"The micro-organism is always right, your friend and a sensitive partner........."

"If you take care of your microbial friends they will take care of your future"

Dr. D. Perlman
SYNOPSIS

Antarctica is the fifth largest continent on the Earth and is the only continent with a pristine environment. The permanent ice-cover, extreme low temperature, strong winds, low humidity and non-availability of water, make the continent unique and uninhabitable. The average annual temperature of the continent is maintained between -20°C to -30°C and in summer the temperature reaches to a maximum of +5°C. Most of the lakes and ponds are covered with thick ice sheet during the winter months. But, during the summer months, the ice melts. The climatic conditions support only few life forms such as lichens, algae, fungi, cyanobacteria and bacteria. All these life forms are bound to be unique due to their ability to survive and divide under the prevailing harsh climatic conditions. Bacteria are known to be extremely versatile and can adapt to any climatic condition and Antarctica is no exception. Therefore, sampling of bacteria from Antarctica may result in identification of novel and unique species and such species may have biotechnological application. Till now, only 63 species of Gram-negative bacteria belonging to 34 different genera and 34 species of Gram-positive bacteria belonging to 16 different genera have been identified from various habitats of Antarctica. In the present study, an attempt has been made to study the diversity of Antarctic bacteria from one particular habitat, the cyanobacterial mats collected from different lakes and ponds of McMurdo dry valley region, Antarctica.

Chapter 1 is a brief review on the bacterial biodiversity of Antarctica. It highlights at the start of the chapter, the importance of biodiversity, in general and the need for exploration and conservation of biological species, including bacteria. The history of Antarctic Microbiology is also briefly presented so as to unveil what little is known about the bacteria of Antarctica, with respect to their taxonomy, phylogeny and
biotechnological potential. This background, in fact, leads to the following four objectives, namely:

1. To isolate and identify the bacteria present in association with cyanobacterial mats up to the species level.
2. To determine the phylogenetic position of the unique species isolated from the mat samples.
3. To establish the physiological grouping on the basis of their enzymes involved in biodegradation and to correlate these enzymatic activity with nutrient regeneration.
4. To evaluate the biotechnological potential of these microorganisms.

Chapter 2 deals with the Materials and Methods employed, the source of the various chemicals used and the bacteria used.

In Chapter 3, a detailed account of the experimental results is given. The initial part of this Chapter from Section 3.1 to 3.7 deals with the results obtained in identifying a particular bacterial species. To this end, a brief introduction is provided and the results are highlighted and discussed species-wise, so as to establish the identity and phylogeny of the species.

A total of 76 bacteria were isolated from 12 different cyanobacterial mat samples collected from different lakes, ponds and streams of McMurdo region, Antarctica. Preliminary characterization resulted in grouping of all the bacteria in to 5 genera belonging to Gram-positive bacteria and 2 genera belonging to Gram-negative bacteria.
1. **Identification of Kocuria polaris** sp. nov.

Strain CMS 76or\(^T\), an orange pigmented bacterium isolated from cyanobacterial mat samples from L9 pond of Wright valley, located in McMurdo Dry Valleys, Antarctica. Based on the phenotypic and phylogenetic characteristics, CMS 76or\(^T\) was identified as a member of the genus *Kocuria*. It exhibited a 16S rDNA similarity of 99.8% and DNA-DNA similarity of 71% with *K. rosea* (ATCC 186\(^T\)). However, phenotypic and phylogenetic traits confirmed that CMS 76or\(^T\) and *K. rosea* are well differentiated. CMS 76or\(^T\) differed from *K. rosea* in that it was psychrotrophic, less tolerant to NaCl, arginine dihyroolase positive and nitrate reduction negative. MK-6 (H\(_2\)), MK-7(H\(_2\)), MK-8(H\(_2\)) and MK-9(H\(_2\)) were the four major menaquinones and it had increased quantities of iso-C\(_{17:0}\) and iso-C\(_{18:0}\) and decreased quantities of iso-C\(_{14:0}\), iso-C\(_{16:0}\) and C\(_{18:0}\). It is therefore proposed that CMS 76or\(^T\) be classified as *Kocuria polaris* sp. nov. The type strain of *K. polaris* is CMS 76or\(^T\).

