{T} and JA247) of phototrophic, purple non-sulfur bacteria capable of growing at low temperatures (5\textdegree C) were isolated from the Himalayas. The two strains showed positive phototaxis and grew over a relatively wide temperature range (5-40\textdegree C). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain JA194\textsuperscript{T} clustered with members of the genus \textit{Rhodobacter}. Strain JA194\textsuperscript{T} showed
highest 16S rRNA gene sequence similarity with *Rhodobacter sphaeroides* DSM 158$^T$ (99%). However, DNA–DNA hybridization experiments between *Rhodobacter sphaeroides* DSM 158$^T$ and strain JA194$^T$ revealed a level of relatedness of only 67%. The DNA base composition of strain JA194$^T$ was 66.67 mol% G+C (by HPLC). Based on 16S rRNA gene sequence analysis, morphological, physiological, Fourier transform infrared fingerprinting and DNA–DNA hybridization studies, strain JA194$^T$ (=KCTC 5602 =JCM 14598) is sufficiently different from other *Rhodobacter* species to merit its description as the type strain of a novel species, for which the name *Rhodobacter megalophilus* sp. nov., is proposed.

### 3.4 Polyphasic characterization of *Rhodobacter sp. JA296$^T$*

An ovoid to rod-shaped, phototrophic, purple non-sulfur bacterium (JA296$^T$) was isolated from a brown-coloured microbial mat from the brackish water of Bhitarkanika mangrove forest, Dangmal, Orissa, India. Cells of strain JA296$^T$ were Gram-negative and motile, forming chains of four to eight cells. The colour of the cell suspension grown under anaerobic conditions in the light was yellowish green. Bacteriochlorophyll \( \alpha \) and the carotenoids spheroidene and spheroidenone of the spirilloxanthin series were present as photosynthetic pigments. The bacterium was a facultative anaerobe and was able to grow photo-organo- and chemooorganoheterotrophically. Thiamine was required as a growth factor. \( \text{C}_{18} : 1\omega 7c \) was the dominant fatty acid. Internal cytoplasmic membranes were of the vesicular type. Strain JA296$^T$ did not require NaCl for growth. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strain JA296$^T$ was most
closely related to *Rhodobacter capsulatus* ATCC 11166\(^T\) (95.5% sequence similarity) and clustered with species of the genus *Rhodobacter* of the family *Rhodobacteraceae*, class *Alphaproteobacteria*. On the basis of 16S rRNA gene sequence analysis and morphological and physiological characteristics, strain JA296\(^T\) represents a novel species of the genus *Rhodobacter*, for which the name *Rhodobacter aestuarii* sp. nov., is proposed. The type strain is JA296\(^T\) (=JCM 14887 =CCUG 55130).

### 3.5 Polyphasic characterization of *Ectothiorhodospira* sp. JA430\(^T\)

Strain JA430\(^T\) is a Gram-negative, vibrioid to spiral shaped phototrophic purple sulfur bacterium isolated from anoxic sediment of a saltern at Kanyakumari in a mineral salts medium that contained 2\% NaCl (w/v). Strain JA430\(^T\) grows optimally at 5-6\% NaCl and tolerates up to 12\% NaCl. Intracellular photosynthetic membranes were of the lamellar type. Bacteriochlorophyll \(a\) and carotenoids of the spirilloxanthin series are present as photosynthetic pigments. Major cellular fatty acids are C\(_{18:1}\) \(\omega7c\), C\(_{16:0}\), C\(_{19:0cyclo}\omega8c\) and C\(_{16:1\omega7c/C_{16:1\omega6c}}\). Strain JA430\(^T\) exhibit photoorganoheterotrophy and chemoorganoheterotrophy and has requirement for *para*-Aminobenzoic acid, pantothenate and pyridoxal phosphate for growth. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strains JA430\(^T\) form monophyletic group in the genus *Ectothiorhodospira*. The highest sequence similarity of strain JA430\(^T\) was found with the type strains of *Ectothiorhodospira variabilis* DSM 21381\(^T\) (96.1\%) and *Ectothiorhodospira haloalkaliphila* ATCC 51935\(^T\) (96.2\%). Morphological and physiological characteristics discriminate strain JA430\(^T\) from other
species of the genus *Ectothiorhodospira*, for which we describe this as a novel species, *Ectothiorhodospira salini* sp. nov. (=NBRC 105915 =KCTC 5805).

### 3.6 Polyphasic characterization of Blastochloris sp. JA248\(^T\)

A new Gram-negative, motile, bacteriochlorophyll \(b\) containing purple non-sulfur bacterium, strain JA248\(^T\) was isolated from phototrophic enrichments of an yellow green epilithic biofilm sample collected from Gulmarg, India. The genomic DNA G+C content of the strain JA248\(^T\) was 63.8 mol%. A phylogenetic tree based on the 16S rRNA gene sequence analysis showed that strain JA248\(^T\) had highest similarity to members of the genus *Blastochloris* and were closely related to *Blastochloris sulfoviridis* DSM 729\(^T\) (98.4%) and *Blastochloris viridis* DSM 133\(^T\) (98.3%) of the class *Alphaproteobacteria*. Strain JA248\(^T\) was characterized based on polyphasic taxonomy and the distinct phenotypic and molecular differences based on DNA-DNA hybridization (DDH; relatedness of <46.5% with the two species of *Blastochloris*), multilocus sequence analysis (MLSA), phenotypic and chemotaxonomic evidences separate strain JA248\(^T\) from other species of the genus *Blastochloris*, for which the name *Blastochloris gulmargensis* sp. nov., is proposed for the new strain. The type strain is JA248\(^T\) (=JCM 14795 =DSM 19786).

