## 6. Summary

Microbes are ubiquitous in nature, expanding their presence in all components of biosphere which can be studied broadly using two methods- (1) blind study, and (2) targeted study. In this work targeted study was taken into consideration. Here, screening starts from the very first step and is narrowed down to targeted organism. Glutaminase was selected as target molecule due to its wide application in food and pharmaceutical industry. Production of Glutaminase at industrial scale requires the enzyme to be active at high salt concentration, high pH, high temperature and glutamine-specific. Soil samples were collected from Mangrove ecosystem (Southern coast of India) and Glutaminase producing bacteria were isolated from the samples. 77 positive isolates were obtained and 2 isolates giving maximum activity on plate were identified as *A. baumannii* and *A. guillouiae* using 16S rDNA sequencing. Glutmainase gene was amplified from *A. baumannii* MSP4-16, cloned, expressed and purified from *E. coli*. Glutaminase from MSP4-16 showed high thermo stability, high optimum pH and a haloactive nature which makes it a good commercial molecule.

As there was no previous record of *A. baumannii* from Mangrove ecosystem, we tried to study the difference between the environmental isolate MSP4-16, type strain ATCC 17978 (clinical isolate) and other ten *A. baumannii* isolates. Comparative analysis shows that environmental isolate MSP4-16 shows very near genomic feature with ATCC 17978. Few differences in genomic features may be due to environmental stress.

One of the major problems associated with *Acinetobacter* species is their identification. This study implies to revisit the Genus using latest taxonomic techniques available along with commercial identification methods which include biochemical study and chemotaxonomy, VITEK® 2 identification, 16S rRNA gene sequencing, MALDI characterization, and whole genome taxonomic analysis. In this study, 27 species of *Acinetobacter* were analyzed; 24 among them were type species, 2 environmental isolates and one synonym. An extended biochemical characterization and antibiosis study was done. Biochemical properties were very similar to the already reported studies while an extensive antibiosis analysis was an addition to the previously available data. In chemotaxonomic study, polar lipids and fatty acid profile were studied. There is very little

description of polar lipid and fatty acid profile of the Genus. Also the available data was not generated under similar condition. Polar lipid analysis was done in this study and it was found that DPG, PE, PG and PC are major lipids for all the species with few exceptions for PE. This was first time where such an extensive study was done for polar lipid analysis and hence, this should be included in the Genus description of *Acinetobacter*. Fatty acid profiles were generated for all the 27 species under similar conditions and it was found that major fatty acids were 18:1ω9*C*, 16:0, Summed feature 3, 12:0, 12:0 2OH, 12:0 3OH and Summed feature 2. Pathogenic bacteria identification using VITEK® 2 cards has long been in practice and it is one of the foremost technology used in clinical diagnostics. We analyzed all 27 species for both substrate utility and identification as there was no previous data for such comparative analysis. It was found that in identification perspective VITEK® 2 was able to identify only one species correctly. It was found from our analysis that there are differences in VITEK® 2 profile in *Acinetobacter* and the database needs to be updated accordingly.

16S rDNA analysis is one of the most important methods for identification and describing novel species. One of the major problems in this method is of incomplete sequences in databases. In this part of work, we have sequenced complete gene (nearly 1500 bases) so that a reliable phylogenetic analysis can be obtained. After sequencing the complete gene, NJ, MP, ML phylogenetic trees were constructed which showed that the whole Genus can be divided into three groups: Group one *A. calcoaceticus* group, Group two *A. soli* group, and Group three *A. bereziniae* group. This grouping came into picture only after using complete gene sequence which shows its importance instead of partial sequencing. The same sequence data was used to generate ARDRA banding profile using 17 different enzymes. Out of these 17 enzymes, EcoRI and SspI showed similar digestion pattern throughout the Genus and can be used for Genus identification. Secondary structure prediction was also done using 16S rRNA gene sequences. CENTROID FOLD web server was used for this purpose in which it tried to predict a minimum energy state structure with pseudoknots. There was lot of variability in the predicted structures of various species. Hence, in current scenario 16S rRNA secondary structure for species description and identification should not be used.

Apart from 16S rRNA gene analysis, MALDI is one of the current and futuristic technology for species identification. Practice for identification of bacterial species using MALDI is merely two decades old but recent with new computer algorithm has found its place as reliable technology. It is quick and less labour intensive compared to any method in practice. MALDI analysis of 27 species using ethanol/ formic acid extract and direct cell loading was done. Mass spectrum profiles obtained were used for species identification and it was found that most of the species were identified correctly using both methods barring some exceptions. Pseudogel analysis using MALDI profile gives a clear picture of unique band for each species of the Genus which can be used as its identification trait. Dendogram obtained using MSP and PCA gives a relational analysis between the species which is quite different from the one obtained from 16S rRNA gene analysis. This may be due to difference of subject under study. Instead we can use CCI for relational analysis which gives a much better picture. This is the first and foremost criteria for description of species of Genus *Acinetobacter* under similar condition using MALDI and the data obtained can be used to update the database for future identification.

With growing technology and availability of complete genomes, use of these genome sequences as identification tool has recently grown. In this study, we have analyzed few of the benchmarking methods used for species description and identification. Digital DNA-DNA hybridization has potential to replace wet lab DNA-DNA hybridization whereas ANI can be used for species delineation. Other methods such as SpeciesFinder, rMLST, KmerFinder, Reads2Type and PathogenFinder show promise in species description and identification but this still needs to be further improved as in the background single gene or a Kmer is being used instead of whole genome. On the basis of the present study, emendations are proposed for the Genus *Acinetobacter* and its twenty-four species which were part of this study

So, in final words what is the conclusion of whole thesis? We as a member of taxonomist society have to come out of closet and work in a more applied manner. We are not saying that people are not doing it but we need to change our approach and stretch out ourselves for the final product either in a self-sustained or in a collaborative manner which currently lacks in our society. Microbes are storehouse of millions of molecules which are yet to be harnessed by mankind and being a taxonomic microbiologist, we belong to that group

which have first interaction with any novel organism. Identifying the actual capabilities of an organism at very first stage can give us more options of novel molecules to choose from. We further need to address the question why an organism is unclassified, unidentified and misidentified in a serious manner. There are many more Genus other than *Acinetobacter* which are taxonomically unstable and need serious revisit like this present study. Instability can be due to many reasons which can be summarized as follows:

1) Not following set rules for identification and sometimes lack of defined rules for identification,
2) Logical up gradation of rules which give new horizon to study rather than confusing the whole scenario,
3) Lack of basic understanding of microorganism and their predicted evolution,
4) Lack of skilled workforce in this field. Skill should not only be of hands but also of novel thoughts,
5) Right interpretation of the data is necessary and always being sceptical about the results gives space for new thoughts and research.

The most important of all do taxonomy as passion rather than just routine work or in simple words I would like to quote myself "Do taxonomy to learn, don't learn to do taxonomy".