Antifungal and Antibacterial activity of Actinomycetes isolated from the Sundarbans Mangrove forest soil and water from Bay of Bengal

A project submitted

By

Md. Fahad Rahman

ID: 14146010

Session: Spring 2014

To

The Department of Pharmacy

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy (Hons.)

Dhaka, Bangladesh

October, 2018

Dedicated to my parents for their constant love and support

Certification Statement

This is to certify that the project titled "Antifungal and Antibacterial activity of Actinomycetes isolated from Sundarbans Mangrove forest soil and water from Bay of Bengal" submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Md. Samiul Alam Rajib, Senior Lecturer, Department of Pharmacy, BRAC University. Throughout the project, I have given appropriate credit where I have used the language, ideas or writings of another.

Signed,

_____

Countersigned by the Supervisor,

_____

Acknowledgement

I would like to begin by thanking the Almighty Allah from whom I got the strength to complete this project work. This project would not have been possible without numerous individuals who are recognized here.

Firstly, I would like to thank my parents. I could not have achieved this without their constant support and love.

I would like to express my deepest gratitude and respect to my most esteemed supervisor Md. Samiul Alam Rajib (Senior Lecturer, Department of Pharmacy, BRAC University), without whom I could not have completed this project. His constant guidance and support towards my research based project helped me grow as a research student as well as a future researcher.

I also want to express my Gratitude to Dr. Eva Rahman Kabir (Chairperson, Department of Pharmacy, BRAC University), for giving me the chance and support to do this project in in an individual level.

I would like to express my gratefulness to Dr. Tofazzal Islam (Professor, Department of Biotechnology, BSMRAU) for letting us do our antifungal test in his lab.

I would like to thank Dr. Nihad Adnan (Senior Lecturer, Department of Microbiology, Jahangirnagar University) who I am deeply grateful for providing us with reference strains. I would also like to thank Dr. Raeed Jamiruddin (Assistant Professor, Department of Pharmacy, BRAC University. My sincere thanks to my senior Kazi Nuruddin Al-Masud for helping me during the project.

Abstract

Antibiotic resistance is increasing throughout the world and with time it is becoming more and more severe but the sources of the cure remains limited. Antibiotics losses their effectiveness with time and the necessity of getting new antibiotic becomes a dire need. In present day this antibiotic resistance has reached a certain level that many scientists are thinking that the “Pre-Antibiotic” era might return. The only way to solve this problem is to get new and novel antibiotics. Marine environment and less-explored or unexplored terrestrial environment holds the answer. The reason or the main objective of this study is to get effective antibiotic producing microbe from marine sources and unexplored soil sources. In this study soil samples were collected from the Sundarbans and water samples from the Bay of Bengal. After this the samples were grown in yeast malt broth as sub-culture process to get pure colonies after that we got 15 different colonies and they were named as sample no. 1, sample no. 2 and so on to sample no. 15. After that their growth was observed in nutrient agar, potato dextrose agar and in yeast malt broth too. Perpendicular streaking method was used for antifungal and antibiotic test. Only sample number 3 which is a soil sample showed both antifungal and antibacterial activity of normal to weak level. Other soil samples and all of the water samples did not show any activity.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Acronyms

YMB	Yeast Malt Broth
NA	Nutrient Agar
PDA	Potato Dextrose Agar
PKS	Polyketide Synthase
GPA	Glycopeptide Antibiotics
NRPS	Non-ribosomal Peptide Synthetase
APH	Aminoglycoside Phosphotransferase
HMG	3-Hydroxy 3-Methylglutaryl
HMG CoA	3-Hydroxy 3-Methylglutaryl Coenzyme A
QQC	Quorum Quenching Compounds
WEEV	Western Equine Encephalitis Virus
PCR	Polymerase Chain Reaction
HTS	High Throughput Screening
HDAC	Histone Deacetylase
REE	Rare Earth Elements
SMURF	Secondary Metabolite Unknown Regions Finder

Chapter 1. Introduction

1.1.Background

The threat of antibiotic resistance is increasing worldwide and the number of resources that are used to get antibiotics for this problem is decreasing. Actinomycetes are a group of gram positive bacteria that has contributed for a very long time in this area. At first, antibiotics were only derived from terrestrial actinomycetes but the rediscovery of same material has turned the attention of modern science toward marine actinomycetes (Wezel et al., 2013).

Now a days actinomycetes are not only collected from soil sources but also from marine sources like marine invertebrates, marine plants and even from the very sea water itself (Gonzalez & Anderson, 2004). Different parts of the earth is very famous like Lake Baikal, South China Sea, Baltic Sea for providing actinomycetes with the capability of producing novel antibiotics. Even after all this it seems that the rate of getting new antibiotics is not coping up with the uprising of antibiotic resistance (Zheng & Zeng, 2000). Modern science has also found that actinomycetes also contain many inactive gene clusters that have not been used till day and if those are reactivated the discovery of new antibiotics is entirely possible (Zhu et al., 2013). The alternative solution to this antibiotic resistance could be CRISPR but it is still under research and it is not going to be used anytime soon. So our hope is all this natural resources to fight against resistant pathogens

Here the purpose of the research is to isolate and characterize actinomycetes from sample collected from Bay of Bengal and the Sundarbans along with finding their activity against fungal and bacterial strains.

1.2. Objectives

The purpose behind this research is to find out the antifungal and antibacterial activity of actinomycetes. For this reason following steps were done:

- ✓ Isolation of pure colonies by using selective media called Yeast Mault Broth.
- ✓ Making of glycerol stocks.
- ✓ Observing growth on other medias.
- ✓ Use of perpendicular streaking method for antifungal and antibacterial test.

1.3. Literature Review

1.3.1 Introduction

Actinomycetes are an order of actinobacteria. They are very diverse in nature and some of them are very difficult to classify especially the genus *Nocardia.* They are like the hybrid of bacteria and fungi because of their peculiar behavior. They are gram positive in nature but some of their species have complicated cell wall structure especially *Mycobacteriaceae*,which makes it impossible for gram staining. In our research topic we are focused on the *Streptomyces* genus of actinomycetes because it is the only genus that has huge contribution in modern medical science. Streptomyces are gram-positive in nature. In our research topic we are focusing on the activity of Streptomyces against fungal and bacterial pathogens.

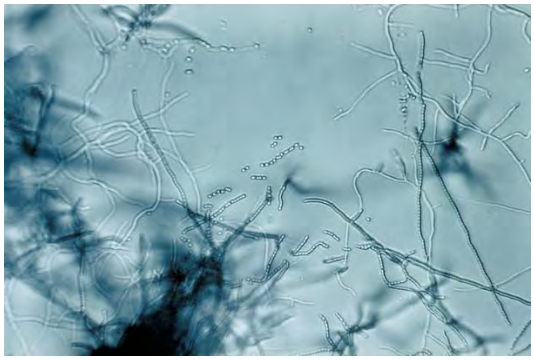


Fig 1.1. Side culture of a *Streptomyces* species (obtained from Wikipedia)

1.3.2. Present situation of Antibiotics

Even in this modern day natural resources are the only source of antibiotics to fight of diseases but the phenomenon named as antibiotic resistance is becoming more and more severe with time. Novel antibiotics are the new type of antibiotics that can fight against the resistant strains and actinomycetes are the main producers of novel antibiotics. To find out these new and novel antibiotics research is focused on unexplored locations or habitats (Manivasagan et al., 2013). The main reason behind this is to increase the possibility of finding new species of actinomycetes so that we could get new antibiotics. Actionbacterias are the producers and suppliers of antibiotics to pharmaceutical companies because of their ability to produce secondary metabolites of a wide varieties. Actinobacterias are quite famous for their ability to produce antibiotics to fight off pathogens (Jensen et al., 2006). It is only recently that marine actinomycetes have gained recognition as a source of antibiotics that are novel and the source of anticancer and antitumor agents with peculiar structures and properties.

1.3.3. Previous Situation

At the beginning research was focused on soil actinomycetes and when the research on soil actinomycetes went to its peak level the biologically active metabolites produced from soil inhabiting actinomycetes started to decline mostly because the results were almost same when scientists started to get same compounds over and over again. In the meantime antibiotic resistance was heading to a new level of danger (Meline et al., 2010). Only because of this reason modern research to find new actinomycetes to produce new antibiotics has shifted toward marine sources. More than 70% of earth's crust is covered by water. Within this 70% deep sea region is 92-93% and the only 7-8% goes for coastal region area. The deep ocean has very harsh environment due to its high pressure, low oxygen level, pitch black darkness and obviously low temperature. Even with all this deep sea is teeming with life whether it's the microbes or the giant animals. Now why we are choosing deep sea actinomycetes? Because in this huge ocean this actinomycetes fight for their food and the only way to kill their competitors is to create new antibiotics (Ekwenye & Kazi, 2007). Deep sea corals, marine invertebrates or vertebrates all contain actinomycetes in their system and the ocean itself is

the container of wide range of actinomycetes species. Not only antibiotics but as a new source for enzyme inhibitors, antitumor agents and antiviral agents deep ocean is very promising. Since it is well known, actinobacteria especially *Streptomyces* are considered to be the main source for producing diverse structured secondary bioactive metabolites for pharmaceutical application, particularly antibiotic agents and antitumor agents (Bister et al., 2004). Though most of the actinomycetes are terrestrial origin the ocean itself is containing the marine counterpart of *Streptomyces*. Actinobacterias are widely distributed in marine ecosystem and it's diverse marine organisms. In past new species of marine actinomycetes were even discovered from the mariana trench with depth up to10,898 meters (Asolkar et al., 2008). Terrestrial actinomycetes are known for playing important role in ecological recycling of compound that are organic in nature and also for producing other natural bioactive compounds, which are desired to protect the host organisms from pathogenic attack. However in the deep ocean environment their biogeographic distribution, role in ecology and history of evolution are not very well known (Fenical et al., 2009). This is what makes the marine actinomycetes the perfect source for novel antibiotics because here vast amount of active secondary metabolites come from the genus *Streptomyces.* The distribution of marine actinobacterias is very wide they are present in molluscs, mangroves, fishes, seaweeds, sponges besides sediments and seawater. These microorganisms are gaining attention not only for their characteristics or ecological perspective or taxonomy but also for their ability to produce antibiotics, immunosuppressant agents, enzymes, antitumor agents, pigments and enzyme inhibitors etc (Protasov et al., 2017). So we can see that, due to worldwide constant need for novel antibiotics to fight off pathogenic attack in host body or to neutralize strains that show antibiotic resistance or to use it as a drug for anti-tumor chemotherapy, deep ocean actinomycetes are rising as a new source of antibiotics and other bioactive natural products.

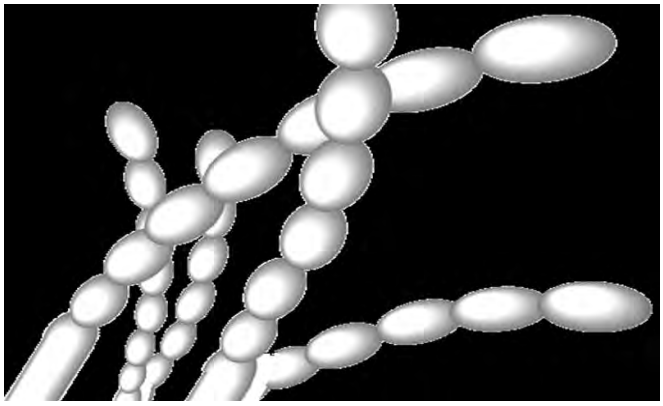


Fig 1.2. Soil Actinomycetes *Streptomyces griseus* (obtained from Wikipedia)

1.3.4. Mystery of cryptic gene clusters

Filamentous actinomycetes specially the bacteria and the fungi order of actinobacteria are the main source of antibiotics because they are known for producing 90% of all used and known antibiotics. It is not like that one species of Streptomyces is only capable of producing only one type of antibiotic compound (Zhu et al., 2013). Modern research shows that most of the Streptomyces species that were used to produce antibiotics have gene clusters in them. Genome sequencing of actinomycetes confirmed the presence of many other biosynthetic gene clusters with the potential of creating new antibiotics that didn't exist before (Altermann & Klaenhammer, 2005). For example, for many decades it has been thought that *Streptomyces coelicolor* only produces four different antibiotics like actinorhodin, calcium dependent antibiotic, undecylprodigiosin and plasmid encoded mythelenomycin from the research of david hopwood and his colleagues (Hopwood et al., 1999). After fifty years of researching intensively it came like a complete wonder when *S.coelicolor* revealed an entirely new set of gene clusters and one of these gene clusters is likely to produce cryptic polyketide called antibiotic. This phenomenon alone proves that other species of actinomycetes also have these latent arsenals within them that can give mankind new antibiotics. Therefore it seems that the potential of actinomycetes as a source for producing novel antibiotics is way more bigger than which was originally anticipated (Horinouchi & Beppu, 1994). It has initiated extensive research into the field of applied genomics to find out this cryptic, sleeping or silent antibiotics. So we can finally see that, there are many other yet unidentified antibiotics out

there for us to discover, those were missed due to the less production of gene clusters that identifies them in the first place.

1.3.5. Antibiotic Resistance

Though antibiotic resistance is not new to modern science but the problems caused by it seems increasing more and more with time. Diseases that were once curable by antibiotics are returning with resistant characteristics (Jensen et al., 2005). Bacteria can trigger mechanisms when they face same antibiotic twice or more and this causes change in the mechanism of the attack created by bacteria like change in their genetic level resulting in expression of enzymes that can degrade antibiotic capabilities or more hard to degrade cell walls or the ability to expel antibiotics from within the bacteria or decreasing permeability on the cell wall of bacteria (Bao et al., 2013). Marine microorganisms are used continually to get new types of secondary metabolites of unique and novel compounds like characteristics. They are used for new types of drug discovery. Bacteria, fungi can adapt themselves when they sense or feel the change in their surrounding environment. Marine environment is known for its richness of having a huge diversity of microbes that can produce unique secondary metabolites that can destroy or inhibit other microorganisms like actinomycetes. Still in these modern era the production of antibiotics from actinomycetes is in its infant level. Only 10^5 out of 10^{25}-10^{26} in 1m of global soil was screened for finding of new antibiotics and all this happened since last 50 years (Zhang et al., 2009). The problem of antibiotic resistance to multiple drugs caused bay microbes is a global problem and because of that modern research is focused on getting new and powerful bioactive compounds form new sources. Bacteria that are multidrug resistant are characteristically nosocomial but in one case methicillin-resistant *Staphylococcus aureus* is also present in settings of community as a result there is a dire and continuous demand of getting new antibiotics from new sources (Huang et al., 2007). All this reasons are leading the research to finding new antibiotics form new sources especially from new and unexplored habitats because the lesser known the area the bigger and greater chance of getting novel antibiotics. Marine ecosystems are characterized by their diverse characteristics like salinity which is very high, pressure, low level of temperature and continuous changing in oxygen level. All these factors have created something on these microorganisms and because of that they differ hugely from their terrestrial counterparts and clears the possibility to obtain

new types of antibiotics for the marine source (Lee et al., 2003). Marine derived sediments may contain a great variation of culturable actinobacteria. Modern research showed that marine actinobacterias are more effective than earth variations. The main key for such diversity can be because of hydrographic feature present in marine environment. The report of WHO given in 2014 showed that the effects and danger level of diseases caused by multiple drug resistant strain is increasing at an alarming level throughout the world and scientists are thinking that if these continues then in future there will be no treatment that are from antibiotic in origin (Anderson et al., 2005). After careful accounting it has been found that the classes of approved drugs that aware released in market from the time of 1981 to 2014, almost 69% of these drugs are either natural products or products that were derived from natural origin. Isolation, culturing and characterization of pure microbiological strain is a key to finding natural products with potential to cure diseases that are resistant to old drugs (Hirsch et al., 2004). Many products that belonged to the class that were obtained from microbial biotechnology are in current use and with time the expiry date of this strains are coming to an end because we cannot use these drugs or the strains that produce these drugs forever, actinobacteria contribute for 40% production of bioactive secondary molecules and nearly 80% of those drugs come from the genus *Streptomyces* (Sigmund et al., 2003). The potential of the actinomycetes to produce these new antibiotics is still unknown but mostly it lies in their genetic level because within their DNA lies several gene clusters that can produce antibiotics but not all of them are active, they also contain gene clusters that are inactive or not producing antibiotics or secondary metabolites. Research is going ion about how to activate the inactive gene clusters to get new types of antibiotics to solve this multi-drug resistant crisis (Wezel et al., 2013). The continuous decrease in isolating or culture of strains or their secondary metabolites from traditional sources like soil is happening because of getting same antibiotics or resistance to multiple soil derived secondary metabolites. On the other hand ocean has large portion of the earth and the possibility of getting new actinomycetes and new secondary metabolites is more likely on ocean than earth. Several researches on marine actinomycetes have solidified this prediction of getting new antibiotics from marine sources (Tanaka et al., 2010).

1.3.6. Treating tumor and inflammation

In case of treating tumor and inflammation anctinobacterias play an important role. In the past actinobacterias were only used to treat bacterial diseases and the actinomycetes were isolated from soil sources. Mosdern research showed that actinomycetes that were obtained from marine sources not only acts as antibacterial drugs but also prevents the formation of tumor and initiation of inflammation (Li et al., 2000). For future esearch and to get new drugs initiatives were taken to increae expedition in marine area and on the same time research focusing on plants and animals will also be increased. Marine seaweeds is one of those things that remain unexplored in many areas only in some areas it remains underexplored. This seaweeds said to be contain actinomyctes of great uses. Because many scientists are focusing on experimenting on seaweeds obtained from baltic sea, north atlabtic sea and peninsula costs (Imamura et al., 1993).

1.3.7. Substances that are host of actinomycetes

1.3.7.1. Coral reefs

Coral reefs are another thing in the sea that contain actinomyctes in it.They are also considered as the ‘rain forest’ of ocean. They are a source of unique prosucts that are biosynthetic that has unique biomedical potential. Though very little is known about coral reef and its associated actinomycetes research focuses on coral reef obtained from china sea coral ecosysytems is opening new doors in search of new antibiotics (Pham et al., 2015). Research shows that the *Streptomyces* obtained from coral reef is barotolerant because they have the capability to grow in very high hydrostatic pressure. The interaction of hosts of atreptomyces with marine ecosystem is also a subject of research because it has been thought that the behaviour of host with environment can affect the microbe within the host (Raina et al., 2010). Scientists are also thinking that these type of interactions can also give rise of new strains inside them. New researches revealed that some natural products that were thought to be of marine invertebrate origin is now found out to be produced by symbiotes. All this research also makes us aware that the proper distribution of *Streptomyces* is important for both the marine environment and for the ecological balance between animals of ocean and the ocean

itself and most of all it assured the finding of new products of symbiotic origin than lives within coral reef in the marine environment (Peng et al., 2013).

