INTRODUCTION
Chapter-1

Introduction

Naturally occurring process, the green house effect that aid in heating Earth’s surface atmosphere. It results from the fact that certain atmospheric gases, such as Carbon dioxide, Water vapor and Methane are able to change the energy balance of the planet by absorbing long wave radiation emitted from the Earth’s surface. The methane gas occupies second place in Green house. Methane in atmosphere has increased by more than 150% to termites, landfills, coal mining, and oil and gas extraction. The anaerobic conditions associate with rise paddy flooding results in the formation of methane gas. At between 50 and 100 million tones of methane a year, rice agriculture is a big source of atmospheric methane, possibly the biggest of man made methane sources.

1.1 Rice and methane

Rice is a staple crop food for nearly half of the world’s population. Unfortunately, anoxic conditions in the Wetland soil rice paddies are ideal for microbes that produce methane, which traits only Carbon dioxide in terms of the green house effect. As the world’s population continues to grow, rice production will follow, bringing more climate warming methane with it. The recent findings noticed that optimizing crop yields could provide more food and surprisingly, curtail production of methane. Rice production currently accounts for
approximately 13 percent of global methane emissions. The scientists suggest that developing varieties of rice capable of storing more carbon, perhaps in additional shoots, could help mitigate methane production. Ronald L. Sass of Rice University and Ralph J. Cicerone of the University of California, Irvine, note in an accompanying commentary that population increases and recent climate change make such efforts necessary. "The food demands of an increasing world population and the disruptive effect of global warming," they write, "both challenge the agricultural science community to pay attention to how these two environmental pressures interact and to accelerate efforts to develop higher yielding, farmer-friendly rice that emits less methane."

With an increasing world population, reductions in rice agriculture remain largely untenable as on methane emission reduction strategy. However, through a more integrated approach to rice paddy irrigation and fertilizer application substantial reductions remain possible. Many rice varieties can be grown under much drier conditions than those traditionally employed, with big reductions on methane emission without any loss in yield. Additionally, there is the great potential for improved varieties of rice, able to produce a much larger crop per area of rice paddy and so allow for a cut in the area of rice paddies, without a cut in rice production. Finally, the addition of compounds such as ammonium sulphate, which favour activity of other microbial groups over that of the Methanogens, has proved successful under some conditions.

Methane and its oxidation product methanol, have occupied an important position in the chemical from which many products are produced. More recently, the role played by
methanol as a potent "Green House" gas and has aroused considerable attention from environmentalists and climatologists alike. The role for C1 compounds has, of course, been quite incidental to the myriad of microorganisms on this planet that have adapted their lifestyle to take advantages of these readily available ambient sources.

Methane, a renewable energy sources that will always be with us, is actually a difficult molecule to activate. So any microorganism that can affect this may point the way to catalytic chemists looking for controllable. Methane oxidation, methanol, formed as also a ubiquitous and has also encouraged the growth of prokaryotes and eukaryotic. The warm water logged soil of rice paddies provides ideal conditions for Methanogenesis and though some of the methane produced is usually oxidized by Methanotrophs in the shallowing overlying water, the vast majority is released into the atmosphere.

1.2. Methylotrophy

1.2.1. Model system: Methylotrophy

Methylotrophy is the capacity to aerobically utilize single-carbon (C1) compounds as a sole source of carbon and energy. This consists of three stages: 1.) oxidation of C1 compounds to formaldehyde, 2.) dissimilation of formaldehyde to CO2, and 3.) assimilation of formaldehyde into biomass. What is remarkable about this capacity is that nearly all such C1 compounds are oxidized to formaldehyde as a central metabolite, which itself can be burned for energy or drawn into assimilatory metabolism.

This presents an intriguing problem: fast growth requires a high flux through formaldehyde, but the pool must remain low or else it would pickle the cell. It has been calculated that if
formaldehyde production went unchecked by utilization for just one minute, the cell would fill to over 100 mM formaldehyde. Thus, methylotrophs must maintain a healthy balance of production/consumption, while also efficiently proportioning carbon to assimilatory and dissimilatory metabolism?

1.2.2. So why study methylotrophy?

There are multiple aspects to methylotrophy that make it a wonderful model system. On the practical side, methylotrophs play a key role in the global cycling of C₃ compounds (such as methane) and offer intriguing biotechnological opportunities for the production of commodity chemicals from methanol. As mentioned above their physiology revolving around the internal production and consumption of a toxic intermediate, formaldehyde, is fascinating and involves the action of multiple unique metabolic modules that are not commonly present in other organisms.