2. **Identification of Arthrobacter flavus** and **Arthrobacter roseus** spp. nov

The yellow pigmented and red pigmented CMS 90r and CMS 92r were isolated from E4 and L4 ponds of Wright Valley of McMurdo Region, Antarctica. These bacteria confirmed to the phenotypic and chemotaxonomic characteristics of the genus *Arthrobacter*. Further, the 16S rDNA gene analysis indicated that CMS 19y\(^T\) is closely related to *Arthrobacter agilis* (97.91%). DNA-DNA hybridization studies also indicated 77% homology between CMS 19y\(^T\) and *A. agilis*. But, it differed from *A. agilis* in that it was psychrophilic, non-motile, yellow pigmented, exhibited rod-coccus growth cycle, had higher degree of tolerance to NaCl and was oxidase positive. In addition, it had a distinct fatty acid composition compared to that of *A. agilis*. It is, therefore, identified as a new species and was named as *Arthrobacter flavus* sp. nov.
CMS 90r and CMS 92r exhibited identical properties at phenotypic and genotypic level and probably are clonal in origin and therefore, CMS 90r was taken as the representative strain and characterized in detail. CMS 90r^T based on its phenotypic characteristics and 16S rDNA sequence was identified as a member of Group I Arthrobacter. It shared a 16S rDNA similarity of 98% with A. oxydans and A. polychromogenes. Further, DNA-DNA similarity between CMS 90r^T and A. oxydans and A. polychromogenes was less than 70%. It also differed from all the reported 17 Arthrobacter species with A3α-variant peptidoglycan in that they possessed a unique peptidoglycan (Lys-Gly-Ala3), had galactose, glucose, ribose and rhamnose as the cell wall sugars and did not utilize glycine, threonine, lysine, tyrosine and histidine. They specifically differed from A. oxydans and A. polychromogenes in that they were psychrotrophic, red pigmented, did not utilise nicotine, did not hydrolyse either starch or aesculin and also contained the fatty acids C_{16:1} and C_{18:2}. It is proposed that CMS 90r^T be classified as Arthrobacter roseus sp. nov.

3. Identification of Planococcus antarcticus and Planococcus psychrophilus sp. nov.

Thirteen orange-pigmented bacteria were isolated from cyanobacterial mat samples collected from four different lakes (Balham lake, E4 and SF1 ponds and Lake Canopus) in McMurdo Antarctica. Twelve of the isolates, which were coccoid in shape, were very similar and possessed all the characteristics of the genus Planococcus and represented a new species which was assigned the name Planococcus antarcticus sp.nov (CMS 26or^T). Apart from the phenotypic differences, P. antarcticus differed from all the reported species of Planococcus by more than 2.5 % at the 16S rDNA sequence level and exhibited very little similarity either with P. mcmeekinii (30-35 %), P. okeanokoltes (26-29 %) or CMS 53or^T (15-25%), the three species with which it is closely related at the 16S rDNA sequence level (2.5 - 2.9%).
CMS 53or\textsuperscript{T}, the thirteenth isolate, was also identified as a new species of \textit{Planococcus} and was assigned the name \textit{Planococcus psychrophilus sp.nov.} (later amended to \textit{Planomicrobium psychrophilus}). This species was distinctly different from all the reported species, including the present new species \textit{P. antarcticus}, with respect to a number of phenotypic characteristics. Based on the 16S rDNA sequence, it was closely related to \textit{P. okeanokoites} (98.1 \%) and \textit{P. mcmeekinii} (98 \%) but based on DNA-DNA hybridization the similarity was only 35-36\%.

4. **Identification of \textit{Leifsonia rubeus} and \textit{Leifsonia aureus} spp. nov.**