1.3.7.2 Lichens

Actinomycetes are one of those species that are present widely on terrestrial area as weel as other ecological areas like ocean. Now a days scientists are using lichens as a source for getting actinomycetes as well as a new source to isolate and culture of novel antibiotics to prevent the multiple-drug resistant disease crisis (Gonzalez et al., 2005). Generally lichens are the type of species that are like the mixtures of green algae with fungi or with cyanobacteria. Though the components that are symbiotic in nature well described but the microbial group that consists of this community is still not properly understood (Basillio et al., 2003). They are the type of species that can be found in regions like arctic areas and also in the tropical areas with huge diversity growing on stones, epiphytes, arid soils and in plants too. They were described widely about their capability to produce secondary metabolites or other activities that are biological in nature. Cold desert of Antarctic areas are covered with lichens of tundra origin and they were and still been experimented widely (Jensen et al., 1999). Recently there were reports that actinomycetes genus of *Micromonosproa* was isolated from this type of arctic environment lichens. Now scientists are considering the lichens as a third source for getting new and novel antibiotics , the first source was soil origin while the second source is very popular source marine or ocean in origin (Ayuso et al., 2004) . Scientists collected lichens form hawaiian areas and form mountains of alaska and after proper research they have been found out that lichens can be used as good source of actinomyctes in order to get new and novel types of antibiotics. Currently scientists are working on lichens collected from arctic, tropical and marine environment to find new species of actinomycetes and many research revealed that species of actinomycetes obtained from lichens contains a significant potential to produce antibiotics that are novel in nature (Hunneck, 1999). Though the mechanisms were known and all these experiments were lab based where experimental conditions may have altered the outcome slightly but scientists are hoping that large scale production can correct all these errors.

1.3.7.3. Sponges

The sponges are the organisms which was extensively studied for the purpose of finding a cure. The genus *Streptomyces* and the Genus *Micromonospora* seemed to be largely present the sponge community of Lake Baikal. In case of other animals of Lake Baikal they were less studied than their sponge community (Selvin et al., 2009). There were many exploration that were the first in case of amphipods. These amphipods and other macroinvertebrates play a crucial role in the ecological balance of Lake Baikal. Recently these amphipiods are facing a great challenge due to their lifestyle and environment changing but a very little is known about how these amphipods fight for their survival and how they fight for food against other competitors and their mechanism of protection against other pathogens (Protasov et al., 2017).

1.3.8. Places to get Actinomycetes

1.3.8.1. South China Sea

Even a few days ago number of researches conducted on marine actinomycetes were very low but with time the number and frequency of marine actinomycetes research is increasing. South China Sea is one of those regions where experiment occurs heavily on actinomycetes of those area. The class known as actinobacteria is considered as a great source for drugs like enzymes, antibiotics and biotechnological products like recombinant products (Yang and Song, 2017). At the beginning of getting drugs from *Streptomyces* the rates were very high but with time the rate of getting new drugs from *Streptomyces* is decreasing. On the other side of equation multiple drug-resistant diseases are rising at an alarming rate. Because of all these reasons scientists all around the world are focusing their research on species of rare actinomycetes because the chance of getting novel and entire new class of drugs is very high when experiments are conducted upon rare species of actinomycetes (Berdy, 2005). Actinomycetes of terrestrial origins are giving same drugs over and over again and as a result modern research is focused on marine derived actinomycetes. Many major ocean parts of planet earth is now the target of scientists to get new drugs. Marine actinomyctes possess their own unique characteristics like unique biochemical metabolism and extraordinary defense technique (Liu et al., 2003). All these activities rise due to their survival by fighting and competing against other microbes of ocean and as a result they produce their own secondary metabolites which

are known to us as novel or new antibiotics. From 2006 to 2016 these ten years is the timeline when a lots of new and novel antibiotics were isolated from actinobacterias that are of marine in origin. Again the South China Sea is one of the important regions that provided actinobacterias with the capability to produce new antibiotics and lots of its part is still under investigation so that we may find new types of novel antibiotics (Gou et al., 2012). The south china ocean is like any other marginal ocean but it has enough sediments that have the potential to hold actinomycetes with a huge variety of taxa. We all can get the importance if this sea by looking at the research of Chinese researchers who have successfully isolated 28 genera in total with 13 new families including entirely new 14 new species and every single one of them has incredibly diverse type of metabolites (Chen et al., 2013). For example, *Micromonospora rosaria* and *Streptomyces niveus* are those two drugs that showed good inhibiting activity against *Staphylococcus aureas* and NCI-H460 cancer cell respectively. Many other species isolated from these region showed activity against *Mycobacterium phlei* and the number of strains that did this is four. There are other five strains that gave excellent activity against methicillin resistant *Staphylococcus aureus*. The novel alpha pyron was obtained from nocardiopsis species that belonged to the South China Sea and this alpha pyron proved to be HIV protease and it selectively inhibits COX-2 form giving its action (Schaberle TF, 2016). Another strain known as *Salinispora* species has the potential to produce new and novel drugs like rifamycin synthesizing strains. All those studies uncovered the true face of south china sea which is providing the basis only for south china oceans bioprospection. All those studies concluded and proved one thing for sure is that, marine actinomycetes of South China Sea provided novel antibiotics in the past, they are also providing novel antibiotics now and will be providing novel antibiotics for the necessity of mankind in the near and distant future (Zhang et al., 2017).

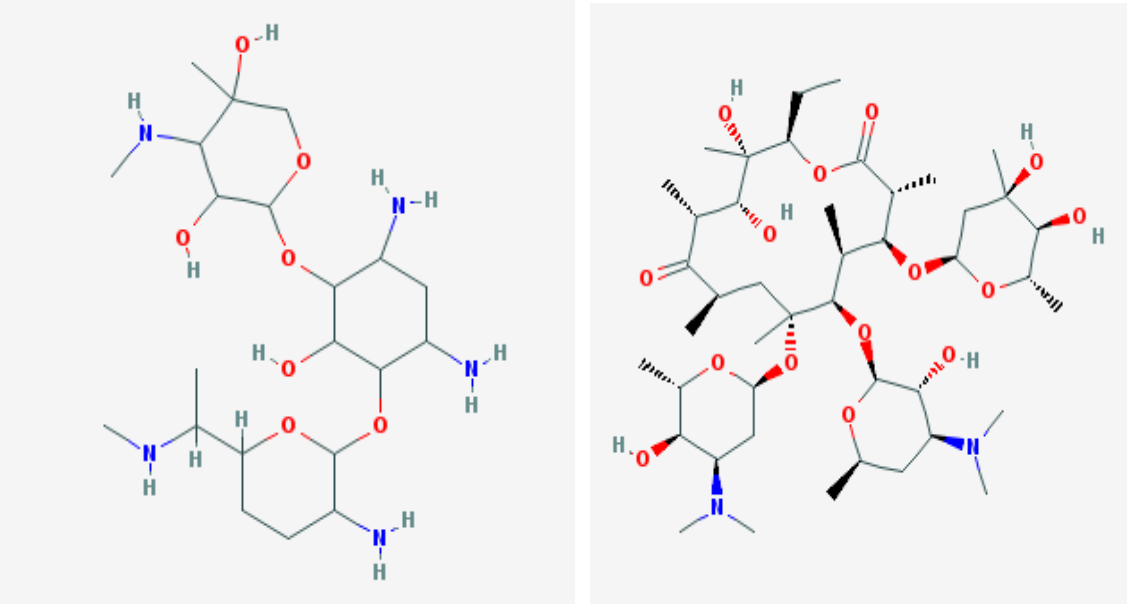
The actinomycetes belong to a group of well known filamentous bacteria that are gram-positive in nature. They also play a huge role in the balance-keeping of soil nutrients. In case of experimenting on soil originated actinomycetes mostly was done on rhizospheres and also on actinomycetes obtained from plants, animals that are marine in origin etc (Moore et al., 2012). Why actinomycetes are so different than other microbes when it comes to producing secondary metabolites? The answer lies in their rapid metabolism which is caused by extreme type of chemical natured diversity. There are a wide variety of methods to characterize

actinomycetes like morphological, molecular-fingerprinting and as well as chemo-taxonomical etc (Toske et al., 1995). In chemo-taxonomic analysis fatty acid analysis is widely used to characterize actinomycetes. Amplification of repetitive sequences in molecular-fingerprinting is also widely used in higher level of analysis. Sometimes strains of actinomycetes are characterized by targeting metabolic potential genes that are involved in the production of secondary metabolites by actinomycetes. In the species of actinomycetes modular polyketide synthase, iterative polyketide synthase and non-ribosomal peptide synthase is responsible for producing secondary metabolites (Yang & Sun, 2016).

Many researches all around the world is happening to find out about actinomycetes ability to fight as an antitumor and antimicrobial activity. For this reason samples of actinomycetes were collected from soil, from intestines or from epidermis of marine animals and plants respectively (Rahavee, 1974). Compared to soil actinomycetes counterpart their marine counterparts proved to be more useful when it came to fight back new and stronger resistant microbes and that's why they are considered as a weapon of humanity's hope in the battle against multi-drug resistance disease. All this factors have raised the attention toward marine actinomycetes to a new level. Thus, the actinomycetes of marine in origin are becoming more and more important to be a new and potential source for growing new bioactive agents (Tamura et al., 2013). Infectious diseases cause one third of deaths worldwide every year in addition to that multi-drug resistance is also becoming a new threat to mankind and to mankind's future. The morality rate is already very high sue to this diseases and in the same time people with weak immunodeficiency problem are also at a great risk. Most of the people around the world that are suffering from this immunodeficiency problem also suffer from chronic infections (Qin et al., 2009). This is not only matter of poor countries because both poor developed and underdeveloped countries and their people are suffering from this types of nosocomial disease. There are a wide variety of nosocomial diseases around the world that are causing these problems. Surgical wounds, respiratory tract infection and urinary tract infection all are nosocomial disease and we see people everywhere who are suffering from this types of diseases (Wiley et al., 1984).*Staphylococcus aureas*, *Bacillus subtilis*, *Proteus vulgaris* and *Pseudomaonas aeruginosa* are few of the huge number of bacteria that are responsible for this. All these bacteria are not only responsible for nosocomial diseases or infections but also responsible for increasing the mortality rates for human by causing

community acquired diseases (Ward et al., 2006). Now a days lung infection is also becoming a major problem to human health all around the world. The mycobacteria known as *Mycobacterium abscessus* is the root cause for this global problem. It has the capability to cause diseases like pulmonary diseases as well as diseases like cystic fibrosis, postsurgical infections but what makes this disease formidable is it is very hard to cure and as well as very hard to eradicate (Hodgen et al., 2012) . Children all around the world suffers or suffered at least once in their lifetime from diarrhea and the microbe that causes this problem is Escherichia coli. Though it is present in our body and remains neutral or sometimes gives positive actions sometimes it acts abnormally and creates conditions like this. Not only diarrhea but Escherichia coli is also responsible for urinary tract infection. Along with *E.coli* there is another species that can cause diarrhea and that is *Pr.vulgaris*. In case community diseases and community acquired diseases staphylococcus is one of the bacteria that affects all from hospital admitted patients to people that are in prefect health condition (Lazzarini et al., 2001). It is also responsible for a wide range of diseases like skin infection, central nervous system infection, bone-joint infection, soft tissue infection, skeletal muscle infection, urinary tract infection and respiratory tract infection. Pneumonia is another disease that is caused by bacteria. According to the research of world health organization (WHO), 47 countries out of 112 countries is heavily affected by this disease (Liao et al., 2016). In the case of chemotherapy of different kinds microbial resistance is becoming a throne to people's health. All these reasons and facts prove that right now mankind is in dire need of a cure that has the capability fight back the raising threats that are emerging because of resistant strains. So scientists are focusing their research on microbes specially bacteria that have features like diverse chemical structure and as well as diverse biological activities. The genus known as *Micromonospora* is one of those bacteria species that has the potential to fight back against multiple drug resistance diseases and the microbes that are causing this diseases (Chen et al., 2013). Gentamicin, Megalomicin, Sagamicin, Halomicin, Mycinamicun, Everninomicin and Mutamicin are few examples. Presently many research is being conducted upon *Macromonospora auratinigra*. The drugs that are in existence is becoming and proving to be inadequate in the face of this phenomenon known as multi drug resistance (Hodges et al., 2012). There are many regions in these earth that are still untouched by humans and might

also contain huge diverse ecological area filled with organisms that may be our only hope to fight against this diseases.



A **B**

Fig 1.3.A Gentamicin(left) Fig 1.3.B Megalomicin(right) (obtained from NCBI) (Hodges et al.,2012)

1.3.8.2. Baltic Sea

It is a well recognized matter is that the microbes associated with marine organisms specifically the marine sponges and corals in addition to coral reefs are without a doubt an excellent source to obtain natural products in order to cure multi-drug resistant diseases. Because of all this marine animals mostly marine invertebrates are getting more attention as a possible and potential source of novel antibiotic compounds (Wiese et al., 2015). Soft coral bacterium *Alcynium digitatum* has huge antimicrobial potential in this field. Baltic Sea is the place from where it was first obtained. The Baltic Sea is one of those hugely diverse area that contains many different animals and as well as microbes to solve this problem. Marine sponge, algae and corals all are count as a potent source of novel natural compounds and as a producer of substances that could act like a source enriched with novel compounds (Imhoff et al., 2011). Experiments on marine bacterial communities and the products that are antimicrobial in nature produced by them is also getting toward bacteria that are related with sponges, molluscs, algae

etc. Studies on hard corals already gave enough evidence that these things can permanently use for production of novel anti-microbial compounds. Gene sequence analysis, culture based methods, antimicrobial susceptibility testing and last of all the biochemical testing all are being used by researchers all around the world in order to search the perfect producer of novel antibiotic compounds (Theil et al., 2007). Except actinobacerias there were many other bacteria like *bacteoidetes*, *firmicutes* and *betaproteobacteria* and all of these gave excellent antifungal activities and antivacterial activities. The species *Dendrophytha* also showed antifouling mechanisms. This species was collected from port shelter of hong kong which falls under south china sea which was previously described as a heaven for natural resources that has the potentials to produce new and novel antibiotics compound in the future (Peng et al., 2013). These microbes ive or inhabit the larval stage settlement of tubeworm known as *hydroids elegans*. *Alcynium digitatum* is also known as corpses fingers and this species belongs to the group known as alcyonidae family which is a common boreal species. It can also be found from Northwest Europe to North America (Ritchie K, 2006). They are also well researched in the terms of taxonomy, reproduction and also distribution. There were very few studies about this species but the number of research on this topic is increasing with time. The aim of studies like this is to find out how to isolate and identify bacteria from soft coral and also find out about their capability to produce novel antibiotic compounds (Lampert et al., 2008). Most of the researchers use the method where many colonies are picked randomly rom many mediums that are differential in nature so that they could get a diversity of maximum level. Most of the researches that were conducted on the samples that were collected from Baltic Sea showed good results. In many studies species like *Paenibacillus*, *Lysinibacillus* were found out to be the dominant type of *Firmicutes*. *Paenibacillus* and *Exiguobacterium* is more associated with black corals of Baltic Sea than any other marine invertebrates (Altschul et al., 1990). Even after all this our knowledge on the ability of marine actinomycetes to produce novel antibiotic compound is poor. We also cannot fully understand the potential of coral related bacteria to produce antimicrobial compounds and the role of soft coral associated bacteria is still unknown, mostly (Gray et al., 2011). A wide range of bacteria over many phyla has antimicrobial potential and scientists are thinking that they also play an important role in the life cycle of corals.

1.3.8.3. Colombian Caribbean Sea

When it comes to production of new and novel antimicrobial compounds marine actinomycetes are considered as one of the most promising sources. Colombian Caribbean Sea is another important region of the world that has huge biodiversity and in many studies scientists used samples like sediments, algae and other marine invertebrates. Their aim was to find out actinobaterial strains from these marine samples (Garcia et al., 2014). The second half of the twentieth century is the time when the major concerns about agriculture was the pollution of our environment by excessive use of pesticides and other highly poisonous agrochemicals. Soon it was found out that all these pesticides and other chemicals like this do not only cause environmental pollution but also increasing the ability of pathogens to resist the actions of antibiotics (Armstrong et al., 2001). And the presence of these types of pathogens in crops is a global concern so both the academy and industry level people are working effortlessly and trying to find out a permanent solution to this global problem. Colonies of both types of bacteria like benefic and pathogenic act as social natured communities and in the mean time they are also able to use density dependent method for their gene expression and this type of phenomenon is commonly known as quorum sensing (Cane & Ikeda, 2011). This quorum sensing system has the ability to regulate the metabolic processes of bacteria along with development of bacteria and also developing virulence in bacterial cells. The method is based on working through signaling molecules and the concentration of these signaling molecule increases with the increasing of bacterial cells (Bull et al., 2005). Once the concentration of these growing cells as well as concentration of signaling molecules reaches at a specific or highest peak different types of signals produces a cascade which gives the result like gene expression and other different types of pathogenic effects. The ability to express virulence in many strains are dependent on this system or process. Many bacteria that are plant pathogenic in nature depends mostly on this system to produce disease in plants (Arahal et al., 2008). Most of all this process is widely recognized for controlling the production of toxoflavin in some species of *Burkholderia*. In case of rice rotting and wilt the production of this phytotoxin acts as a key factor. Now scientists are trying to find a solution of this problem and they are eyeing the derivation of antimicrobial compounds from microorganisms which is largely known as the main source of antibiotic compounds (Bourne et al., 2009). At the first time microorganisms that are terrestrial in origin

were considered to be the source of cure but now scientists are trying to use different things and new approach like the marine sources, which is becoming very famous for the last few years in the field of microbiology. Moreover the phyla of actinobacteria of marine source is very famous for producing antimicrobial compounds and that is the only reason which makes them more appropriate candidate against the agrochemicals (Aoki et al., 2011). The species *Streptomyces kasugaensis* is a bacteria that is terrestrial in nature and it is very famous for producing antibacterial compound kasugamycin which acts against bacteria *Burkhulderia glumae* and it also acts as an antifungal agent and acts against fungus *Magnaporthe grisea*. The previous two antibacterial and antifungal compounds were also isolated from marine *Streptomyces rutgersensis.* Therapeutic efficacy mode of antibiotics is attributed by inhibition of the growth of bacteria in in vivo when concentration of antibiotic exceeds minimum inhibitory concentration (MIC) (Brana et al., 2014). However the attenuation of growth can still occur below the level of minimum inhibitory concentration level and a variety of bacterial virulence factors can also be expressed which can ultimately lead to the stage where the ability of pathogen to create disease is ceased. This type of activity of microbes is also known as sub minimum inhibitory concentration level effects and the type of compounds that creates these effects is known as quorum quenching compounds (QQC). These quorum quenching compounds can also be used against the expression of virulence factors by phytopathogens because it naturally inhibits this process (Amato et al., 2007). Here the activities are the production of enzymes, the degradation of signaling molecules and the inhibition of enzymes that are responsible for production of signaling molecules or it can also inhibit the production of the receptors that activates quorum sensing. There are also some bacteria that can inhibit the activation of receptors of quorum sensing process of pathogen type strains (Gust et al., 2003). In addition to this there are also some species of actinomycetes that can or has the ability to inhibit the virulence factors that are regulated by *Plasmodium caratovorum* in quorum sensing system. There are some secondary metabolites of actinomycetes known as Piericidin A and Glucopiericidin A and these substances showed their ability to control the pathogens that generally affects plants (Singh et al., 2011).