It is the apparent evolutionary history of methylotrophy, however, that is perhaps the most compelling reason to pursue these organisms for our studies. First, methylotrophy clearly does not have a monophyletic origin. Examination of a phylogenetic tree of bacteria shows that methylotrophs are found in multiple clades of bacteria with non-methylotrophic sister taxa. However, the genes required for methylotrophic growth are highly conserved amongst methylotrophs, are found in large gene clusters, and have phylogenies that are often incongruent with that of the "organismal" 16S rDNA tree. These observations are consistent with methylotrophic lineages having arose multiple times, apparently as a consequence of horizontal gene transfer (HGT, genetic exchange between microbes -sometimes from different clades entirely) of methylotrophy gene clusters.
1.2.3. *Methylobacterium extorquens AM1*

*M. extorquens* AM1 is the best-understood methylotroph. It is an alpha-proteobacterium closely related to rhizobia and is a facultative methylotroph (can grow on C1 and multicarbon substrates). The figure to the right of a clover leafprint applied to a methanol-containing plate illustrates its niche as a leaf epiphyte (and yes, leaves really do make methanol - lots of it).

Approximately 100 methylotrophy genes have been identified thus far in *Methylobacterium* and the genome sequence (~8 MB) is nearly completed. Furthermore, a stoichiometric model of central metabolism has been generated and tested, expression microarrays and proteomic methods have been developed, and a series of genetic tools have been developed. These advances have made *Methylobacterium* a powerful model organism.

Methylotrophy refers to the ability of an organism to use C1 compounds as energy sources. These compounds include methanol, methylamines, formaldehyde and formate. Several other, less common substrates may also be used for metabolism, all of which lack carbon-carbon bonds. Examples of Methylotrophs include the bacteria *Methylomonas* and *Methylobacter*. Methanotrophs are a specific type of Methylotroph that are also able to use methane (CH3) as a carbon source by oxidizing it sequentially to methanol (CH3OH), formaldehyde (CH2O), formate (HCOO⁻) and finally carbon dioxide (CO2) initially using the important enzyme methane monooxygenase. An oxygen is required for this process, all (Conventional) Methanotrophs are obligate aerobes.

Reducing power in the form of quinones and NADH is produced during these oxidations to produce a proton motive force and therefore ATP generation. Methylotrophs

5
and Methanotrophs are not considered as autotrophic, because they are able to incorporate some of the oxidized methane in to cellular carbon before it is completely oxidized to CO₂ (at the level of formaldehyde), using either the serine pathway (*Methylosinus* and *Methylcystis*) or the ribulose monophosphate pathway (*Methylococcus*), depending on the species on Methylocotroph.

In addition to aerobic Methylocotrophy, methane can also be oxidized anaerobically. This occurs by a consortium of sulfate- reducing bacteria and relatives of methanogenic archa working syntrophically.

Methanogenesis is the biological production of Methane . It is carried out by methanogens , strictly anaerobic archaea such as *Methanococcus*, *Methanocaldococcus*, *Methanobacterium*, *Methanothermus*, *Methanosarcina*, *Methanosaeta* and *Methanopyrus*, The biochemistry of methanogenesis is unique in nature in its use of a number of unusual cofactors to sequentially reduce Methanogenic substrates to Methane. These cofactors are responsible (among other things) for the establishment of a proton gradient across the outer thereby driving ATP synthesis. Several different types of Methanogens occurs, which differ in the starting compounds oxidized . Some Methanogens reduce carbon dioxide (CO₂) to Methane (CH₄) using electrons (most often) from hydrogen gas ( H₂) chemolithoautotropically . These Methanogens can often be found in environment containing fermentative organism.
The tight association between Methanogens and fermentative bacteria can be considered to be syntrophic because the Methanogens, which rely on the fermentors for hydrogen, relieve feedback inhibition of the fermentors by the build up of excess hydrogen that would otherwise inhibit their growth. This type of syntrophic relationship is specifically known as interspecies hydrogen transfer.