### 3.7 Polyphasic characterization of Rhodopseudomonas spp. JA310\(^T\) and JA531\(^T\)

Four strains (JA310\(^T\) JA531\(^T\) JA447 and JA490) of red to reddish brown pigmented, rod shaped, motile and budding phototrophic bacteria were isolated from soil and fresh water sediment samples from different
geographic regions of India. All strains contain BChl a and carotenoids of spirilloxanthin series. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that all strains clustered with species of the genus *Rhodopseudomonas* in the class *Alphaproteobacteria*. Strain JA531\(^T\), is genotypically (>80% homology based on DNA-DNA hybridization) and phenotypically closely related with each other and the three strains were distinct from strain JA310\(^T\) (47.6% homology based on DNA-DNA hybridization). Further, all the strains have less than 62% genome similarity with the type strains *Rhodopseudomonas palustris* DSM 123\(^T\), *Rhodopseudomonas faecalis* JCM11668\(^T\) and *Rhodopseudomonas rhenobacensis* DSM 12706\(^T\). The genomic DNA G+C (mol%) content of the strains JA310\(^T\) and JA531\(^T\) are 63.8 and 62.4. On the basis of phenotypic, chemotaxonomic and genotypic data, it is proposed that strains JA310\(^T\) and JA531\(^T\) be classified as two novel species of the genus *Rhodopseudomonas* with the species names *Rhodopseudomonas parapalustris* sp. nov. and *Rhodopseudomonas harwoodiae* sp. nov., respectively. The type strains of the proposed new species are; JA310\(^T\) (=NBRC 106083 =KCTC 5839) and JA531\(^T\) (=NBRC 107575 =KCTC 5841).

Chapter 4 provides discussion on the results obtained with respect to habitats, random sampling, selective enrichment, isolation, rapid typing and polyphasic characterization of anoxygenic phototrophic purple bacteria. The discussion also included the description of 7 novel species, i.e. *Rhodobacter maris* sp. nov. JA276\(^T\), *Rhodobacter megalophilus* sp. nov. JA194\(^T\), *Rhodobacter aestuarii* sp. nov. JA296\(^T\), *Blastochloris*

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*guilmargensis* sp. nov. JA248\(^T\), *Ectothiorhodospira salini* sp. nov. JA430\(^T\). *Rhodopseudomonas parapalustris* sp. nov. JA310\(^T\) and *Rhodopseudomonas harwoodiae* sp. nov. JA531\(^T\). Updated parameters for the description and delineation of new taxa of anoxygenic phototrophic bacteria with an importance of few techniques such as Fourier Transform Infra Red (FTIR), polar lipids, Fatty Acid Methyl Esters (FAME), DNA-DNA hybridization, and Multi Locus Sequence Analysis (MLSA) of polyphasic taxonomy were also added in the discussion. Purple bacterial species from India is compared with that of other countries. Spatial distribution and wide occurrence of strains of specific genera of phototrophic bacteria, such as *Marichromatium, Rhodobacter* and *Rhodopseudomonas* was revealed.

Objectionable reclassification of *Rhodobacter changlensis* in the genus *Rhodobacter*, legitimacy of type strain *Rhodopseudomonas palustris* ATCC 17001\(^T\) in the genus *Rhodopseudomonas* and basis for proposal of *Rhodothalassiaceae* fam. nov. and *Rhodothalassiales* order nov. are discussed. Finally, Predominance, geographical distribution and possible evolutionary divergence of purple bacteria is also discussed.

**Chapter 5** gives brief conclusions of the work with respect to results obtained, as follows:

- Phototrophic purple bacteria are widely distributed in the most of the habitats tested.
- Thirty five (35) strains of phototrophic purple nonsulfur bacteria and 24 strains of purple sulfur bacteria were isolated.
Strains of the genus *Marichromatium* of purple sulfur bacteria, and *Rhodobacter* and *Rhodopseudomonas* of purple nonsulfur bacteria could be cultured abundantly from marine and fresh water habitats respectively.

First report of strains of the genera *Rhodomicrobium*, *Blastochloris* and *Rhodothalassium* from India

Eight (8) strains of purple nonsulfur bacteria and one (1) strain of purple sulfur bacteria were subjected to detailed characterization using polyphasic taxonomy.

Description of novel species, *Rhodobacter maris* JA276<sup>T</sup> sp. nov., *Rhodobacter megalophilus* JA194<sup>T</sup> sp. nov., *Rhodobacter aestuarii* JA296<sup>T</sup> sp. nov., *Blastochloris gulmargensis* JA248<sup>T</sup> and *Ectothiorhodospira salini* JA430<sup>T</sup> sp. nov. is validly published.

Description of two novel species, *Rhodopseudomonas parapalustris* JA310<sup>T</sup> and *Rhodopseudomonas harwoodiae* JA531<sup>T</sup> is under revision.

Geographical mapping of isolates was done.

Most of the strains isolated were preserved by lyophilization, while the 16S rRNA gene sequences are deposited with EMBL.

The type strains are available at DSMZ /ATCC /JCM /KCTC/ NBRC/ABRC and are accessible to public.

Future prospects of the present work are also included in the discussion.

Chapter 6 consists of references used and cited in the entire thesis.