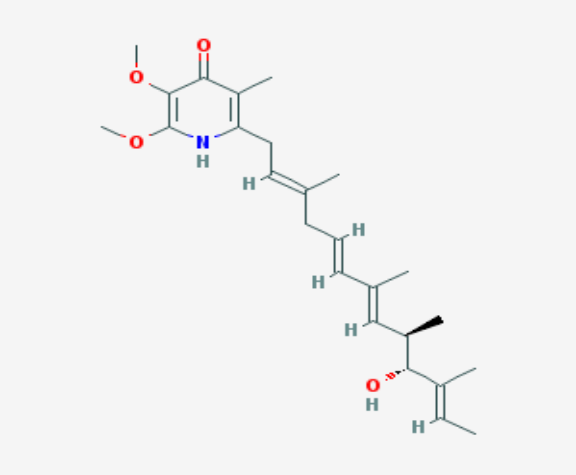


Fig 1.4. Piericdin A (obtained from NCBI) (Singh et al., 2011)

However there are metabolites from microbes that are known for their perfect activity as quorum quenchers. Most of them give their activity or effects by disrupting the pathogenic communication and acts as an antipathogenic compound. Linear dipeptides from marine actinobactria also known as Pro-Gly and N-amino alpha proline is active against quorum sensing influenced virulence factors produced by *Pseudomonus aeruginosa*. Despite all these efforts to isolate novel and new compound from marine envronment the rediscovery of same old existing compound is not gone completely (Hughes & Fenical., 2010). This is only occurring due to the same old use of approaches that are classical for selection of microbial strains based only on antimicrobial or taxonomical information. When studies are made based in chemical diversity lacks data for their biological activity which also prevents from their further research and application (Stroch et al., 2005). That's why the bioprospecting of bacterial strains for isolation of new novel compounds is moving toward more integrated levels. This method combines phylogenetic data and bioactive data as quick and faster alternative to identify and characterize known and metabolites that are remaining in a sample mixture. All these strategies proved to be suitable for performing multivariate analysis to get accurate results and also can be used to selectively identifying new and novel bioactive compounds. Which can only mean that all these methods can be applied to improve the methods to discovery of new drug molecules (Rahman et al., 2010).

1.3.8.4. Chile

There are many marine regions of earth that are famous for producing new and novel antibiotics. One of these regions is Chile. The coast of Chile has a favourable type of ecosystem many researches in past that were focused on the coast of Chile showed extraordinary results and all those results encouraged more and more scientists from around the world to focus on this region (Undabarrena et al., 2016). Marine ecosystems are an established source of marine actinomycetes. The sponges, corals, pufferfishes, sea cucumbers of this area is very ideal for finding novel antibiotics. The coast of Chile was mentioned in many journals for the efficacy and better results of the samples that were obtained from this part of globe (Lam KS, 2006).

1.3.8.5. Baikal Lake

The new emergence of pathogenic bacteria resistance to multiple drug therapy increases the dire necessity to get or discovery of new and novel bioactive compounds. There are so many unexplored habitats all around the world that can be used to get new antibiotics to help us solve this problem like unknown or unexplored deep sea region, unknown wetlands, unexplored caves etc. Many of these sources proved to be housing actinobacteria which is the type of bacteria that has been providing new and novel bioactive compounds for years (Protasov et al., 2017). The Baikal Lake is one of these important places to get new and novel bioactive compounds. Many experiments were conducted upon the cultivated strains of actinobacteria of these locations. There were also isolation of *micromonospora* and *pseudonocarsia* from these Baikal Lake's invertebrates. The genus *Streptomyces* found to be the dominant species of this region (Barazi et al., 2002).There were some number of correlations that were observed to the strains and their collection from sources and with the depth of sample collection site. There were many samples whose analysis gave annotation of several new bioactive compounds from which new explanation of their biological activities can be made (Armstrong et al., 1976). The found characteristics of these samples and their sources suggest that the numbers of actinobacteria that were isolated may not be so random as it seemed previously and the have the possibility to represent the interactions that are long term symbiotic in nature. Still in this modern day natural products or the source of drugs that are obtained from natural sources remain on the number one position including antibiotics,

hormones, enzymes and other drugs etc (Costa et al., 2013).The emergence of microbes that are resistant to these drugs is making this situation more and more difficult and also raising the matter that is to obtain new and novel bioactive compounds from these sources. Though there were some negative or bad reviews about actinomycetes and the complain of getting the same bioactive compounds from them actinomycetes still remain as the major source of getting new and novel bioactive compounds that has the possibility to fight against resistant strains or resistance microbes. This phenomenon of creating novel compounds by actinomycetes is a part or better told the result of their diverse and unique type of metabolism. Eight thousand types of novel bioactive compound of sixteen thousand is obtained from actinomycetes (Smith, 1975). The genus *Streptomyces* alone provides eighty percent of world's demand for bioactive compounds. From early twentieth century soil was the first choice to get actinomycetes to get new and novel bioactive compounds but the percentage of getting new compounds were decreasing because there were problems like getting the same type of bioactive compounds over and over again and in the meantime microbes were becoming resistant to the bioactive compounds that were derived from soil inhabiting actinomycetes (Ling et al., 2015). That's why scientists and research organizations all over the world shifted their focus to marine counterparts of actinomycetes rather than their soil counterparts. The sources of marine environment that contains actinomyctes were things like marine invertebrates, sponges, coral reefs and sometimes lichens though the latter is becoming more and more famous as new source of getting acyinomycetes. Scientists are also thinking to make lichens as the third known source after soil and marine sources of actinomycetes (Gonzalez et al., 2005). New species of the genus known as *Micromonospora* was collected from marine insects. Even source like termite nest proved to be a source of getting *Pseudocardia*, *Nocardia* and *Streptomyces* like genus. The actinomycetes that are sponge related is also becoming a hot topic for the isolation of new and novel bioactive compounds. There were many research on marine invertebrates compared to those researches the number of research on marine sponges is still low but due to the severity of multiple drug resistance by microbes the experiments on marine sponges is increasing (Hall T, 1999). Now scientists are also increasing their research on fresh water originated sponges. Actinomycetes isolated from aquatic environments especially from marine environment is getting famous for derivation of antibacterial, antiviral, enzyme inhibitors etc. As previously described lake

Baikal is one of those regions to get these novel and new bioactive compounds (Haste et al., 2012). Lake Baikal is UNESCO declared heritage site and it is located on eastern side of Serbia, Russia. It is one of the oldest lakes on the surface of earth approximately twenty five to thirty million years of old. This lake also famous for containing twenty percent of whole world's unfrozen fresh source of water. Lake Baikal is also famous for being the deepest lake on planet earth almost sixteen hundred and forty two meters. The lake also contains more than twenty five hundred animals and almost eighty percent of them is endemic (Kozhov M, 1963). The lake is characterized by its fluctuation of temperature which varies with the depth of the lake and the water of this lake is well oxygenated. All these factors are responsible for creating a different type of ecosystem on the lake. There were also proofs that many species that are residing today in lake Baikal also evolved from ancient times (Fang et al., 2015). Which is also responsible for creating s unique and very old ecosystem in the lake. Among the macroinvertebrates the amphipods are the dominant ones of the ecosystem of Lake Baikal. Lake Baikal is also very famous for containing forty five percent of the whole world's amphipod fauna. Two hundred and forty six species and seventy eight subspecies are endemic in nature (Conti et al., 2016). They inhabit the ecological niches of the zones that are abyssal. The amphipods of Lake Baikal live on by feeding on dead detritus and other organisms. There also self cleaning that is occurred by the deep region amphipods of Lake Baikal which is very important because it keeps the marine environment of Lake Baikal well and clean for the safety of other species and the lake Baikal itself. The lifestyle and types of feeding style of different organisms have given rise to a very different and unique ecosystems on Lake Baikal (Duncan et al., 2015). Both the abiotic and biotic factors possesses a uniqueness in themselves and all this uniqueness have made lake Baikal and its living organisms a very promising source of novel and new bioactive compounds that can be obtained from them in near future. There were many studies that were focused or founded on the species of Lake Baikal's endemic origin (Bowman et al., 1988). All those studies or researches have proved one thing and that is there lies a high abundance of actinomycetes inside Lake Baikal sediments, water, sponge and other communities of microbes. All these observations are suggesting one thing only and that is that in the near future lake Baikal can become a very big source for novel species of actinomycetes (Farnaes et al., 2014). There also findings that suggested that actinomycetes collected from amphipods possess high antifungal activity. Which also makes the assumption

that was made previously that there must be some sort of symbiotic interactions between amphipods and bacteria. There were also findings about strains that had antifungal activity from amphipods and it lead scientists to believe that the amphipods are the consumers who consumes substances like organic type matter and other substances like detritus and all these things are inhabited by fungus as decomposers (Fang et al., 2015). The fungus enter the gastrointestinal tract of the amphipods when they are taking food and creates effects that might be undesirable for the physiology and metabolism of the amphipods. It is a very well known matter that fungi are responsible for most of the aquatic diseases of marine organisms. There are many types of crustaceans that are able to develop resistance against these fungal infections and scientists are thinking that these crustaceans might be the source of getting new antifungal disease (Sarker & Nahar, 2012). Described by gill turns the shrimp species of *Palaemom macrodactylus* has the ability to prevent the attack if fungi known as *Lagenidium callinestes*. Scientists found out that the cause if resistance of the shrimp was due to the presence of a bacterium in its body known as *Alteromonas* sp. This bacteria was collected from the surface of the embryo of the shrimp and they were able to produce the antifungal compound known as 2,3 indolinedione or better known as tribulin (Kieser et al., 2000). A similar type of phenomenon lead to the discovery of 4 hydroxyphenethyl alcohol which is better known as trisol which is also an antifungal compound. This compound was isolated from the North American lobster known as *Homarus americanus*. Both of thse collected antifungal compounds suggests only one idea and that is the interaction of bacteria and crustaceans which is very adaptive in nature. Also this was the first report (Protasov et al., 2017) that tried to understand and explore the amphipods of lake baikal and also the first journal reporting the process of isolation of actinomyctes belonging to the genera of *Pseudonocardia* and *Micromonospora*, In the previous study generas like *Streptomyces* and *Nocardia* actinobacteria was isolated from the species known as *Brandita* sp (Axenov-Grivanov et al., 2015).All these findings proved that if proper research is conducted then many new antifungal and antibacterial compounds can be isolated from the hugely diverse aquatic ecology of Lake Baikal. Which is very important when the threat of multiple drug resistance phenomenon is increasing globally (Stadler & Dersh, 2016).

1.3.8.6. East China Sea

Gorgorian corals are becoming a major source fir isolation of actinomyctes and their unique novel and new bioactive compounds and south china sea is very important place that is known for its housing of many species of gorgorian corals. Gorgorian corals plays not only a huge role in marine ecology but also act as a reservoir for other marine organisms like shrimps, crabs, fish, hydroids, gastropods, bryozoans and other marine invertebrates (Gho & Chou, 1994). Gorgorian corals do not share the similarity of having calcium exoskeleton like their other counterparts. Even after not having any armored protection they seem to be fine in marine environment because they do not have many predators and in the same time they release secondary metabolites that were previously accumulated in their body. These settlements play an important part in preventing the settlement, grazing and feeding of barnacles on the body of gorgorian corals (Qi et al., 2006).

The research of (Orland & Khishmaro, 2009) showed that with the release of secondary metabolites they also might be releasing other antibiotics to protect themselves in the hostile marine environment. Previously many studies have proved that many species of bacteria that occupy the corals are capable to create antibiotics that protect them and their residence which is also known as corals. With time a huge number of studies have revealed that these microorganisms that are inhabiting on corals might be pathogenic in nature. There were other assumptions like these microbes might be protecting the corals or taking nutrients from the coral to live or they are a source that is providing essential nutrients to the coral itself etc (Rowher et al., 2002).

Among the microbe community actinobacteria is the community that is inhabiting a large number of corals which has been used as a good source of novel and new bioactive compounds of unique characteristics. Actinomycetes are responsible for almost half of the drugs of database of antibiotic literature (Jiang et al., 2007). Marine actinobacteria present a source of huge potential that could save the lives of thousands all around the world by producing is unique characteristics of secondary metabolites. In current years actinobacterias that are of marine origin have drawn attention from scientists all around the world (Lampert et al., 2008). However the number of research related to collection of actinomycetes from marine source is still low.

In order to get a better understanding of gorgorian corals and their residing actinomycetes (Zhang et al., 2013) have conducted this research. In their study they showed that five new species of gorgorian corals were found from South China Sea and they were also able to find a huge diversity of actinobacteria residing on those gorgorian corals. It seemed that it was the first attempt to the record that tried to find information about gorgorian corals residing actinomycetes. More than fifty eight point five percent of actinobacterial isolates that were collected from South China Sea shoed activity against other pathogenic bacteria.

All these findings suggest that the presence of actinobacteria and their own produced secondary metabolites might be the mean of protection of these gorgorian corals against other species and hostile marine environment. That is why actinomycetes collected from marine source is becoming a very popular source of medically applied secondary metabolites and it is increasing the number of research that are focused on how to get antibiotic compounds from actinomycetes (Zotchev, 2011).

For the discovery of natural products with bioactive characteristics marine actinobacteria are emerging as a new unexplored source. Cantabrian Sea is very important location that is known for its huge diverse marine ecological system. Eighty seven different types of invertebrates that were residing in coral reefs were collected from this region for the checking of antibacterial activity in them (Vizcaino et al., 2016). All these samples were collected from the depth difference of fifteen thousand to forty seven thousand meters. Here with coral reefs samples were collected from arthropods, annelida and molluscs.

Though we are living in modern day the natural source still remains to be primary source for getting products related with biomedical products. We also use products that are to be further used through biotechnology from natural sources. Now scientists are using new and different types of trends to find compounds that are novel and new which can also be used as pharmaceutical compounds like as antibiotics for fighting human pathogens. Estimation tells that almost sixty percent of deep sea and more than two thousand meter of depth is covered by water. All the water area of these region has enormous pressure with pitch black darkness and very low oxygen level and all these factors make this sea very worthy for getting new and novel bioactive compounds (AT, AC & Goodfellow, 2000).

1.3.8.7. Central Cantabrian Sea

The coral reefs are considered to be the most productive type of marine ecosystem because the type of diversity found in this coral reefs said to be higher than earth tropical forests (Haefner, 2003). I case of products with huge biomedical relevance coral reefs are one of the reservoir for these type of products and it was previously estimated that some natural compounds are indeed produced by the microbes specially the marine invertebrates that are inhabiting all these coral reefs (Ryan & Dow, 2008). Previously there were some confusions and misconceptions about the origin of product like there were some products that thought to be derived of invertebrated but later found out that those were obtained from microbes with symbiotic characteristics and as a result the term that is known to be 'symbiotic-products'emerged (Giddings & Newman, 2013). There were very little known about coral bacteria but many studies showed that actinobacteria population isolated from soft corals as well as from corals that are known as 'stony corals'. All those studies were based on corals that were of tropical water corals in origin so very little was known about corals of cold water area. What makes tropical area corals different than cold water corals are factors like cold water corals live on areas like dark areas and they do not contain any endosymbiotic algae in their system (Morgan, 2005).

Recently the activities caused by humans are increasing than ever before and all these activities are threatening the life cycle of these cold water corals. There were some reports that were associated with corals associated with soft corals and most of them were related to corals of ocean of northeast atlantic. Both approaches of culture dependent and culture independent was carried out. Recently it was found out that at the depth of more than forty seven hundred meters actinobacterias associated with corals and other invertebrate species. Recently a new type of novel antibiotic known as *Myceligenerans cantabricum* from th depth of fifteen hundred meters in Cantabrian Sea.

It has become a well established matter that the Streptomyces genus of actinobacteria has become very famous for the production of their unique natured secondary metabolites that are being used for pharmaceutical appliances like antibacterial and antifungal agents. At first most of the Streptomyces obtained was from terrestrial sources and at the early of twentieth century it became evident that actinomycetes also exist in marine environment and since then

expedition marine borne actinomycetes has started (Fieldler et al., 2014).There were reports of collection of actinomycetes at the depth of ten thousand meters of mariana trench. Since terrestrial actinomycetes were found at first place too much research was conducted on this topic and it was found out that terrestrial actinomycetes decompose matters that are organic in nature and also produces secondary metabolism which protects both them and their hosts. In this case we have very little knowledge about marine actinomycetes and recently we have found out that they also produce secondary metabolites to protect themselves and their hosts but there is surely a lots of things that we do not know (Nava et al., 2014).

The research conducted by (Vizcaino et al., 2016) showed that they found actinomycetes that produced secondary metabolites that can fight against the human pathogens that also had antibiotic resistance. However most of the metabolic related potential of actinomycetes is still unknown because the metabolic actions cannot be expressed no matter how hard it is tried in laboratory condition which also represents the characteristics of silent or dormant gene cluster in actinobacterias.

However it is undoubtedly true that scientists have made extraordinary achievements in the field of marine actinomycetes and all these advancements have opened a new possibility for its future use like in cosmetics, industrial products and in clinical medicines etc.

Recently there are many studies going on about how to activate the dormant or silent gene clusters in actinobacteria. The report of (Reen et al., 2015) found something that is able to activate the silent gene clusters inside actinobacterias.

The news of actinomycetes existence and their ability to produce secondary metabolites to fight back human pathogens is not new but the diseases are becoming multidrug resistance and it is becoming more and more severe with days (Levy, 2002). Diseases and their progenitor pathogens that were once thought to be curable by antibiotics are becoming more and more powerful and old antibiotics seem to be not working against this diseases anymore. When one bacteria or other microbe fights against same antibiotic it develops new mechanisms with time and these mechanisms act by disabling the actions of antibiotics making them completely useless like not letting the antibiotics in their body part or making pumps to express out the antibiotics or making enzymes to degrade the antibiotics like beta lactamase breaks down beta lactum ring of penicillin related antibiotics. It has been said that

the drug resistance charactertistics of microbes may be a part of their genetic trait or it also can be achieved by the acquired means. Marine actinobacterias and their hosts and possible hosts are continuously explored for the search of new and novel types of bioactive compounds. Microbes like fungi and bacteria survive by fighting against their competitor and also by adapting with their surroundings and we all know that actinomycetes is like the hybrid of bacteria and fungi and it also has survival and defensive tactics. These tactics are responsible for production of secondary metabolites. These secondary metabolites are created by them in response to stress and due to other stimuli and many of these secondary metabolites have shown their bioactive properties and used in production of pharmaceutical products (Shetty et al., 2013).

1.3.9. Regulators of antibiotic production in Actinomycetes

Marine environment is hugely filled with life and actinomycetes and other bacteria constitutes a large part of its microbial community but among all other microorganisms actinomycetes are the only phylum that has important ecological and biotechnological role in this world (Okami et al., 1988).

Actinomycetes are the holder the position of world's most prominent position for their ability of producing novel and new bioactive compounds. More than seventy percent of our natural products come from actinomycetes. Even after all this the production of actinomycetes is still in its infant level. The diversity of actinobacteria is ahyge chance for screening of new and novel bioactive compounds. Coral reefs, soil sediments and sponges are very rich sources to get actinobacteria. Actinomycetes are also good producers of antibiotics like tetracycline, macrolide, phenazine, plyenes, lactones, glycopeptides and others (Ahmad et al., 2013).