One red pigmented and six yellow pigmented psychrophilic \textit{Leifsonia} species were isolated from a cyanobacterial mat sample from L9 pond in Wright Valley, McMurdo, Antarctica. The results indicated that the six yellow pigmented bacteria were clonal in origin and therefore, CMS 81y was chosen as the representative strain. Both the isolates (CMS 76r\textsuperscript{T} and CMS 81y\textsuperscript{T}) were assigned to the genus \textit{Leifsonia} based on their phenotypic and chemotaxonomic characteristics. In addition, both the isolates were related to the reported five species of \textit{Leifsonia} (\textit{L. aquatica}, \textit{L. poae}, \textit{L. xyli} subsp \textit{xyli}, \textit{L. xyli} subp \textit{cynodontis}, \textit{L. naganoensis} and \textit{L. sinshuensis}) at the 16S rDNA sequence level by about 95 to 96\% and between themselves they differed by 2.5\%. CMS 76r\textsuperscript{T} and CMS 81\textsuperscript{T} also differed from one another with respect to many other phenotypic characteristics and exhibited only 30\% homology at the DNA-DNA level, thus, indicating that they are two different species. Further, these two isolates also showed many distinct differences with the reported species of \textit{Leifsonia} (\textit{L. aquatica}, \textit{L. poae}, \textit{L. xyli} subsp \textit{xyli}, \textit{L. xyli} subp \textit{cynodontis}, \textit{L. naganoensis} and \textit{L. sinshuensis}) with respect to their phenotypic characteristics, biochemical properties, chemotaxonomic features, sensitivity to various antibiotics and at the level of 16S rDNA similarity clearly indicating that CMS
76r\textsuperscript{T} and CSM 81\textsuperscript{T} are two new species of *Leifsonia* and have been assigned the names *L. rubea* sp. nov. and *L. aureus* sp. nov.

5. **Identification of *Sporosarcina mcmurdo* sp. nov.**

Six bacteria isolated from L8, E4 and L4 ponds Wright Valley located in McMurdo Dry Valleys, Antarctica exhibited identical properties and therefore, CMS 21w\textsuperscript{T} was chosen for detailed characterization. The strain CMS 21w\textsuperscript{T} is a Gram-positive, rod-shaped, motile bacterium that forms terminal or sub-terminal spores. The mole\% G+C content of the DNA was found to be 44\% and the major fatty acid was anteiso-C\textsubscript{15:0} and iso-C\textsubscript{16:1}. Based on the phenotypic and phylogenetic characteristics CMS 21w\textsuperscript{T} was identified as a member of the genus *Sporosarcina*. It exhibited a 16S rDNA similarity of 93 to 96\% with the species of the genus *Sporosarcina* and more closely with *Sporosarcina globisporous* (96.11\%) and *Sporosarcina psychrophilus* (95.97 \%). However, phenotypic and phylogenetic traits confirmed that CMS 21w\textsuperscript{T} and *S. globisporous* and *S. psychrophilus* are well differentiated and CMS 21w\textsuperscript{T} was identified as a new species of the genus *Sporosarcina* and was named as *Sporosarcina mcmurdo* sp. nov.

6. **Identification of *Psychrobacter psychrophilus*, *Psychrobacter vallis* and *Psychrobacter aquaticus* spp. nov.**

Fourteen Gram-negative, coccoid, non-motile bacteria were isolated from L3, SF1 ponds of Wright Valley, Adam’s glacier stream and Lake Canopus. Based on the SDS-PAGE analysis all the 14 isolates were grouped in to 3 groups. One isolate from each group (CMS 30, CMS 39 and CMS 56 respectively) was studied in detailed. Based on the phenotypic, chemotaxonomic and 16S rDNA analysis, the three isolates were assigned to the genus *Psychrobacter* and were observed to be closely related to *P. proteolyticus*, *P. glacincola*, *P. frigidicola* and *P. urativorans*. DNA-DNA hybridization.
between Antarctic isolates and *P. proteolyticus*, *P. glacincola*, *P. frigidicola* and *P. urativorans* indicated a homology of 10 to 50%. Thus, the three isolates were assigned the status of a new species and were named as *Psychrobacter psychrophilus* sp. nov. (CMS 30), *Psychrobacter vallis* sp. nov. (CMS 39) and *Psychrobacter aquaticus* sp. nov. (CMS 56).

7. Identification of *Pseudomonas orientalis*, *Pseudomonas polaris* sp. nov. and *Pseudomonas brenneri*

Thirty one Gram-negative, rod shaped, motile bacteria belonging to the genus *Pseudomonas* were isolated from L1, L3 ponds of Wright Valley, Lake Canopus, Adam’s glacier stream1 and 2 and Canada glacier stream of Miers Valley located in McMurdo Dry Valley region of Antarctica. Based on the SDS-PAGE analysis, all the isolates were grouped in to 4 groups represented by CMS 35 (Group I), CMS 38 (Group II), CMS 45 (Group III) and CMS 64 (Group IV), respectively.