A second group of Methanogens use methanol (CH₃OH) as a substrate for Methanogenesis. These are chemoorganotrophic, but still autotrophic in using CO₂ as only carbon source. The biochemistry of this process is quite different from that of the carbon dioxide reducing Methanogens. Lastly, a third group of Methanogens produce both methane and carbon dioxide from acetate (CH₃COO⁻) with the acetate being literally split between the two carbons. These acetate-cleaving organism are the only chemoorganotrophic Methanogens. All autotrophic Methanogens use a variation of the acetyl-CoA pathway to fix CO₂ and obtain cellular carbon.

Naturally occurring substances with Indole nucleus possessing growth promoting activity are referred to Auxins. In many infected plants IAA increases (Hyperauxiny) but in a few cases, definitive decreases in Auxin. Any changes in Auxin level has a direct effect on the physiology of the plant and efforts show that the symptoms of the host are due to Auxin change rather than to the pathogen.
1.3. Auxin (IAA)

Auxin is a growth hormone which induces the germination of plants. Auxin accumulation is attributed to:
1. Rapid synthesis by the infected plant.
2. Synthesis by the pathogen.
3. Operation of new pathways of IAA synthesis such as release of IAA protein complex
4. Inhibition of Auxin destruction catalysed by IAA oxidase and Peroxidase.

1.4. Rhizobial Inoculant formulations

Worldwide, legumes are grown on approximately 250 million hectares and fix about 90 Tg (or $9 \times 10^{13}$ g) of dinitrogen ($N_2$) per year. *Rhizobium* spp. and *Bradyrhizobium* spp. are major contributors to overall $N_2$ fixation through the legume-rhizobium symbiosis and have been used for over a hundred years as beneficial microorganisms. For example, the improvement of crop production through mixing of soil containing naturally-occurring rhizobia with seeds became a recommended practice in the USA by the end of the 19th century. Since then considerable improvement in this inoculant technology has been made, resulting in the successful commercialization of rhizobial inoculants by several companies around the world.

Development of a successful inoculant involves several critical elements such as strain selection, selection of a carrier and mass multiplication, formulation of the inoculant,
and packaging and marketing. Stringent quality assurance at various steps of production ensures the production of consistently high quality inoculants.

Formulation is a crucial aspect for producing inoculants containing an effective bacterial strain and can determine the success or failure of a biological agent. Formulation typically consists of establishing the active ingredient (i.e., microorganism) in a suitable carrier together with additives that aid in the stabilization and protection of the microbial cells during storage and transport, and at the target site. Whether a product is new or improved, it is imperative that the formulation be stable during production, distribution, storage, and transportation. The formulation should also be easy to handle and apply so that it is delivered to the target in the most appropriate manner and form, protects the agent from harmful environmental factors, and maintain or enhance activity of the organism in the field. Another important consideration is the cost-effectiveness of the formulation.

1.5. Biotechnological applications with Methylotrophs

Methylotrophs are ubiquitous microorganisms that can use C1 compounds (methanol, methylamine, methane, etc.) for growth. Some Methylotrophs (especially the Methanotrophs, those that can utilize the gas methane, which possess a special enzyme, Methane MonoOxygenase, MMO) can degrade pollutants (aromatics, trichloroethylene), but most strains available in the labs are not very resistant in real contaminated conditions. We have been isolating new, more robust Methylotrophs from a variety of environments that can serve in bioremediation directly, or in co-remediation, or as vectors for degrading enzymes (oxygenases).
Methanotrophs are amenable for biotech applications: they grow on a cheap and clean substrate (methane), can be genetically manipulated and, for one of them, *Methylococcus capsulatus* strain Bath, the genome sequence is available. For these reasons we are constructing engineered Methanotrophs with the aim of producing recombinant proteins and other added-value products.

1.6. Methanesulfonic acid utilizers

Some methylotrophic bacteria can grow utilising methanesulfonic acid (MSA), a very simple sulfur compound constantly produced by natural reactions in the atmosphere. We have isolated and characterised a number of these organisms and are studying the genes that in all of them permit the utilisation of MSA.
The present findings are aimed to give a novel approach for the growth promotion of plants with the help of *Methylo trophs*.

The following are the Objectives of the present study.

- to isolate Methylo trophs from Various sources
- to characterize Methylo trophs
- to optimize the media and growth factors
- to estimate the growth hormone produced by Methylo trophs
- to prepare bioinoculant using Methylo trophs
- to promote growth to plants by Methylo trophs
- to analyze agrobacterial characters of plants.
- to check the role of Methylo trophs in plant defense mechanism