1.3.9.1. Goadsporin

The genus of Streptomyces have the ability to produce secondary metabolites more than thirty different types. However the presence of cryptic or better known as silent gene clusters are disabling the production of more new types of secondary metabolites. The nature of the expression of these biosynthetic genes is conditional and requires different culture conditions for expression (Onaka et al., 2017). There were development of a huge number of methods to solve these problem and it was found out that goadsporin is the secondary metabolite isolated

from the *Streptomyces* sp of TP-A0584 and this compound has the ability to activate the biosynthetic gene clusters in and sporulation in many other Streptomyces species (Igarashi et al., 2001).

The class of natural products especially the bioactive compounds isolated from Streptomyces like streptomycin has a played a huge role in modern medical science. Recently the pharmaceutical companies worldwide is shutting down their production of antibiotics from natural sources. The only reason behind this is the rediscovery of same existing bioactive compounds. The same bioassay guided screening is becoming less efficient and not giving expected results at all. Even new products are isolated question remains about their stability (Cragg & Newman, 2013).

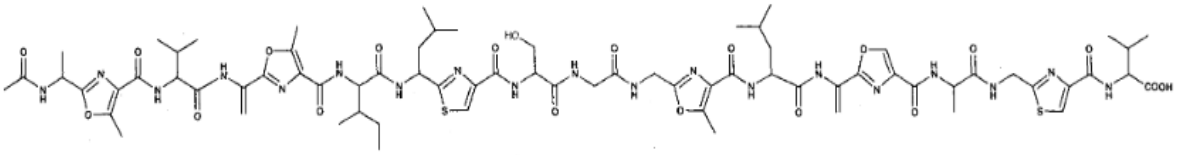


Fig 1.5. Goadsporin structure (Onaka et al., 2001)

Most of the pharmaceutical companies have their own libraries of synthesizable compounds and gives priority to the screening assays that are able to give large amount of new products. Even after all this factor natural drugs remain a very attractive site due to the factor like the core nature like chemical structures and other bioactive properties of natural drugs can only be found from nature not inside of laboratory. That's why for identification of novel and new medicinal candidates with a diverse chemical structures it is important to do screening of natural products. Since the beginning of 2000s, many different novel methods for analysis of genomes have been developed and all this methods are responsible for revelation of many important news about the history of antibiotic production, for example, by advance level of genome analysis it has been found out that the genus *Streptomyces* is able to produce thirty different types of secondary metabolites, suggesting that the genus *Streptomyces* has the potential to produce thirty different types of secondary metabolites with potential bioactive properties from each of its strain. After many attempts only a few secondary metabolites were found in culture broth (Nett, Ikeda & Moore, 2009).

That is why novel and new techniques like changing the condition of nutrients in production medium, co-cultivation with other microorganisms or genetic modification and recombination of biosynthetic gene clusters or simply chemical elicitors are needed for accessing the gene clusters that are able to produce new and novel bioactive compounds (Scherlack & Heartweck, 2009).

1.3.9.2. Autoregulators

A hormone like component named autoregulators are widely distributed in the system of Streptomyces species and they are responsible for the production of secondary metabolism by Streptomyces species. Many research is going on about the existence of this mysterious compound though some Streptomyces species are missing this component while a majority of them have this component (Hrinouchi & Beppu, 2007).

The most fascinating feature of combined cultivation is both of the microbes grow depending on the scale of culture and thus the preparation of mycolic acid containing bacteria in large amounts is easier than the preparation of compounds that are chemical in nature. Though the concept of co-cultivation is not entirely new (Betrand et al., 2014). The efficacy and efficiency of the use of co-cultivation for the production of secondary metabolism is yet to be determined. There are speculations that co-cultivation of mycolic acid containing bacteria along with actinobacteria may be able to stimulate the production of secondary metabolism. Moreover here are no relationships based on the structure of compounds that were isolated from co-cultivation (Hoshino et al., 2015).

Actinobacteria are the type of organisms that are widely known for its co-existence with other species like, protozoa, insects, and even in plants, soil environment. A series of experiments have showed that the type of secondary metabolism produced by actinomycetes changes with type of microbe that it is co-existing with. They can also change their pattern of producing secondary metabolites. Thus it is still in mystery that why actinomycetes produce many types of secondary metabolism. Sometimes it is thought that they are producing these different typed of secondary metabolites due to survival purpose or to protect the host organisms which we can see in case of corals that get their protection from the actinomycetes that live within them and protects them from hostile environment and predators (Bertrand et al., 2014).

It is very difficult to get a clear picture of the energy into the process of the production of secondary metabolites. In combination culture secondary metabolism production id triggered by contact with other type of species and this also provides a technique by which one specific organism recognizes another organism in the outer world.

1.3.10. Situation of antivirals from Actinomycetes

Natural products are the source that provides new products which can be used immediately or the modifications of chemical structures can be used as drugs in the future. But to date there were very few work related to antiviral compound discovery from actinomycetes (Delekta et al., 2013).Arthropod-borne viruses or better known as arboviruses are the reason behind the infection vased disease reemergence. They are increasing both in urban areas and worldwide due to traveling which is ultimately leading to epidemics created by arbovirus (Miller et al., 2001).

At the same time people are using these arboviruses as their defense. For example, United States of America is using arboviruses that can readily attack the central nervous system as a weapon. The genus known as Alphavirus of the Togaviridae family is housing almost thirty total different insect-borne, positive stranded RNA, enveloped viruses and one-third of these viruses have the potential to cause disease in animals and humans worldwide (Griffin, 2001).

All of the encephalitic alphaviruses including eastern, western and the Venezuelan encephalitis directly attack central nervous system resulting in destruction of neurons and inflammation of central nervous system. These extremely virulent pathogens have the power to increase fatality in humans above seventy percent and the survivors can suffer from long-term and severe neurological sequelae (Aguiler, 1970).

There are no antiviral drugs or a perfect cure for arvovirus infections at this moment but there are investigational drugs that are still on trial level that are tested against eastern equine encephalitis virus or western equine encephalitis viruses, these drugs are formalin-inactivated. The use of these drugs to laboratory personnel who are working with these infectious agents. In the meantime scientists are trying to develop an alternative, chimeric, attenuated, DNA dependent alphavirus vaccines but it seems that their work to give results is still years away (Wolfe, Forence & Bryant, 2013).

1.3.10.1. Present crisis of alphavirus diseases

However the combo of vaccines that are active and antiviral therapy may be able to help us in these situation like stopping an outbreak or intentional exposure of viral pathogens. Although there were numerous amounts of samples that showed activity against alphavirus infections only few of those showed their ability in animal models (Sindac et al., 2012). Thus there is a dire need to make or to find a cure that can target the pathogens effectively and eradicate this disease from the face of the earth (Reichert et al., 2009).

The chemical libraries that contain the collection of huge amount of small molecule components of known structures can be examined based on the order of importance to find out a rich source from where identification of novel materials can be started. The research paper of (Dobry et al., 2013) did something like this.

1.3.10.2. Combinational chemistry approach

The alternative approach takes the advantage of the complicated biosynthetic pathways of living microorganisms which has the potential to produce new compounds of unlimited structured diversity (Verdine, 1996). This approach was used at its early stage and the results were very successful because it helped scientists to identify and as well as to develop new antimicrobial agents. However, the precedence is limited to this approach because natural products were not used previously to develop novel antiviral compounds (Wang et al., 2007). With time huge amount of secondary metabolites were collected from microbes of different regions of earth and all these products are responsible for changing the face of human and animal or veterinary drugs over the previous several decades and these drugs are continuously providing new medicine leads for pharmaceutical development (Walsh, 2004).

1.3.10.3. Use of cell based replicon HTS

Shallow and deep water sediments from marine environment is proving its worth as a rich source of novel microbes whose secondary metabolites provide not only new medicines but also creates the situation where further modifications may improve the future drug crisis scenario. The research of (Delekta et al., 2013) used the cell based replicon high throughput

screen which enabled them to use a drug discovery process for antivirals that are effective against western equine encephalitis viruses (WEEV) and other related arboviruses.

1.3.10.4. Output of HTS

The approach of (Delekta et al., 2013) used the marine species of actinomycetes *Streptomyces kaviengensis* and was able to isolate antimycin which showed very potent activity against western equine encephalitis viruses (WEEV). The acntimycin went through mETC inhibition in order to disrupt WEEV replication. Antimycin was successful in inhibiting WEEV in mice with a narrow therapeutic window.

1.3.11. Other areas for application of Actinomycetes

Modern research is also focusing on the ability of actinomycetes to produce lipopeptide biosurfactants that has the ability to degrade hydrocarbons. These new lipopeptide biosurfactants are drawing the attention of scientists all over the world because of their low toxicity, ecological acceptability and non-hazardous degradation (Raviji et al., 2009).

1.3.12. New source for different types of drugs

with the increasing of resistance in antibiotic effects new drugs are badly needed and chemical synthesis, bioengineering of new drugs are progressing but the nature is still the rich source for new antibiotics. The family actinomycetaceae of actinobacteria is very famous for producing antibiotics which are active against many different pathogenic organisms (Manivasagan et al., 2013). In the past for production of antibiotics the terrestrial actinobacterias were used but now new and more resistant diseases are emerging and that's why scientists are now using marine sources to produce novel antibiotics. Marine actinobacterias produces secondaty metabolites which are the best and a vast amount of these secondary metabolite come from Streptomyces genus. This specific genus is widely distributed in both terrestrial and marine areas throughout the world and gaining interest from scientists for their unoque ability to produce antibiotics. This Streptomyces genus is responsible for producing 80% of natural products alone. Marine actinobacterias have distribuition which is very wide they are present in sponges, mangroves ,seaweeds mollucs , fishes etc (Pham et al., 2015). They are not getting importance only due to producing

antibiotics but also their ability to produce immunesupressant agents, antitumor agents, pigments,enzymes and enzyme inhibitors etc. with time the chance of getting new antibiotic from terrestrial source is decreasing and now marine source is said to be the source of novel antibiotics. Though actinomycetes covers 80% of natural products but rest is covered by species like *Micromonspora*, *Actinoplanes*, *Saccharopolyspora* and *Amycolatopsis*. actinobacterias were collected from free swimming areas, marine vertebrates and invertebrates, deep oceans etc. modern day researchers are getting new types of genera on regular basis and new metabolite producers from oceanic environment that hasn't been reported earlier. A single strain of actinobacteria has the potential to produce 10 to 20 different types of antibiotics (Betancur et al., 2017). Recent research has proven that there are many unknown gene clusters in many known species of actinobacterias which for some reason remain dormant but research is going on to unlock the cascade of these gene clusters. Secondary metabolite or antibacterial compounds like essramycin, caboxamycin, marinopyrroles, dihydroquinones were obtained from actinomycetes. For antifungal effect secondary metabolites like chandrananimycin, tirandamycin etc were found from actinomycetes which are agtive against candida albicans and aspergillus niger (Zhu et al., 2013).

1.3.12.1. For Cancer

Cancer is one of the most serious problems in human history and actinomycetes are also contributing in this case too compounds like caprolactone, chinikomycin, andmarinomycin show activity against cancer cells. These anticancer agents re orally active inhibitor of proteasome which acts as inducer of apoptosis in cells with myeloma cell characteristics. But it seems that research on finding anticancer agents is not as wide as research on finding antibacterial compounds from actinobacterias (Cartuche et al., 2015).

1.3.12.2. For Tumor

With the prevention of new diseases by antibiotics more diseases and tumors are becoming resistant to these agents. Which draws the attention for obtaining antitumor agents from actinomycetes. Before research on actinobacterias for getting antitumor drugs development of new novel antimicrobial and antineoplastic agents were the only source for cure. When

research on actinomycetes begun a large number of new structured molecules have been found (Zheng et al., 2000). This occurred specially focusing on marine actinomycetes because chances of getting new antitumor agents from terrestrial sources were very low. Some antituomor agents like chinikomycin showed desired effect against tumor but produced no effect against virus disease (Jensen & Fenical, 1994). But further research produced agents like aureovertilactam, marinomycin, mechercharmycin which showed powerful effect against both tumor and viral diseases. In case of cytotoxic effect piericidin C7 and C8 were some of the many cytotoxic effect givers (Bernan, Maiese & Greenstein, 1997). In tumor cell proliferation process angiogenesis is an essential step and it is very important in metastasis. If this step can be prevented tumor cell formation can be stopped. Streptopyrroidine is a derivative related benzyle tetrahydropyrole that showed significant anti-angiogenesis activity against human umbilical vein endothelial cells (Trischman et al., 1994).

1.3.12.3. For Anti-inflammation

Actinomycetes were also responsible for producing anti-inflammatory drugs because infectious disease is the number one fatal disease in topical countries and between 1940 to 2004 almost 335 whole new infectious disease emerged. Salinamides A and B form Streptomyces showed good anti-inflammatory and antibiotic effect (Fielder et al., 2014).

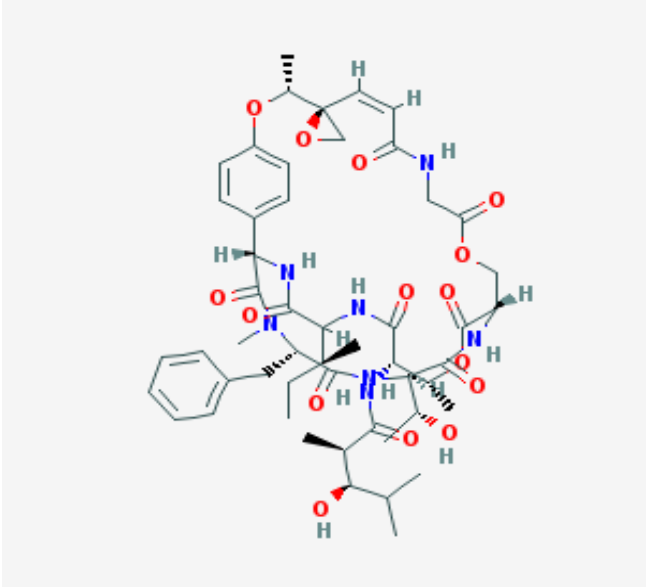


Fig 1.6. Salinamide A (obtained from NCBI) (Hassan et al., 2015)

1.3.12.4. Antimalarial activity

Antimalarial effects are another feature of actinomycetes. Malaria is caused by the genus plasmodium and it alone is responsible for 300 million annual clinical cases and 2 million deaths per year. Among many other malarial parasites P.falciparam is the most lethal one because with time it becomes resistant to any drugs that it has faced before. Trixocarcin A,B and C showed powerful effect against malarial parasites and in addition they show powerful antiviral and antibacterial activity (Karthik et al., 2014).

1.3.12.5. Antiviral Drugs

In order to control viral contamination and transmission with getting chemotherapy for humans and animals viral disease antiviral agents form marine actinomycetes plays a significant role. Benzastatin is one of the few antiviral agents that shows powerful effect against both herpes simplex virus type1 and type 2. Recently marine *Streptomyces* showed activity against white spot syndrome disease of penaeid shrimps in india (Manivasagan et al., 2013)

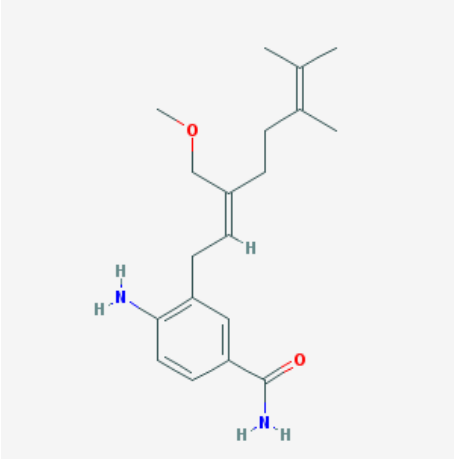


Fig 1.7. Benzastatin A (obtained from NCBI) (Lee et al., 1995)

1.3.12.6. Antioxidant

To prevent natural oxidation of body antioxidants are needed and actinomycetes can also produce antioxidant agents. Dermacozins A-G are the group of antioxidants isolated from Streptomyces species (Manivasagan et al., 2013)

1.3.13. Triggers for antibiotic production

What causes the production of antibiotics from actinomycetes and why specific species of actinomycetes only produce the same old antibiotics? This question become more frequent when a group of researchers led by Dr. David Hopwood showed that one single gene cluster of *Streptomyces coelicolor* contained several other genes with potential to create antibiotics but the obstacle was the other gene clusters were dormant (Zhu et al., 2013). From then it became clear that other species of actinomycetes also have arsenals of unknown antibiotics. Therefore it seems that the potential of *Streptomyces* genus in producing antibiotics is much wider than originally predicted.

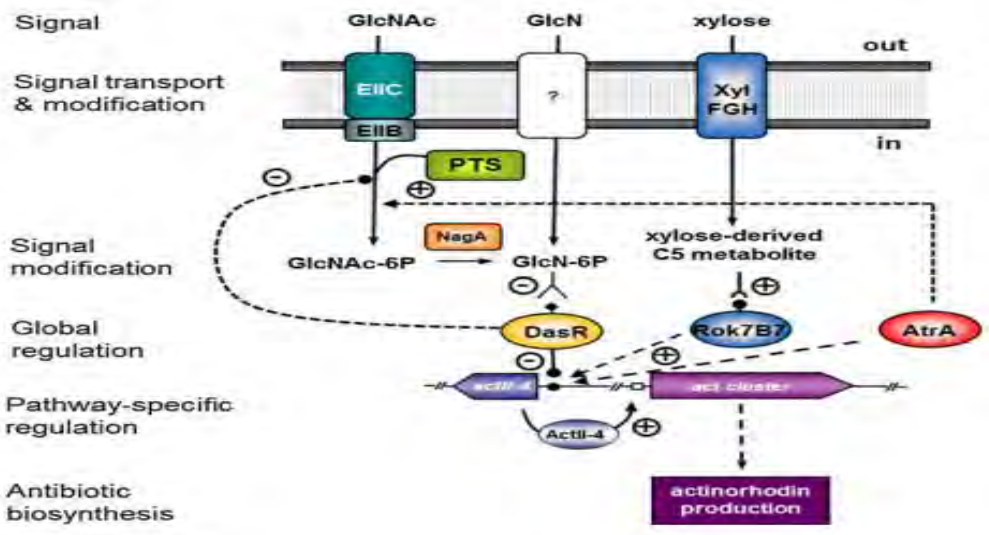


Fig 1.8 Involvement of different triggers in the production of actinorhodin by *S.coelicolor* (Zhu et al., 2013)

1.3.13.1. Ribosome Engineering

Ribosome engineering is a very effective and efficient way to activate the dormant gene clusters in order to produce antibiotics. The effectiveness of this process is proved because this same process was used to activate gene clusters and producing antibiotics in *Streptomyces coelicolor* and *Streptomyces lividans*. It is also responsible for triggering the production of less known actinomycetes species such as *Streptomyces mauvecolor* (Ochi & Hosaka, 2013).