Phylogenetic analysis using 16S rRNA indicated that CMS 35 and CMS 45 are closely related to *Pseudomonas orientalis* sharing more than 99% homology at the 16S rDNA level and 90% at the DNA-DNA level. Thus, these two isolates were identified as psychrophilic strains of *Pseudomonas orientalis*. Isolate CMS 64 formed a robust cluster with the clade representing *P. brenneri* and *P. migulae*. It shared an identity of >99.5% with both *P. migulae* and *P. brenneri* at 16S rDNA sequence level. Further, CMS 64 shared a homology of 55% at whole genome level with *P. migulae*, indicating that it is different from *P. migulae*. Though, the DNA-DNA hybridization was not performed due to the non-availability of the strain *P. brennerii*, based on the identical phenotypic characteristics it exhibited with *P. brennerii*, CMS 64 was identified as an isolate of the species *P. brennerii*. Strain CMS 38 formed a coherent cluster with a clade representing Antarctic isolates CMS 35, CMS 45 and *P. orientalis*. It shared more than 99.5%
similarity at 16S rRNA but at whole genome level it shared only 50 to 65% with Antarctic isolates and other closely related species of the genus *Pseudomonas*. Apart from the above differences, it showed distinct phenotypic differences with *P. orientalis*, CMS 35 and CMS 45 and was therefore, identified as a new species of the genus *Pseudomonas*, for which the name *Pseudomonas polaris* sp. nov. was given.

In this chapter, in addition to establishing the taxonomic position and phylogenetic relationship of the 76 isolates, attempts were also made to group all the 76 isolates based on the enzymes produced by them. The results indicated that all the 76 isolates could be grouped into 11 physiological groups based on the number and the specific enzyme produced. Further, the enzymes from all the isolates were partially characterized for their activity at different temperatures, pH and salt concentrations. The amylase characterized from CMS 21w and CMS 76or exhibited activity from 4°C to 30°C, between pH 6 to 11 in the presence of 1 to 5% salt concentration and could be heat-inactivated above 50°C indicating that the amylase is a cold-active and a heat-labile enzyme. The lipase activity was detected in 31 isolates belonging to the genera *Psychrobacter*, *Pseudomonas* and *Kocuria* and the enzyme showed activity between 4°C to 30°C, pH 6 to 11 and in the presence of 1 to 5% salt. Heat-inactivation studies indicated that the enzyme could be inactivated above 65°C. Extra-cellular protease was detected in 29 out of the 76 Antarctic isolates and the maximum activity was seen in isolates belonging to the genera *Planococcus* (CMS 26or), *Planomicrobium* (CMS 53or), *Arthrobacter* (CMS 19y and CMS 90r), *Sporosarcina* (CMS 21w) and *Pseudomonas* (CMS 64). The enzyme from all the above six isolates exhibited activity between 4°C to 30°C, pH 6 to 11 and salt had no effect on the activity. The enzyme could be heat-inactivated at 50°C. Phosphatase activity was observed in 37 isolates and the isolates belonging to *Psychrobacter* produced the maximum amount. The enzyme was active
4. CMS 26or, an orange-pigmented Gram-positive, motile, coccoid bacterium was identified as \textit{Planococcus antarcticus} and another orange pigmented, rod shaped and motile bacterium, CMS 53or, was identified as \textit{Planomicrobium psychrophilus}.

5. CMS 76r and CMS 81y, a red pigmented and a yellow-pigmented, motile, Gram-positive, rod-shaped bacteria were identified as \textit{Leifsonia rubea} and \textit{Leifsonia aureus}, respectively.

6. CMS 21w, a rod shaped, motile and sporulating bacterium was identified as \textit{Sporosarcina mcmurdo}.

7. CMS 30, CMS 39 and CMS 56, Gram-negative, non-motile, coccoid bacteria were identified as \textit{Psychrobacter psychrophilus}, \textit{Psychrobacter vallis} and \textit{Psychrobacter aquaticus}, respectively.

8. CMS 38, a Gram-negative, motile and rod-shaped bacterium was identified as \textit{Pseudomonas polaris}, two isolates CMS 35 and CMS 45 were identified as new isolates of the species \textit{Pseudomonas orientalis} and CMS 64 as a new isolate of the species \textit{Pseudomonas brennerii}.

9. Based on the activity of the enzymes amylase, lipase, protease and phoshatase, all the bacteria were divided into 11 physiological groups.

10. The biotechnological potential of the new species with respect to the enzymes produced and other biological products such as pigments, fatty acids, and menaquinones was evaluated.