1.3.13.2. Co-cultivation

In order to activate the dormant gene clusters of actinomycetes we first need to understand their biological purpose. Streptomyces species reproduce via forming spores that is dependent on aerial mycelium. This mycelium goes through lysis when stress factor such as lack of food source is applied and more and more microbe starts competition and as a result they produce antibiotics as a self-defense act (Mantaeca et al., 2005). Based on this phenomenon co-cultivation was used to produce antibiotics like alchivemycin from Streptomyces with the help of *Tsukamurella pulmonis*. The acidic cell wall of *T.pulmonis* induced production of secondary metabolites in *Streptomyces* to defend itself against pulmonis (Wezel & Mcdowall, 2011).

1.3.13.3. Rare Elements

Rare elements of earth have been proven effective in activating gene clusters which were previously dormant. Enhanced production of actinomycin, streptomycin and actinorhodin from *Streptomyces coelicolor*, *Streptomyces griseus* and *Streptomyces* antibiotics by scandium and lanthanum (Ochi & Hosaka, 2013). Moreover use of acandium also increased production of actinorhonodin in Streptomyces lividans and enhanced production of amylase and baclysin in *Bacillus subtilis* (Ochi & Tanaka, 2013).

1.3.13.4. HDAC Inhibitors

HDAC inhibitors or inhibtors of histone deacetyles has shown increased expression of secondary metabolites in many actinobacterias. They produce antibiotics by altering the structure of chromosome and alternation of chromosome structure is caused by effects that antagonizes histone acetylation in eukaryotic cells (Sterner & Berger, 2000). Sodium butyrate is a well known HDAC inhibitor that is used to produce antibiotics from *Streptomyces coelicolor* but the exact and detailed mechanism of sodium butyrate is still under investigation.

1.3.13.5. Genome Mining

Now a days genome mining is becoming more and more famous in identifying genes that are able to express secondary metabolites. Processes like secondary metabolite analysis shell, secondary metabolite unknown region finder, cluster sequence analyzer have made this secondary metabolite expression very easy and also caused a rapid decrease in cost for genome sequencing (Caboche et al., 2008).

1.3.14. Producing Biosurfactants

Marine sponges are getting very famous for the production if various natural products because they contain massive numbers of bacteria within them that are useful. Compounds obtained from symbiotic microorganism have the big chance for providing a source that is sustainable when extracting bioactive compounds. In recent years natural biosurfactants from natural sources is becoming more important because of features like simple biodegradability, less toxic, non-harmful to environment and ecologically acceptable (Gandhimathi et al., 2009). Microorganisms are capable of producing a wide variety of biosurfactants. Biosurfactants have amphipathic properties like having hydrophilic and lipophilic parts that can reduce the surface tension at a high degree of aqueous media. From Rosenberg and Ron, biosurfactants are two different types one is like low molecular weight glycoloipids also known as lipopeptides and high molecular weight polysaccharides. Biosurfactants can be produced by microbes in cell culture broth or they have the property to adhere to the cell surface of microbes (Rosenberg & Ron, 2001). Surface active microbes are getting the attention for biomedical and therapeutic purposes like antibacterial, antimycoplasmic and antifungal activity. Strains that causes high yielding in production are perfect for biosurfactant

production. Composition of media such ad growth factors, carbon and nitrogen factors are also important during culture of these microbes (Cameotra & Makkar, 2004). Sponges that are the containers for microbe provides more nourishment to microbes than sea water. Marine microbes are perfect source for producing bioactive products, bioremediation and other pharmaceutical products. The actinomycetes strain *Nocardiposs alba* is very famous for its biosurfactants. Literature study proves that the biosurfactants from *N.alba* plays a very crucial role in breakdown of polycyclic aromatic hydrocarbons (Bultel et al., 1999). There are several method and solvents for extraction biosurfactants from culture like precipitation by acid, centrifugation, ammonium sulfate crystallization, solvent extraction and crystallization. Sometimes solvents like methanol, chloroform and acetone is too dangerous to use for the recovery of some specific biosurfactants if this is the case then cheap and less toxic solvents can be used to extract biosurfactants to minimize or to prevent the loss. Many studies have revealed that glucose can serve as the best source of carbon if the production of biosurfactants that are lipopeptide is desired (Desai & Banat, 1997). During analyzing the carbon source it has been found that paddy straw and other cheap raw materials showed good production. For less emulsification activity NaNo3 can be used, if the desire is to increase the emulsification and growth then yeast extract or peptone can be used. Both gram positive and gram negative is famous for producing lipopeptides that are cyclic. Lipopeptide producing microbes have high diversity which leads to the theory that these lipopoeptides can serve multiple purposes. The limited applications of biosurfactants that are lipopeptide has given limited results like properties of biosurfactants and swarming of bacterias (Mulligan, 2005). It has been said that microbes that can produce lipopeptides may contain potent antimicrobial effects. Due to its easy biodegradability, less toxicity, good foaming property and compatibility to environment natural biosurfactants can be used in different applications (Zajic et al., 1977). Natural biosurfactants can be the very tool to protect our marine environment because oil leaking is dangerous to our ocean and its life these natural biosurfactants have the capability to breakdown these hydrocarbons. There are many theories about host sponges and its biosurfactants but lack of research had proven nothing and it seems that production of biosurfactnts by marine organisms or marine sponges acts more like a defense mechanism. These biosurfactants may be the only and effective tool against the pollution of heavy metals.

Sponge related bacteria and the biosurfactants produced by them and their functionality is still under investigation (Schmidt et al., 2000)

1.3.15. Lichens as an alternative source

Litchens have symbiotic nature and closely associated with fungi. They can be used as an alternative source for actinomycetes. Researches on this case has shown that the number of different srtains isolated from this lichens source is very high than strains of actinomycetes isolated from cold areas (Gonzalez et al., 2005). Litchens have been the source of different taxonomic groups. The richness of the species was immune to the type of substrate of the location point. So there is a high chance that lichens are the specimens from which the isolation of rare and minor genera could be possible. Though the symbiotic components of the lichens have been revealed much is unknown about their microbial community diversity (Petrini et al., 1990).Different tools of a wide variety of taxonomic resolution were used to evaluate the microbial community diversity. Initial morphological characteristics were confirmed by the analysis of the type of fatty acids contained by the species. Rapid evaluation of this microbial community was done by rapid analysis on fatty acids. PCR identification method was used to find out the relation of the previous trains isolated from other sources (Embley et al., 1994). By using this technique misconception about the genera of nocardiopsis and actinomadura which was previously thought of as *Streptomyces*. Analysis of the results obtained from PCR fingerprinting of tRNA amplification provided further information of the diversity of the strain level (Methling et al., 1995). Research conducted by PKS 1 and PKS 2 with NRPS systems showed high detection level of unknown sequence whereas APH and HMG systems showed lower detection of the same sequence (Anderson et al., 2002). Though some of the groups showed limited activity against resistant strains due to limited cultivation conditions further research can improve this situation. The absence of antibacterial activity happened due to the lacking of expression of the biosynthetic processes (Schwarzer et al., 2003).

1.3.16. Antituberculotic activity

In nature actinobacteria are gram positive and they contain guanine-plus-cytosine genomes in their content. They live as saprophytes in different organisms. Actinobacteria are famous for their capability to produce secondary metabolites of diverse types and many of these metabolites have clinical importance (Hussain et al., 2017). This production of different functional and different structured compounds from actinomycetes makes them an important source of new and novel drugs. *Mucobacterium tuberculosis* is responsible for the formation of tuberculosis in human body. It is one of the oldest and pervasive diseases in history (Zin et al., 2007). It has always been a challenge when it came to look for a cure to treat tuberculosis. The treatment for tuberculosis became difficult due to the emergence of new and highly resistant strains because with time non-resistant strains become resistant strains and that is the main problem. The emergence of HIV/AIDS has caused another problem in this scenario because it has worsen the complications in drug-drug interaction in the treatment of tuberculosis. In the treatment of tuberculosis phylum actinobacteria has a long history (Janin, 2007). The strepromycin discovery brought a revolutionary change in the treatment of tuberculosis. Back then the secondary metabolites used to treat tuberculosis mainly came from *Streptomyces griseus*. Other drugs like rifampicin and kanamycin also came from actinobacteria in the tuberculosis treatment therapy (Ashforth et al., 2010). While searching for new secondary metabolies the reisolation of old found molecules or secondary metabolites becomes the main problem. The searchinf of entirely new scaffolds from a wide variety of sources has become the new goal in programs for drug discovery. To increase the chance of getting new molecules now searching is conducted on new locations because at then it becomes highly likely to get new species thus increasing the chances of getting new secondary metabolites (Bull et al., 1992). The research conducted by (Hussain et al., 2017) focused on the northwestern himalayas unexplored habitats. The number of isolates obtained from the research is 125 grown in different medias.

1.3.17. Effects of salinity on antibiotic production

Previous researches showed that in order to grow salinispora actiniobacteria seawater type of medium is necessary. Salts that are available in commercial market or natural seawater can be used for this culture. This research was conducted by (Hodson et al., 2013) to find out the effect of salinity on sponge derived salinispora actinobcteria. *Salinispora arenicola* was used to produce rifamycin as an alternative to terrestrial grown *Amycolatopsis mediterranei*. Rifamycins is the group of antibiotics belong to ansamycind these were first isolated back in 1959 (Jensen et al., 2007) . Rifamycins are very effective agiainst gram-positive bacteria and diseases like mycobacterial infection, leprosy and tuberculosis. They give their action by binding with beta subunit of RNA polymerase which ultimately inhibits DNA dependent RNA polymerase. *Salinispora* requires sodium for its growth. The use of lithium and potassium can also increase growth which is found recently (Tsueng & Lam, 2008). Stress created by salt can heavily affect the nature and formation of both primary and secondary metabolites produced by *Salinispora*. Adapting to marine water conditions can also affect marine bacteria especially in their secondary metabolism. In the research *Salinispora arenicola* was grown in liquid medium of starch-yeast-peptone with supplements of different concentrations of sodium and potassium ions (Malin & Lapidot, 1996). The production of rifamycin was measured by tracking the cycle of growth in shake flask broth culture and salt concentrations were supplied accordingly. In order to perform the analysis of secondary metabolites high performance liquid chromatography with diode array detection was used. Variations were analyzed like time difference of 7 to 14 and 14 to 29 days. Samples were growl separately in low salt and normal sea salt concentrations. Here the main purpose of the research was to find out the effect of salt water in sponge derived *Salinispora arenicola* and its production of rifamycin (Mincer et al., 2005).Other goals were to find out an alternative media other than salt water media and to find out wheather synthetic sea salts can be used for the growth of *Salinispora arenicola* and rifasmycin production. Sodium is the ion that is most abundant in sea water so its no wonder why it is required for the growth. Growth was well in SYP-ASW medium both in 1% concentration and 3% concentration. But the growth was not very good at M413 medium when concentration of sodium was decreased to 0.01% or raised to 6%. Rifamycin production was good in SYP-ASW and M413 mediums on normal salt concentration but no rifamycin was produced when the concentration of salt was decreased to

0.01%, it required 20 days in incubation to produce rifamycin in M413 medium with 0.01% salt concentration. Another pair of secondary metabolites like salinisporamide A was increasing with salt concentration but salinispoaram B was decreasing with increasing level of salt concentration.

1.3.18. Bioprospection of freshwater Actinobacteria

Actinobacteria are a very diverse group of filamentous bacteria. They have the characteristics of both fungus and bacteria and they have guanine-cytosine content of 50% to 70% in their genome (Ventura et al., 2007). Actinobacteria are very famous for their use in the production of industrial grade enzymes and antibiotics, they are also becoming famous for their use in experiment of finding novel secondary metabolites (Goodfellow et al., 2010). The resistance of multidrug resistance pathogens against present antibiotics is the only reason behind the working to get new and novel secondary metabolites (Bae et al., 2016). Most of the multi-drug resistance pathogens that are emerging worldwide are gram negative in nature and bacteria like Vancomycin resistant enterococci (VRE), Methicillin resistant staphylococcus aureus (MRSE) and extended spectrum beta lactamase (ESBL) are producing more and more gram negative bacteria to make this multi-drug resistant situation worse than any other time (Chambers & Delio, 2009). Due to all this factors there is a constant pressure to get new and novel antibiotics from unexplored or less explored sources, because in case of novel secondary metabolites from actinomycetes the lesser explored the area the greater chance for getting new and novel secondary metabolites. Actinomycetes is the only family in bacteria that has the potential to provide mankind with new novel antibiotics (Yuan et al., 2014). It has been reprted that forty five percent of all natural bioactive compounds come from actinobacteria and more than seventy percent of those bioactive compounds are produced by the genus *Streptomyces* of actinobacteria alone (Berdy et al., 2005). Way back in 1940 when the first antibiotic named actinomycin from actinobacteria was isolated many researches was conducted and a huge number of antibiotics were isolated from actinomycetes. Since then several novel species of *Streptomyces* have been reported worldwide to increase the search of novel and naturally occurring antibiotics from actinomycetes (Ray et al., 2015). There were also experiments on actinobacteria of different origin for example, actinobacteria from soil origin, freshwater origin, sea-water origin, plant origin and even from insects actinomycetes were isolated.

However the number of researches based on this origin is continuously decreasing because the rediscovery of dame old actinomycetes species and as a result scientists are giving more importance to the isolation of actinomycetes from unexplored places or systems (Poulsen et al., 2011). The northeast part of india is also known as indo-burma region is very famous for its mega-biodiverse nature and according to Conservation International it is also considered one of the rare hot spots of earth with enriched ecology and unexplored biological sources.

1.3.18.1. Main focus of Bioprospection

The main focus of bioprospection of actinomycetes was on terrestrial actinomycetes and marine actinomycetes and very few bioprospection was conducted on fresh-water actinomycets. There were many biprospection conducted on freshwater actinomycetes (Sibanda et al., 2010). However there were very few cases that showed the potential of fresh water actinomycetes. So the research conducted by (Leo et al., 2018) was mainly focused on freshwater actinomycetes that were isolated from fresh water sediments to check their antimicrobial property and to detect the secondary metabolites produced by them. In their research six different Streptomyces species showed good activity against all the reference strain and more better activity was observed by the methanolic extracts in agar well diffusion method (Leo et al., 2017).

1.3.19. Antimicrobial potential of Actinomycetes of unexplored forest

In the whole world there are about one million compound of natural origin but among these one million only five percent is obtained from microbial origin (Demain et al., 2009). Most of the antibiotics that are persistently used in today's world are of microbial in origin and many of the active microbe strains have made huge contribution in modern medical world and also making contribution in new type of drug discovery and development process. However, due to the lack of knowledge many people all over the world especially people of third class world are using antibiotics without the permission and prescription of physicians and as a result the microbes are adapting new mechanism to make themselves resistant to antibiotics (Wright, 2012). Thus there is the necessity to get or discover new antibiotics or novel secondary metabolites to make counter-offense against the emerging threat of multi-drug resistant pathogens and the life threatening diseases caused by them. Soil is the part of our ecosystem

that contains many important ecological niches and some of them have proved their potential by producing novel secondary metabolites that has great clinical significance (Hong et al., 2009). Actinobacteria represent a huge portion of soil forest and they are also the type of bacteria that has high GC content DNA in their system. They also have diverse physiological characteristics that makes them dominant figure of biotechnological industry because products like enzyme inhibitors, antibiotics, immunomodifier, growth promoting substances and other substances that gets the attraction of biological and biotechnological application (Fiedler et al., 2005). The importance of actinobacteria is clearly observe when we can see that almost five thousand compounds that were derived from actinobacteria and all of those hugely contributed to the development of ninety percent of commercial antibiotics for both clinical and research work (Jose & Jha, 2016). The actinobacteriaceae family members are known producers of polyketide and also nonribosomal polyketide peptide via both type 1 and type 2 polyketide pathway and also nonribosomal peptide synthatase pathway which are the major pathway for producing secondary metabolism in actinomycetes (Singh et al., 2015). The largest Genus of actinomycetes Streptomyces has total eight hundred and twenty six number of species. This genus is very famous for producing novel bioactive compounds for commercial purposes like nystatin, tetracycline, streptomycin and ivermectin etc (Miao et al., 2010). Moreover along with antibacterial medicines actinomycetes is also providing us with antifungal medicines, antitumors, antioxidant and even anticancer like products. At the same time the likelihood of discovering new compounds from actinomycetes is decreasing because of the yielding of old and rediscovered materials (Qin et al., 2009). There are many areas of India that are unexplored and underexplored and one of this places is Assam's forest which can be a promising source to get new species of actinomycetes as well as new and novel bioactive compounds. The study of (Das et al., 2018) showed that the species of actinomycetes that were isolated from Assam's forest showed activity against both non-antibiotic resistant and antibiotic resistant strains. Furthermore the strain PWS52 discovered by them might become a novel antibiotic and might have future applications. From the isolated of their study further experiments can be done in order to get a full idea about their capability against bacteria, fungus and cancer cells (Das et al., 2018).

1.3.20. Glycopeptide antibiotic resistance by Actinomycetes

Glycopeptide antibiotics which are also known as GPAs are the last resort to fight against infections or diseases caused by gram negative pathogens such as staphylococcus aureus, Entrococcus species and Clostridiodes difficile (Perkins & Neito, 1974). Though the first generation glycopeptide antibiotics like vancomycin and teicoplanin were discovered decades ago, they are still being used extensively for the treatment of diseases. Recently some second generation of glycopeptide antibiotics like telavancin, oritavancin and dalbavancine have been approved for clinical use due to their high antimicrobial potency and high type of pharmacokinetic properties (Marcone et al., 2014).

1.3.20.1. First generation GPAs

Among the glycopeptide antibiotics that were derived from soil origin actinomycetes vancomycin and teicoplanin is one of them, their common structural similarity is both of them have heptapeptide scaffold having aromatic amino acids that have gone through extensive oxidative cross-linking and decoration with other types of moieties such as residues of sugar or atoms of chlorine but in case of teicoplanin they contain lipid chain instead of sugar residue or chlorine atoms.

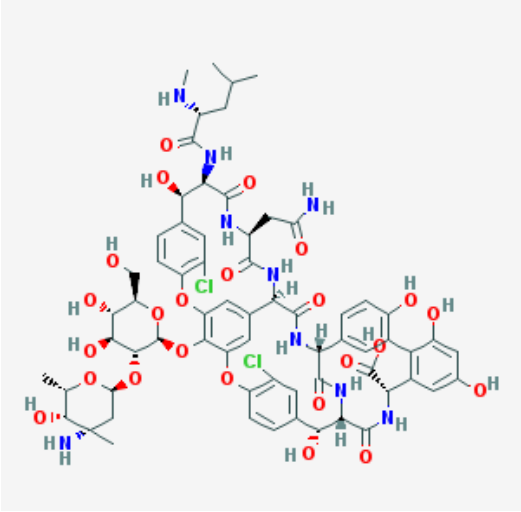


Fig 1.9. Vancomycin (obtained from NCBI) (Bruniera et al., 2015)

Glycopeptide antibiotics give their action by inhibiting peptidoglycan synthesis by binding with D-alanyl-Dalanine terminus of the stem of peptide of the peptidoglycan precursor lipid

2. The binding causes formation of five hydrogen bonds which ultimately locks down peptidoglycan precursor which ultimately leads to the prevention of cross-linking reaction (Cooper et al., 1999).

1.3.20.2. Second generation GPAs

Second generation Glycopeptide antibiotics are more like the semisynthetic derivatives of first generation glycopeptide antibiotic like of Vancomycin and teicoplanin. Their main difference against the first generation Glycopeptide antibiotics lie beyond the D-ala-D-ala pocket where chemical modifications are different than the previous generation. Here these second generation contains attachment of aryl or acyl groups that has the capability to mimic the properties of the lipid chain of teicoplanin. In fact the true strength of teicoplanin lies on its hydrophobic tail which makes the bond strong with membrane localized lipid 2 (Binda et al., 2018). According to (Dong et al., 2009), the lipidationn is the key factor between vancomycin and teicoplanin in case of inducing Glycopeptide antibiotic resistance, because vancomycin does not have the lipid chain and as result it induces resistance quickly whereas the use of teicoplanin induces resistance much lately (Marcone et al., 2018).

1.3.20.3. Reason behind induction of resistance

The van gene that causes the induction of resistance comes from no other than the Glycopeptide producing antibiotics themselves. The replacement of alanine by lactate in (D-Ala-D-Ala) causes the production of (D-Ala-D-Lactate) which reduces the affinity of antibiotic to bind with target molecule thus creating resistance (Allen et al., 2003). The research of (Binda et al., 2018) showed that, the type of GPA resistance created by actinobacteria varies with the genus but what they found in common is that the producers of teicoplain themselves are resistant to Glycopeptide antibiotics.

1.3.21. Concepts and methods to access novel antibiotics

With time antimicrobial resistance is getting more and more attention and the threat itself is becoming more and more severe. According to World health organization report of 2018 and the survey of Global Antimicrobial Surveillance System have showed that the strains like *E.coli, S.aureus, S.pneumoniae* etc are getting more and more powerful (Hug et al., 2018). The species *N.gonorrhoeae* is becoming a superbug because it is evolving and its evolution is making it resistant to third and fourth genarations of cephalosporin and fluoroquinolone and it has been targeted by WHO as one of the most dangerous strains on earth (WHO Report 2018). The golden age of antibiotics which is also known as the time period between 1950 and 1960 was the time ahen people of chemistry and microbiology worked hand to hand and as a result huge number of microbes were cultivated for their secondary metabolites and the genus Streptomyces was the best of them (Aminov, 2010). However the getting of new and novel secondary metabolites from actinomycetes is decreasing since then, and between 2001 and 2008 the production of new secondary metabolites from Streptomyces has decreased to less than thirty percent (Silva et al., 2012). The rediscovery of dame old secondary metabolites, production of low concentration and challenging process of isolation has lowered the research on natural products. All this reasons forced scientists to take their search on unexplored areas like deep ocean or unexplored forest parts and this trials eventually paid off when they found out about *Streptomyces coelicolor*, this particular species became known for producing several new metabolites but when scientists found out that the same species is also holding an arsenal of gene clusters of which expression of secondary metabolites cannot be expressed in traditional culture methods (Li et al., 2009).

1.3.21.1. Exploring new habitats

Because of too much extraction of secondary metabolites from actinomycetes of soil origin rediscovery of same compound started to happen and then scientists started to look toward their marine counterparts or relatives (Lam, 2006). By this way scientists are trying to find new source of actinomycetes in extreme environment, non-*Streptomyces* species actinomycetes and endophytic species. These new sources are described below:

1.3.21.1.1. Extreme Environment

From extreme environments such as dessert and mountain areas sampling have started and this process also brought good result. For example a new species of *Streptomyces* genus was isolated from indian desert and that was able to produce anthraquinone which has weak activity against both gram-positive and gram-negative but also showed moderate to good activity against fungal samples (Al-Dhabi et al., 2016). The screening of Egyptian desert also revealed new strains that showed good activity against most of the pathogens and 12.5% of those new isolates showed excellent activity against both gram-positive and gram-negative strains and also against yeast strains (Arasu et al., 2008). However, the real problem was the lack of correlation because without comparing these new products to other known or unknown product we cannot actually determine the real value of these new secondary metabolites. non-*Streptomyces* actinomycetes from sahara desert named *Saccharotrix* also showed activity against gram-positive and gram-negative strains. Many more non-streptomyces were isolated from areas like sebhka lake of Algeria and from Algerian sahara (Rabie et al., 2011). The hope is all these new isolates showed activity ranging from moderate to extreme level against gram-negative, gram-positive or yeast strains. Another compound named actinomadurol which showed great activity against *Staphylococcus aureus* and pseudominae hauseri but was not much effective against cancer cell lines even at high concentrations (Wilson et al., 2009). Recently water sources that are thermal in nature are being explored for acyinomycetes strains where there is high chance to find actinomycetes especially that are non-streptomyces. The Streptomyces species isolated from Saudi Arabia showed much stronger antibiotic activity profile (Brimble et al., 2009). Now cave systems are also being explored for new actinomycetes species and this type of expedition led to the identification of 15 new Streptomyces species but the only problem is that none of this findings possess any crude extract results that were compared with standard antibiotic activity.

1.3.21.1.2. Endophytic Actinomycetes

Another potential source of new antibiotics could be endophytic antibiotics because there are three hundred thousands of plant species and each of them has the possibility to hold at least one or more type of endophyte (Strobel & Daisy, 2003). The main problem here is the rate of their growth and the rate of their prosuction level which is very lower than already established Streptomyces species. The percentage of Streptomyces found from plants only represent twenty six percent of all known actinomycetes. The plat or rhizosphere originated Streptomyces were 8.1% effective against B.subtilis, 9.1% effective against K.rhizophilia and 1.7% were effective against C.albicans but none of them were effective against E.colim,all this are the results of plant root originated streptomyces (Matsumoto and Takahashi, 2017). The rhizosphere originated Streptomyces were more effective than their plant root counterparts like 17.1% were effective against B.subtilis, 8.9% were effective against K.rhizophilia, 9.8% were effective against C.albicans and 8.1% were effective against *E.coli* (Santana et al., 2016). The most interesting actinomycetes was *Streptomyces pulvurus* because that showed activity against both *S.aureus* and *B.subtilis.*

1.3.21.1.3. Symbiotic Actinomycetes

Bacteria and insects are very closely related to each other because almost every insects carry bacteria in them. Insects and bacteria have a symbiotic relationship among them which is developed by the process known as co-evolutionary adaptation. Insects carry or bacteria reside in a specific part of insects. For example, the attine ants live in a mutual relationship with the fungi known as *L.gongypholorus* and scientists have hypothesized that the actinobacteria that the ants collect from soil keeps the fungi in bay (Barke et al., 2010). A termite related actinomycetes named as Amycolatopsis is responsible for producing macrotermycin which is strong enough to prevent the attack of *B.subtilis* and *S.aureus* (Clurdy et al., 2011). There is another termite related natural product called natalamycin which has very broad spectrum of antifungal activity. Rebeccamycin and several of its analogue shoed very high antitumor activity and they can also inhibit mammalian topoisomerase 1(Kim et al., 2017).

So finally, we can say that underexplored or unexplored areas hold very big arsenal that might help us in the war against multi-drug resistance pathogens (Pettit, 2011). More or less all

newly isolated Actinomycetes show strong level activity against both gram positive bacteria and fungi. Though the findings against gram-negatives remain scarce enriched and diverse terrestrial sources could be our hope to find a cure against gram-negative bacteria which is happening due to the competition among microbes to find nutrients for their survival (Wilson & Brimble, 2009).

1.3.22. Acrtinomycetes an Inexhaustible source

The use of materials that are outside of human body or chemicals to improve or to maintain human health is very old. The greatest achievement of twentieth century is to mankind's ability to discover and develop new drugs to kill harmful pathogens in human body, thus, a tremendous amounts of lives were saved. Natural source still remains as the main source of getting new medicines. From 1981 to 2018 in the last 38 years thousands of drugs were approved by Food and Drug Administration (FDA) and almost 65% of those drugs either directly came from natural source or those were developed based on the drugs from natural source (Newman et al., 2014). The antibiotics or secondary metabolites produced by microbes sometimes possess unique bioactivity and untapped potential that could the research of antibiotics for many more years in the future. Back in 1942 when penicillin was first discovered it saved millions of lives in both World War 2 and throughout the world (Berdy J, 2015). Since then many new discoveries were made and many families related to penicillin were discovered thus, the golden age of antibiotics started. Ever since then the production of new antibiotics started to decline and the cost related to antibiotics development process from concept to market place is increasing. Thousands of drug gets into concept level but less than 10 gets through human trial stage and for final application (Norman, 2016). The way resistance to antibiotics is increasing and the way people of third countries where people are using antibiotics with proper knowledge is making the situation worse, if this continues then this could lead us back to the so called "pre-antibiotic era"(Morgan et al., 2011). Actinomycetes are a group of heterogeneous bacteria and there are almost 355 genera of actinomycetes known to date. They are extremely diverse and distributed in both marine and terrestrial sources. Many of them are harmless to animals and other plants but very few of them are harmful pathogens. The genus Streptomyces of actinomycetes has a huge contribution in modern medical science (Hansen et al., 2016). They have produce many

clinically important antibiotics like streptomycin, actinomycin and streptothricin. The discovery of gentamicin back in 1963 opened the research about so called "rare-actinomycetes" which lowered the work on genus Streptomyces (Barka et al., 2015). Compounds that were isolated from this group are fortimicin, teicoplanin, rosamicin**.** Then there were discovery salinosporamide which has the potential for development of anti-cancer drug was isolated from the *salinispora* genus of actinomycetes. Two-thirds of all known drugs are produced by actinomycetes and the genus Streptomyces is the largest producer of antibiotics of them all almost 61% and "rare actinomycetes" provide 16% of total antibiotics (Waksman et al., 2010).

1.3.22.1. The work of Kitatsu Institute

Since its birth this organization started working on soil dwelling microbes especially soil actinomycetes. In early 1970 Satoshi Omura of kitatsu institute discovered *S.avermectinius* which produced the secondary metabolite known as avermectin. This avermectin may be the safest drug that has the ability to treat a wide range of human disease and other conditions (Bentley et al., 2002). For which Dt.Omura was awarded Nobel Prize in 2015. Further work on this avermectin produced derivatives that were used to treat river blindness and lymphatic filariasis as well as other parasitic diseases (Takahashi et al., 2002). This award of nobel prize was the third related to antibiotic after flaming in 1942 and Waksman in 1952. The finding of ivermectin was only possible due to the belief of Dr.Omura because he believed that microbes are the source that has the potential to produce limitless secondary metabolism and they do not produce useless metabolism (Omura et al., 2018). Their original goal was use to get a product with anti-helmintic properties but when they discovered ivermectin which had multiple other use too besides anti-helmintic properties. Dr. Omura also decided to start a new novel method to isolate new compounds which was called "physicochemical screening" or PC screening. The main goal of this method was having no goal and continuously identifying new compounds and assessing their physical and chemical properties and also to make this products available for other for research purpose. Due to the use of PC screening method the kitatsu institute got almost 500 new products in its early years (Takahashi et al., 2017).

1.3.22.2. Novel compounds obtained by PC screening

The PC screening process is carried out by collecting the ethanolic extract, analyzing it in mass-spectrometry or ultra-violet analysis, then comparing the peak with the peak of a standard compound if the comparison resulted in good results then the obtained products are further analyzed by high resolution mass spectroscopy or nuclear magnetic resonance spectroscopy. By using this process the institute was able to isolate novel compounds like lactacysin, avermectin, setamycin, staurosporine from the secondary metabolites of actinomycetes (Omura et al., 1977). All of those novel antibiotics are stored in Kitatsu Microbial Library (KML) from where this isolates can be used in time of a global crisis.

1.3.23. Actinobacteria and their beneficial traits to plants

Actinobacterias are gram positive bacteria with a high percentage of guanine and cytosine content in their genome. They are prolific producers of a wide range of bioactive compounds (Berdy, 2005). Though there are some reports that actinomycetes acts as a growth factor for plants. Their ability to solubilize inorganic phosphate compound and the ability to produce phytohormones have been well recorded (Lin & Xu, 2013). It had been proved that actinomycetes acts as a growth promoter in plants and it was well-observed n rice, wheat, beans and peas plants. Well known plant growth promoters like Arbuscular mycrrozhial fungi (AMF) is widely used in agricultural industries worldwide (Smith & Smith 2011). The AMF can be grown on plants as a obligate symbiote. This fungi increases the nutrient uptake of the plant and it itself also acts a carbon source. *Funneleformis* is a wide known AMF that has been used in horticulture and bioinoculant for decades. Recently there were reports about AMF associated bacteria that are both gram-positive bacteria like mostly the Streptomyces genus of actinomycetes and also bacteria that are gram-negative in nature (Xavier & Germida, 2003). All these bacteria have shown important plant growth properties and they are now getting more and more attraction for future use. However there were very few research papers that have worked on this type of topic. So the main goal of (Lasudee et al., 2017) was to do some research on this case. Their target was to isolate the *Funneliformis mossae* from amctinomycetes and use it as a plant growth factor both in vitro and in planta by using thai jasmine rice and mung beans. Thay have also given special attention to the microorganisms affect on the growth of the rice plant on times like drought and soil with low nutritional value.

Their taxonomical positions were also analysed. Cultivable actinobacteria that are associated with AMF is a good tool to promote plant growth and other plant related activities. The research of (Lasudee et al., 2017) successfully isolated six different actinomycetes species from *F.mosseae*. All these isolates fall under the Genus Streptomyces which is also famous for producing a wide array of secondary metabolites (Schrey et al., 2012). These actinobacteria did not grow under nutrient agar culture which proves that these actinomycetes are endophyte in nature. Though there were no report that tells us about the interaction about the actinobacteria and mycorrhizal symbiosis like there were reports about the genus Pseudomonas and mycorrhizal symbiosis. Recently some new reports came that showed that, in presence of actinobacteria the growth or formation of mycorrhizal spore occurs at a very high rate (Frey-Klett et al., 2007). They (Lasudee et al., 2017) also mentioned that there were no growth without enrichment stage in extract solution of soil. Enrichment is very common thing that is used to increase the population of target bacteria. Soil extract was used to isolate soil actinomycetes from soil (Hamaki et al., 2005). Conventional culture medias with high nutritional level can cause failure in the culture of most of the bacteria in nature. The addition of soil extract has the potential to increase the number of cultivable actinobacteria that are associated with mycorrhizal spores on the plate to a detection level.

1.3.24. Immunomodulatory activity of Endophytic Actinomycetes

Endophytic microbes are the type of microbes that live on the tissues of plants but do not cause any negative effects. Some of the endophyte bacteria are also used to promote plant growth and to control plant pathogens (Santoyo et al., 2016). Endophytic actinobacteria exist on tissues of plant and considered as a secured source for new bioactive compounds. Teaplants is one of the plants that play a huge role in the life of Asians especially the Chinese people but very little is known about the endophytic actinomycetes that exist on the tea plants. Normally actinomycetes can be found on soil or marine environment but tree or plant parts are the new source from where the isolation of new bioactive compound could be possible (Sardie et al., 1992). In recent years endophytic actinobacteria have been isolated from plants like rice, wheat, peas etc. Now there are talking going on about the applications of these endophytic actinomycetes for the control of pests and diseases as well as to develop products like antitumor, antimicrobial and anti-parasitic activities (Cao et al., 2005). Endophytic

actinobacteria plays an important role in making the plant salt resistant and also in making the plant keep growing in harsh environmental condition.

1.3.24.1. Role of tea

Tea plays an important role in our life as a non-alcoholic and refreshing beverage, it also plays an important role in human health and lifestyle like curing digestive disorders and also lowering the risk of cardiovascular disease (Persson et al., 2010). In Yunnan province in china there are two most important tea plants like Zijuan and Yunkang-10 from which a special drink called dark tea is produced. In case of secondary metabolite products from tea catechins are the number one and then comes polyphenol which is very important for health benefits (Khan & Mukhter, 2007). Anthocyanin is another compound from tea that has special role to keep our body healthy, which is also widely distributed in higher plants like vegetables, flowers, tea plants, fruits and cereals etc. The net content of anthocyanin is very high in Zijuan variety, it is almost three times more than any other Chinese variation (Joshi et al., 2009). That is why zijuan is considered to be a very enriched tea cultivar for anthocyanin. However the number of studies based on this particular field of plant endophytes, whereas most of the studies have been done on endophyte fungi and how to protect plants from disease (Yang et al., 2009). The endophytic fingi known as *Colletorichum gloeosporiodes* collected from fresh tea leaves seems to very effective against tea plant pathogens like *Pestalotiopsis theae* and *Colletorichium camelliae* , here the inhibitory factors that are doing this might be fungal chitinase and protease (Rabha et al.,2014). Thogh endophytic fungi had received more or less attention over the year but the endophytic actinomycetes received no attention at all and the interaction between endophytic actinomycetes and plants still remain to be studied. The future of making bioresource library depends on investigation on cases like this. In the study of (Wei et al., 2018) we found out that, the antimicrobial community of zijuan and yunkang-10 is quite different from one another.

1.3.25. Rare actinomycetes from Atacama desert soil

Back in 1945 when Alexander Fleming received Nobel prize he warned everyone about the misuse of antibiotics because he thought that this type of misuse would lead us to the threat of multi-drug resistance disease. Nobody gave any attention to his warnings and also people's lack of knowledge about use of antibiotics has created this multi-drug resistance situation (Zaman et al., 2017). Most of the multi-drug resistance pathogens are associated with gram negative bacteria which are also responsible for high mortality rate and death. In the meantime mankind already observed the sharp decline in the production of antibiotics and it seems that we are heading towards an age of pre-antibiotic era (Wilyard, 2017). Even in all this we good news like our understanding of the microbial community and application of new technologies associated with genomic sequences might open a new door for us to get new and novel chemical entities (Ziemart et al., 2016). Among the prokaryotes, the filamentous bacteria belonging to the group actinobacteria of the phylum has the unique capability to produce secondary metabolites, among these organisms the genus Streptomyces is responsible for providing two-third of the whole world's antibiotics. Inspite of this the costly researches which lead to the rediscovery of same and known compound has lead the discovery rate of new products downward (Wright et al., 2014). However the realization of capability of actinobacteria to produce ten times more secondary metabolites that are known to modern medical science has once again raised scientists hope all around the world. Gifted actinobacteria especially the ones that have proven that they are containing multiple dormant biosynthetic gene clusters are getting more attention than ever. This gifted actinomycetes are being studied for rare and new chemical entities and also with modern biotechnological techniques (Carro et al., 2018). Here scientists are referring to strategies to awaken this dormant gene clusters so that we could get new antibiotics. All this processes are getting benefitted from proper screening to get the right strain and polymerase chain reaction which is also known as PCR and also by rapid dereplication of already known metabolites from complicated biological extracts. All these observations raise only one question and that is "where and how to activate gifted novel actinomycetes strain?". Ecological studies conducted by Dr.Stan Williams and his colleagues showed that the production of antibiotics from actinomycetes is influenced by factors like P^H, availability of matter that are orgainic in nature, water and temperature (Williams et al., 1972). Their discovery of actinomycetes that

are acidophilic and acidotolerant at the same time from acid coniferous soil is one of the point. This kind of findings has lead or scientists believe that to get new and novel secondary metabolites we always must seek actinomycetes from diverse or extreme environmental condition which is also the basis of novel biochemistry. All the supports that are needed for hypothesis comes from the bioprospection campaigns that were designed to isolate novel compounds from marine actinomycetes (Bull et al., 2005). Based on this method many new compounds were discovered outside of actinomycetes like discovery of abyssomicin which is a polyketide antibiotics from *Verrucosispora maris,* discovery of salinisporamide which is an anti-cancer drug from *Salinispora tropica* and dermacozines from *Dermacoccus abysii.* Extensive studies on salinispora strains showed clear evidence of coupling between chemical and taxonomic diversity (Jensen, 2010). Non-polar desert soils account for a third of landmass of the planet , previously actinomycetes were collected from places like Mojave desert, Mongolian desert, Sahara, Talkimakan and thar deserts.

1.3.25.1. Harvesting of Actinobacteria from Atacama Desert

The location and the environmental condition of this desert were reviewed in several articles like (Silva et al., 2008; Bull et al., 2016). At first there were less amount of research that were conducted on the microbes of this place due to factors like soils acidity, hot air, low organic carbon and presence of oxidants that are organic in nature. With time this misconception was replaced and it was found out that the microbes of this place were capable to adapt themselves with the harsh environment (Mckay et al., 2003). Actinomycetes isolation of Atacama desert was first carried out more than 50 years ago, even after that there were no extensive research based on this topic. So recent bioprospection, isolation and screening has led to the discovery of actinomycetes that are diverse. The bigger the numver of species or generas or families the greater chance of getting new secondary metabolites and also to get new drug leads (Idris et al., 2016). Dereplication id the process by which two phenotypically similar species can be detected this became very helpful recently because of this the chances of rediscovery of existing compounds are getting low because it eliminates any species previously discovered. Before a microbe is used for further screening standard ‘kill’ assay is used to find out that specific microbes activity against gram-negative and gram-positive pathogens (Bull et al, 2016). There was an effective way to apply dereplication process in streptomyces species by

(Williams et al., 1969) who made groups of actinomycetes based on the color or pigments formed by them in oatmeal gar and also by their ability to produce melanin gar in yeast extract and malt extract media. The screening assay of color group representatives has increased the hit rates and subsequent discovery of new antibiotics. In the last decades we were able to isolate many new actinomycetes from higher altitude and hyper and extremely hyper srid natured soil from Atacama desert (Busarakam et al., 2014). All these expeditions and isolation of new strains proved one and only thing only and that is in harsh environmental condition the chances of getting new and novel antibiotics increases. The new compounds that were isolated from the Atacama Desert showed good antibacterial activity against all known reference strains like *E.coli, B.subtilis, B.cerius* etc.

1.3.26. Chitinolytic functions in Actinomycetes

Actinobacteria are an order of gram-positive bacteria and they have large genomes which are bigger than five megabases. All of the species of actinomycetes lead a very complex life cycle including the genus Streptomyces like producing mycelium. They also have high Guanine and Cytosine which is also known as G+C content in their system (Lewin et al., 2016). These large phylum is widely distributed in both aquatic and terrestrial environment. Though they are known as free-living saprophytes, some of the live in organs or inside of tissues of organisms and some also have symbiotic relationships with plants (Santi et al., 2013). Although they have the capability to create disease in humans and plants but the number of proportion of pathogenic actinomycetes is very low. Actinomycetes play a very important role in solubilizing the cell wall of plants and fungi as well as crustacean shells and cuticles of insects thus contributing in carbon cycle (Charter, 2016). The genome of *Streptomyces coelicolor* contains more than 800 genes that are capable of secreting protein and the genus Streptomyces scrabies when grown with the influence of potato periderm can produce more than 200 extracellular proteins which are basically glycosyl hydrolases. In nature the enzymes secreted by actinomycetes works in degradation of biopolymers like lignocellulose (Book et al., 2014).

1.3.26.1. Chtinolytic properties of Actinobacteria

The second most available biopolymer in nature is chitin and it is largely associated with cell walls of fungi, exoskeleton of crustacean, cuticles of insects and eggs of nematodes (Rane & Hoover, 1992). Chitinolytic enzymes are produced by many things in nature both animals and plants are prodycers of chitinolytic enzymes. In case of the prokaryotes especially the actinomycetes probably are the best producers of chitin decomposers. They have the capability to use chitin or chitosan as important source of nitrogen and carbon sources because they have enormous sets of enzymes that are capable enough to degrade chitin and chitosan (Beier & Bertilsson, 2013). Chitin is present in all organisms it is basically omnipresent and it acts as a marker of environmental nutrient status for the genus Streptomyces. During the early 60s the chitin containing mediums were used to isolate actinomycetes. In addition the plating of different soil or addition of freshwater dilutions also helped the growth of actinomycetes over other organisms like other bacteria colonies and fungi colonies on the plate (Hsu & Lockwood, 1975). When actinomycetes are grown on solid chitin containing media the formation of clear zones surrounding the actinomycetes zone reveals that their growth depend on their ability to solubilize chitin, because the actinomycetes are the organisms which are coded to produce chitinolytic enzymes but that does not mean they are capable of living in chitin containing medium (Liao et al., 2014). Even in this day chitin agar media is used to cultivate and isolation of free living actinomycetes and also the actinomycetes that live by interacting with plants or animals. This media was also used for the recovery of bacteria that are chitinolytic from the sediments of Lake Antarctica. Those type of bacteria have been affected by penguin and other chitin containing organisms like squid and krill for over the last sixteen hundred years (Xiao et al., 2005). The research of (Xiao et al., 2005) collected six bacterial genus from there and three of them proved to be in actinomycetes species.

1.3.26.2. Distribution and studies of chitinolytic Actinomycetes

To find out the abundance of chitinolytic bacteria in a place chitin agar medium is used as a growth medium. For example, from the central lake of Poland the abundance of actinomycetes was found out by using chitin agar medium and it was found out that chitinolytic actinobacteria are not only more available in soil than water (Brzezniska et al., 2009). Many

studies are going on all around the world to find out the effects of chitin on taxonomical and functional system of microbial communities. Divergent effects on soil actinomycetes community by chitin availability is also reported in many literature. Many scientists are thinking that this type of contradictory effect may occur due to factors like type of soil, temperature, level of humidity and the sources of chitin themselves etc. The research of (Cretiou et al., 2014) showed that if chitin is added later on culture medium the population of actinomycetes get decreases, he added chitin in normal agricultural soil and after nine months he found out that the bacterial diversity was reduced at a high rate, (Cretiou et al., 2014). This decrease was very important for the genus Streptomyces though it is a good chitinase producer. Another scientist (Kleik et al., 2013) showed that after sixty days of addition of shrimp shells on agricultural soil decreased the quantification and gene related to actinobacterium ChiA. Another study of (Cretiou et al., 2009) showed that population of actinomycetes decreases because it takes time for adaptation, he added chitin on agricultural soil on spring 2007 and almost after two years on fall 2009 he added chitin again on the soil and he found out a huge increase in the abundance of actinomyctes in chitin amended soil (Cretiou et al., 2009). The concentration of chitin on soil also effects the growth of actinomycetes. He added 2 mg chitin in 1g of soil and in another 1g soil headed 20mg of chitin and he observed that the highest concentration gives enormous changes on the actinomycetes community (Jacquiod et al., 2013). The addition chitinious material on soil can be used as a bio-controller since it increases the suppressiveness of soil toward disease causing pathogens. However this could be harmful for the actinomycetes on soil because actinomycetes are known for protecting plants from other hostile pathogens (Bell et al., 1998). Due to factors like this chitin degrading actinomycetes are used as an ingredient in commercially available fungicides. Many fungi cell all has chitin and these chitin lysing actinomycetes degrades the chitin wall resulting in bursting of inner material killing the fungi. Chitinase actinomycetes also causes the association of arbuscular mycorrhizal fungi which is also known as AMF and by this it also accelerates the production of plant origin secondary metabolites like alkaloids, phenolics etc (Genre et al., 2013).

Chapter 2. Materials and Methods

Even in this modern day natural resources are the only source of antibiotics to fight of diseases but the phenomenon named as antibiotic resistance is becoming more and more severe with time. Novel antibiotics are the new type of antibiotics that can fight against the resistant strains and actinomycetes are the main producers of novel antibiotics. To find out these new and novel antibiotics research is focused on unexplored locations or habitats. The main reason behind this is to increase the possibility of finding new species of actinomycetes so that we could get new antibiotics. Actionbacterias are the producers and suppliers of antibiotics to pharmaceutical companies because of their ability to produce secondary metabolites of a wide varieties. Actinobacterias are quite famous for their ability to produce antibiotics to fight off pathogens. It is only recently that marine actinomycetes have gained recognition as a source of antibiotics that are novel and the source of anticancer and antitumor agents with peculiar structures and properties.

At the beginning research was focused on soil actinomycetes and when the research on soil actinomycetes went to its peak level the biologically active metabolites produced from soil inhabiting actinomycetes started to decline mostly because the results were almost same when scientists started to get same compounds over and over again. In the meantime antibiotic resistance was heading to a new level of danger. Only because of this reason modern research to find new actinomycetes to produce new antibiotics has shifted toward marine sources. More than 70% of earth's surface is covered by water. Within this 70% deep sea region is 92-93% and the only 7-8% goes for coastal region area (Moore et al., 2016). The deep ocean has very harsh environment due to its high pressure, low oxygen level, pitch black darkness and obviously low temperature. Even with all this deep sea is teeming with life whether it's the microbes or the giant animals. Now why we are choosing deep sea actinomycetes? Because in this huge ocean this actinomycetes fight for their food and the only way to kill their competitors is to create new antibiotics. Deep sea corals, marine invertebrates or vertebrates all contain actinomycetes in their system and the ocean itself is the container of wide range of actinomycetes species (Yuan et al., 2014). Not only antibiotics but as a new source for enzyme inhibitors, antitumor agents and antiviral agents deep ocean is very promising. Since it is well known, actinobacteria especially *Streptomyces* are considered to be the main source for

producing diverse structured secondary bioactive metabolites for pharmaceutical application, particularly antibiotic agents and antitumor agents. Though most of the actinomycetes are terrestrial origin the ocean itself is containing the marine counterpart of Streptomyces (Wuet al., 2005). Actinobacterias are widely distributed in marine ecosystem and it's diverse marine organisms. In past new species of marine actinomyctes were even discovered from the mariana trench with depth up to10,898 meters . Terrestrial actinomycetes are known for playing important role in ecological recycling of compound that are organic in nature and also for producing other natural bioactive compounds, which are desired to protect the host organisms from pathogenic attack. However in the deep ocean environment their biogeographic distribution, role in ecology and history of evolution are not very well known (Zhang et al., 2006). This is what makes the marine actinomycetes the perfect source for novel antibiotics because here vast amount of active secondary metabolites come from the genus *Streptomyces.* The distribution of marine actinobacterias is very wide they are present in mollucs , mangroves, fishes , seaweeds , sponges besides sediments and seawater (Zotchev, 2012). These microorganisms are gaining attention not only for their characteristics or ecological perspective or taxonomy but also for their ability to produce antibiotics, immunosuppressant agents, enzymes, antitumor agents, pigments and enzyme inhibitors etc. So we can see that, due to worldwide constant need for novel antibiotics to fight off pathogenic attack in host body or to neutralize strains that show antibiotic resistance or to use it as a drug for anti-tumor chemotherapy, deep ocean actinomycetes are rising as a new source of antibiotics and other bioactive natural products (Wang et al., 2010).

Filamentous actinomycetes specially the bacteria and the fungi order of actinobacteria are the main source of antibiotics because they are known for producing 90% of all used and known antibiotics. It is not like that one species of Streptomyces is only capable of producing only one type of antibiotic compound. Modern research shows that most of the Streptomyces species that were used to produce antibiotics have gene clusters in them. Genome sequencing of actinomycetes confirmed the presence of many other biosynthetic gene clusters with the potential of creating new antibiotics that didn't exist before (Wezel et al., 2013). For example, for many decades it has been thought that *Streptomyces coelicolor* only produces four different antibiotics like actinorhodin, calcium dependent antibiotic, undecylprodigiosin and plasmid encoded mythelenomycin from the research of david hopwood and his colleagues.

After fifty years of researching intensively it came like a complete wonder when *S.coelicolor* revealed an entirely new set of gene clusters and one of these gene clusters is likely to produce cryptic polyketide called antibiotic. This phenomenon alone proves that other species of actinomycetes also have these latent arsenals within them that can give mankind new antibiotics (Tredici et al., 2005). Therefore it seems that the potential of actinomycetes as a source for producing novel antibiotics is way more bigger than which was originally anticipated. It has initiated extensive research into the field of applied genomics to find out this cryptic, sleeping or silent antibiotics. So we can finally see that, there are many other yet unidentified antibiotics out there for us to discover, those were missed due to the less production of gene clusters that identifies them in the first place (Yang et al., 2012)

Modern research is also focusing on the ability of actinomycetes to produce lipopeptide biosurfactants that has the ability to degrade hydrocarbons. These new lipopeptide biosurfactants are drawing the attention of scientists all over the world because of their low toxicity, ecological acceptability and non-hazardous degradation (Selvin et al., 2009)

We report here the activity of marine actinomycetes on antibacterial tests, antifungal tests that were isolated form six different locations of sea water of Bay of Bengal and soil from mangrove forest Sundarbans.

2.1. Required Media

Media required:

- Yeast Malt Broth

Table 2.1. Yeast Malt Broth Composition:

Ingredient	Amount (gm/l)
Peptic digest	5
Yeast extract	3
Malt extract	3
Dextrose	10

- Nutrient Agar

Table 2.2. Nutrient Agar composition:

Ingredient	Amount (gm/l)
Peptone	5
NaCl	5
HM peptone B	1.5
Yeast extract	1.5
Agar	15

- Potato Dextrose Agar

Table 2.3. Potato Dextrose Agar Composition:

Ingredient	Amount(gm/l)
Potato	200
Dextrose	20
Agar	15

2.2. Required Chemical

- 50% glycerol

2.2.1. Preparation of 50% glycerol

To prepare 50% glycerol at first 10ml of 99.9% pure glycerol was taken and it was mixed and shaken with 10ml of distilled water. After autoclaving it was made ready for use.

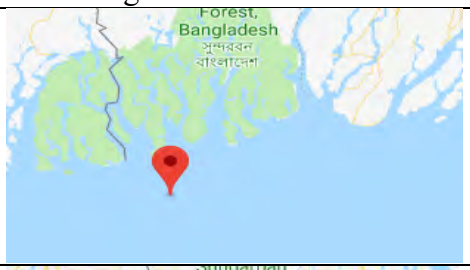
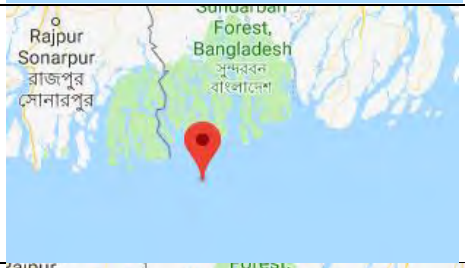
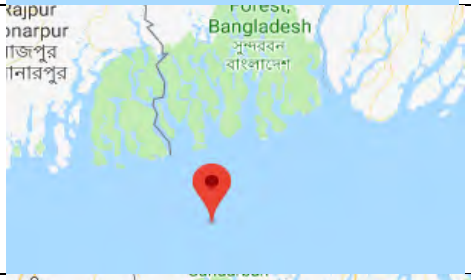
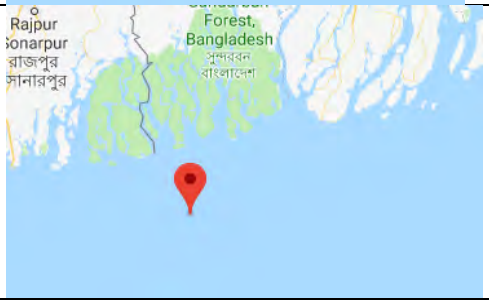
Table 2.4**.** Equipments used for experiment:

Name of instrument and glassware	Function
1. Autoclave machine	Sterilization
2. Incubator	Incubation of solid culture mediums (agar plates)
3. Shaking incubator	Incubation of liquid culture mediums (broth)
4. Analytical balance	Measurement of weight
5. Laminar Air Flow	Maintenance of aseptic environment
6. Water system	Preparation of culture stock
7. Eppendrof tube	For making glycerol stock
8. Micropipette	For withdrawing reagent and media in minute amount

2.3. Collection of Samples

For our research samples were collected from southern part of Bangladesh like the mangrove forest Sundarbans and Bay of Bengal. There were total 6 samples collected from Bay of Bengal and 3 samples collected from Sundarbans. Here are the sample name and coordinates:

Table 2.5. Collection coordinates and names for water samples:

Sample Name	Sample Co-ordinates	GPS Image
At channel mouth	LAT:21° 44' 1000"N LONG:89° 30' 7000"E	
3.5 nautical mile	LAT:21° 47' 2076" N LONG:89° 31' 7034"E	
15.5 nautical mile	LAT:21° 27' 4600" N LONG:89° 31' 8200" E	
18 nautical mile	LAT:21° 25' 7030" N LONG:89° 32' 3491"E	

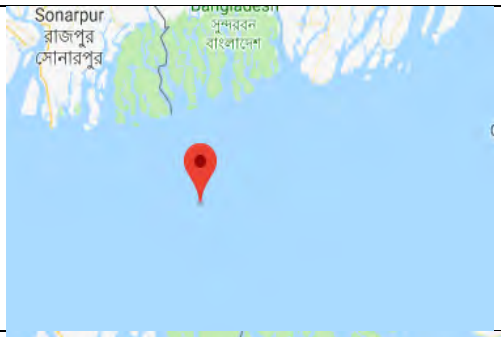
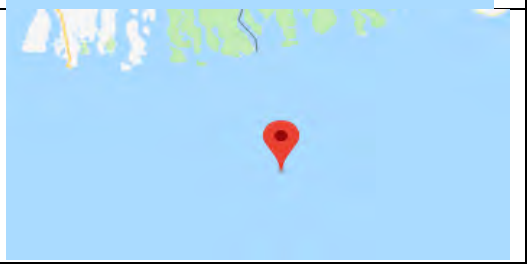
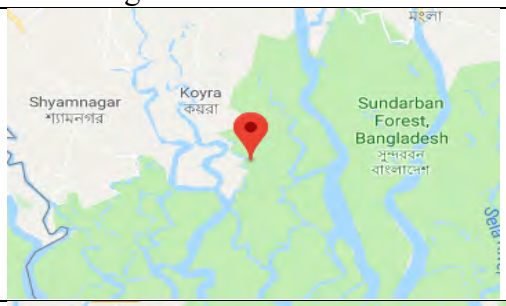
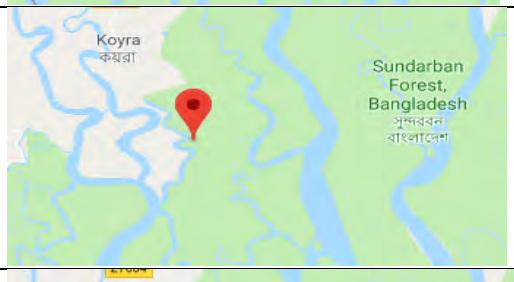
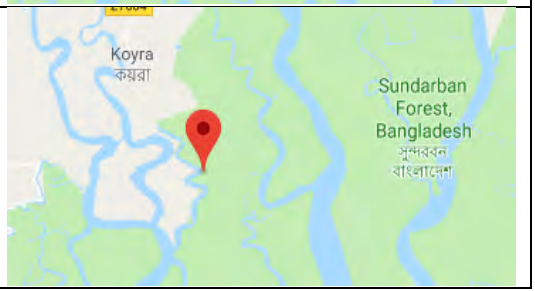
35 nautical mile	LAT:21° 08' 0253' N LONG:89° 30' 6031' E	
38 nautical mile	LAT:21° 02' 5000' N LONG:89° 21' 6000 ' E	

Table 2.6. Collection coordinates and names for soil samples:

Sample Name	Sample Co-ordinates	GPS Image
Soil 1	LAT:22° 25' 4830'N LONG:89° 35' 5020'E	
Soil 2	LAT:22° 25' 3910' N LONG:89° 35' 5810'E	
Soil 3	LAT:22° 25' 7200' N LONG:89° 35' 4440' E	

2.4. Screening and isolation of Actinomycetes

For isolation of actinomycetes, we used the Yeast Malt Broth medium. At first all 9 samples were streaked in 9 different plates, this process were repeated for every 1 week until we got pure isolated colonies. Confirmation of actinomycetes colonies was done by observing the growth of mycelium in colonies. For the growth of actinomycetes we used room temperature (25°-27°) C. For 100 ml of volume we added 3.1 gm of Yeast Malt Broth.

2.5. Observation of growth on other mediums

For observation of growth of our samples on other mediums like Nutrient Agar, Potato Dextrose Agar was used along with Yeast Malt Broth. For 100ml of volume 2.1gm of PDA was used and for 100 ml of volume 3.2gm of NA was used. The observation was done for total 9 days. Before initiating this process we isolated new colonies from previously isolated colonies. Previously we had 6 water samples and after isolating new colonies the total number of water samples became 10. After 9 days of observation we found the growth of mycelia in every samples in all 3 types of mediums. We have also found that the growth in Yeast Malt Broth was very fast and the growth in Nutrient Agar was very slow. We have found our samples growth in Potato Dextrose Agar was less slower than Nutrient Agar but not as fast as growth in Yeast Malt Broth.

2.6. Preparation of Glycerol Stock

Glycerol stock of our samples were prepared by making 50% glycerol (from 100% glycerol). Yeast Mault Both was used to make overnight culture. 1.5gm YMB for 50ml of volume and 15 50ml conical flasks were used for total 15 samples. After overnight culture 500µl of overnight culture and 500µl of 50% glycerol was placed into each 1.5ml eppendrof tube. For each sample 5 stocks were made in 5 eppendrof tube. Identification numbers were used to identify samples in eppendrof tube.

2.7. Anti-Fungal activity

The antifungal test of our samples was done in Bangabandhu sheikh Mujib Agricultural University. It was too risky to perform in our university. All 15 samples were sent to there. Where it was tested against wheatblast strain *Magnaporthe oryzae*. Perpendicular streak method was used for this test. In this method at first single streaking of actinomycetes was done in 15 different Nutrient Agar plates for 15 different samples and after keeping in incubator for 4 days at 27°C reference strain was streaked into 90° angle of actinomycetes streak. Then the samples were kept in incubator for 2 days at 37°C. After 2 days samples were brought out to measure inhibition zone and activity.

2.8. Anti-Bacterial activity

The antibacterial test was done against *E.coli and B.cerius*. Here perpendicular method was used. Same procedure as anti-fungal test. Here at first the reference strains were collected from jahangirnagar university and then 15 Nutrient agar plates were made for 15 samples. In each of this plates we drew our samples streak in the middle, the plates were incubated for 4 days at 27° C and then in one side we streaked E.coli and in another side we streaked *B.cerius.* The streaks were made into 90° angle of our original streak. There were 4 in few cases 5 streaks on per side and they were incubated for 2 days at 37° C.

Chapter 3. Results and Discussion

3.1. Antifungal test

The sample number 3 which is soil 1 red showed antifungal activity against wheatblast strain. Here we are showing the microscopic image of mycelia of sample 3 and the image of sample 3 preventing the growth of wheat blast strain which is *Magnaporthe oryzae*. Here is the image given below:

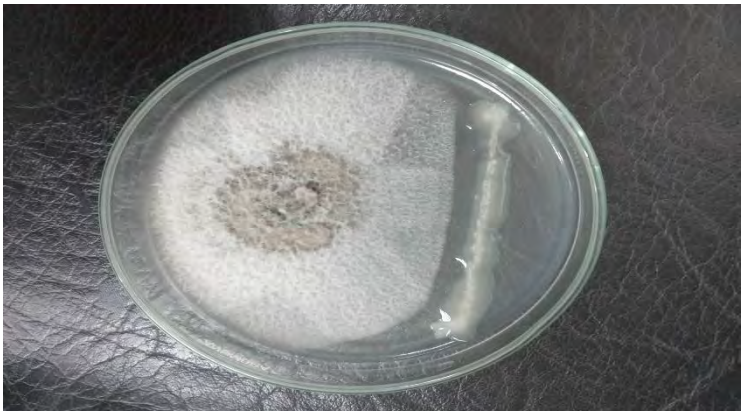


Fig 3.1. Testing against *Magnaporthe oryzae.*

Table 3.1. Mean and standard deviation table for *M.oryzae*:

Sample Name	Average zone of Inhibition (in mm)	Standard deviation
Sample 1	0	0
Sample 2	0	0
Sample 3	5.67	±0.577
Sample 4	0	0
Sample 5	0	0
Sample 6	0	0
Sample 7	0	0
Sample 8	0	0
Sample 9	0	0
Sample 10	0	0
Sample 11	0	0
Sample 12	0	0
Sample 13	0	0
Sample 14	0	0
Sample 15	0	0

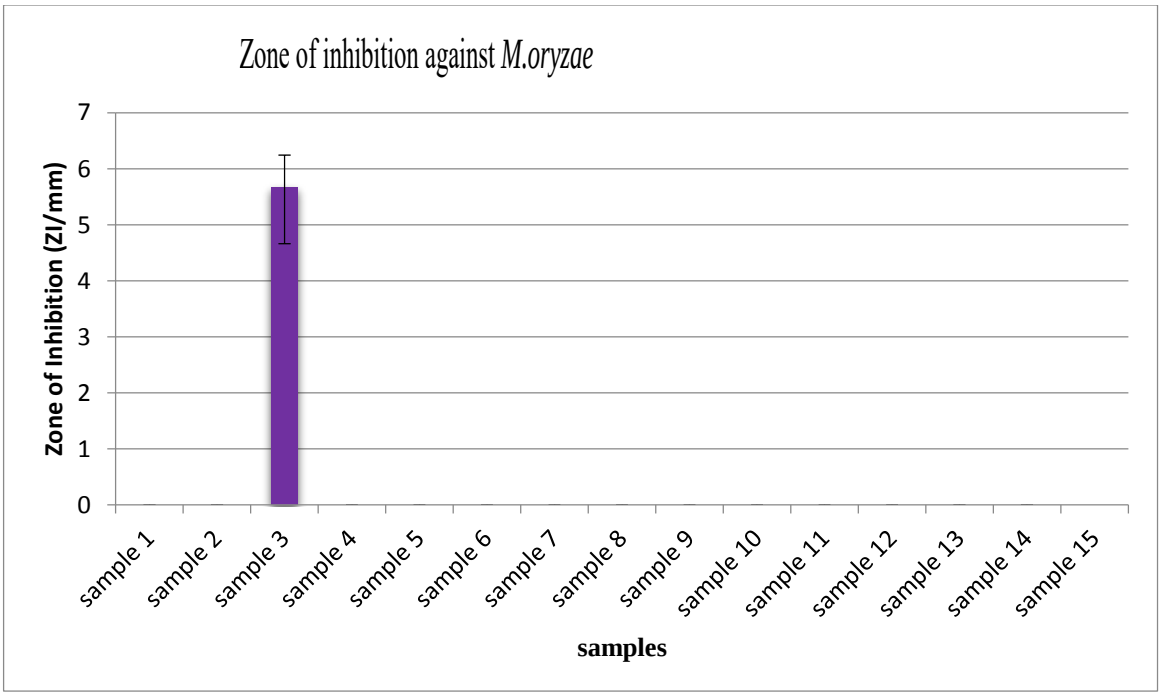


Fig 3.2. Zone of inhibition against *M.oryzae*

Fig 3.3. Microscopic image of the mycelia of sample no. 3 (obtained from BSMRAU)

3.2. Antibacterial test

Samples were tested against *Escherichia coli* and *Bacillus cerius* .

Fig 3.4.A. Sample 1

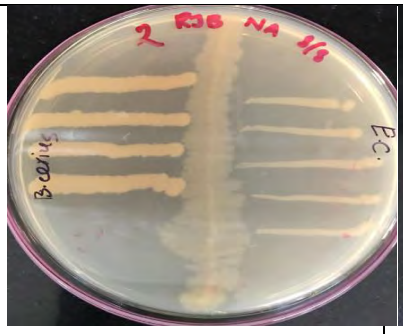


Fig 3.4.B. Sample 2

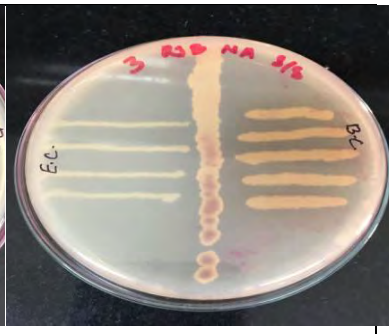


Fig 3.4.C. Sample 3

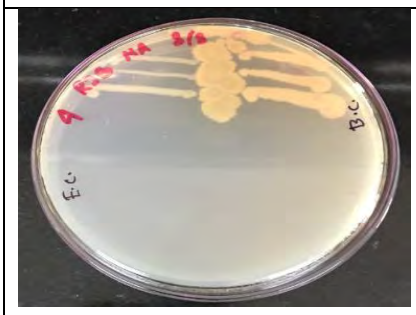


Fig 3.4.D. Sample 4

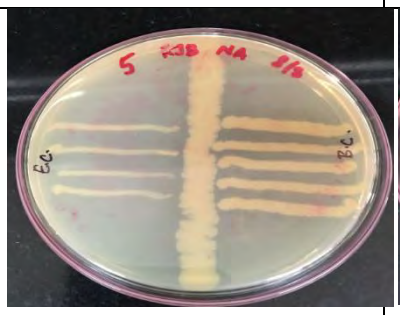


Fig 3.4.E. Sample 5

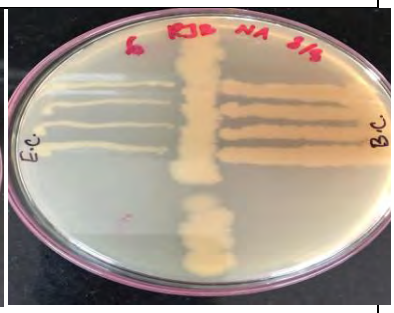


Fig 3.4.F. Sample 6

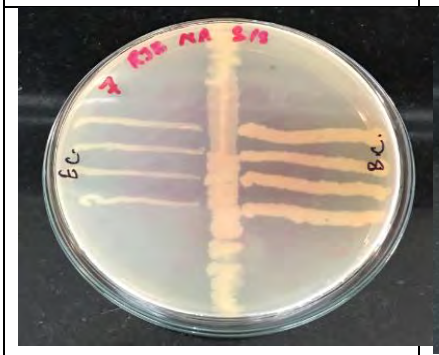


Fig 3.4.G. Sample 7

Fig 3.4.H. Sample 8

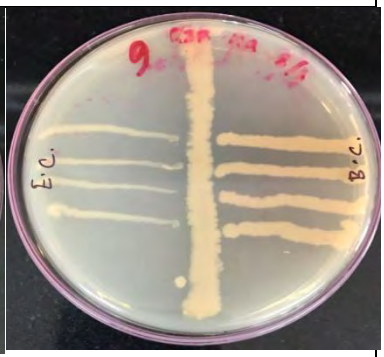
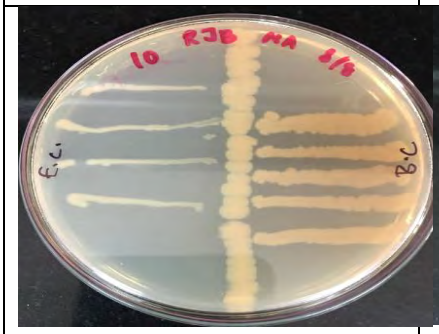


Fig 3.4.I. Sample 9



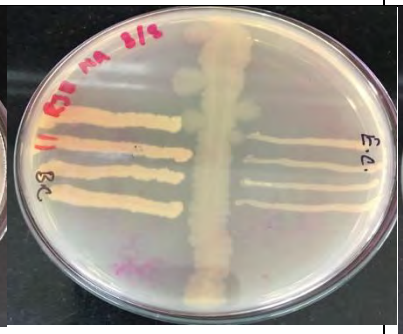


Fig 3.4.J. Sample 10	Fig 3.4.K. Sample 11	Fig 3.4.L. Sample 12

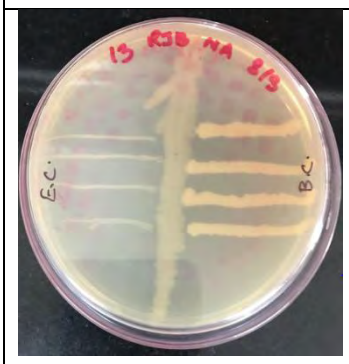
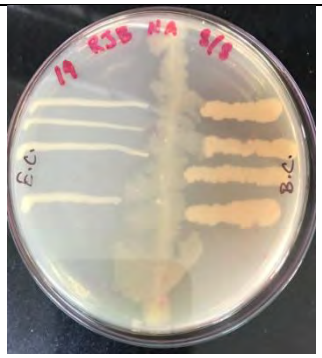
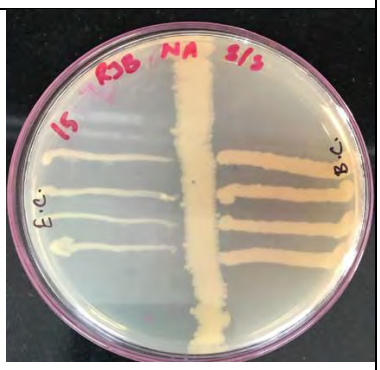

		

Fig 3.4.M. Sample 13	Fig 3.4.N. Sample 14	Fig 3.4.O. Sample 15

Fig 3.4. Antibacterial assay

Table 3.2. Mean and Standard deviation table For *E.coli*:

Sample Name	Average zone of inhibition (in mm)	Standard deviation
Sample 1	2	0
Sample 2	0.57	±0.58
Sample 3	3.34	±1.53
Sample 4	0	0
Sample 5	1.67	±0.58
Sample 6	1.67	±0.58
Sample 7	1.34	±0.58
Sample 8	1.34	±0.58
Sample 9	1.67	±0.58
Sample 10	1.67	±1.53
Sample 11	1.34	±1.53
Sample 12	2	±1
Sample 13	1	±1
Sample 14	1	±1
Sample 15	1	±1

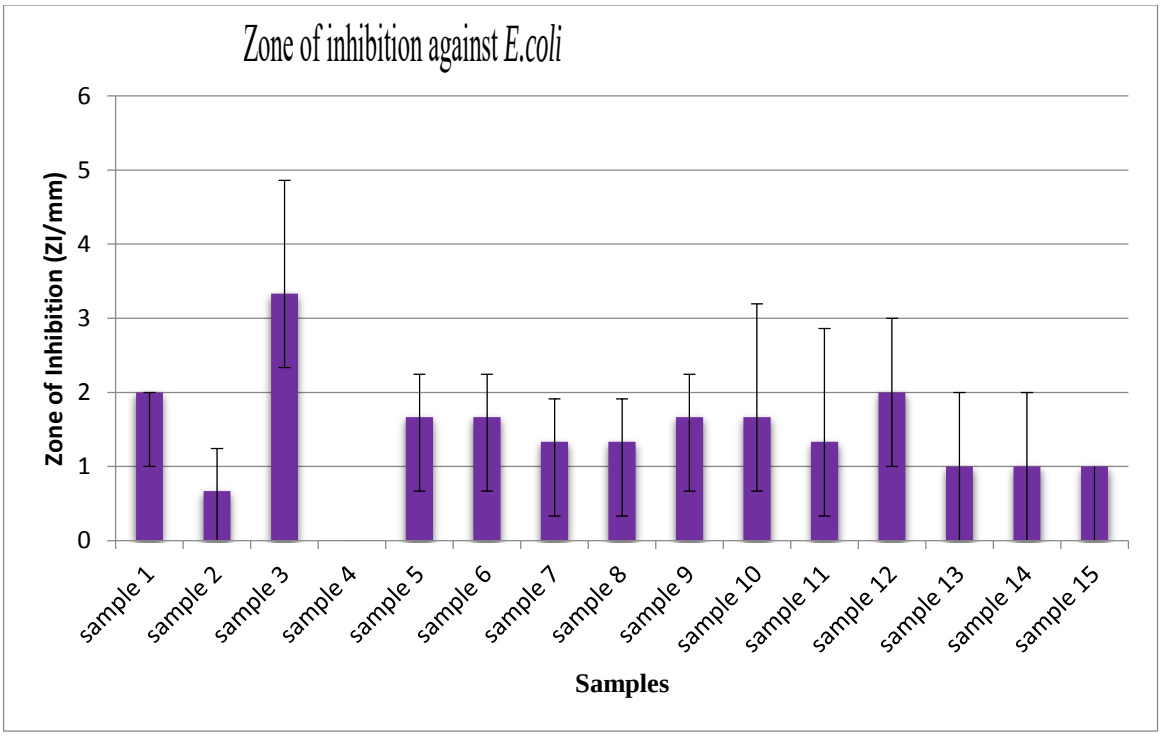


Fig 3.5. Zone of inhibition against *E.coli*

From here we can see that only sample number 3 has shown significant activity against E.coli, creating the inhibition zone of 3.34 mm. sample number 4 showed literally no activity and other samples showed moderately good activity against E.coli.

Table 3.3. Mean and Standard deviation table For *B.cerius*:

Sample Name	Average zone of inhibition (in mm)	Standard deviation
Sample 1	1	±0.1
Sample 2	0	0
Sample 3	6	±1
Sample 4	0	0
Sample 5	0.16	±0.29
Sample 6	0	0
Sample 7	0	0
Sample 8	2.33	±0.58

Sample 9	1	±1
Sample 10	0.67	±0.29
Sample 11	1	±1
Sample 12	1	±1
Sample 13	0.34	±0.58
Sample 14	0	0
Sample 15	0	0

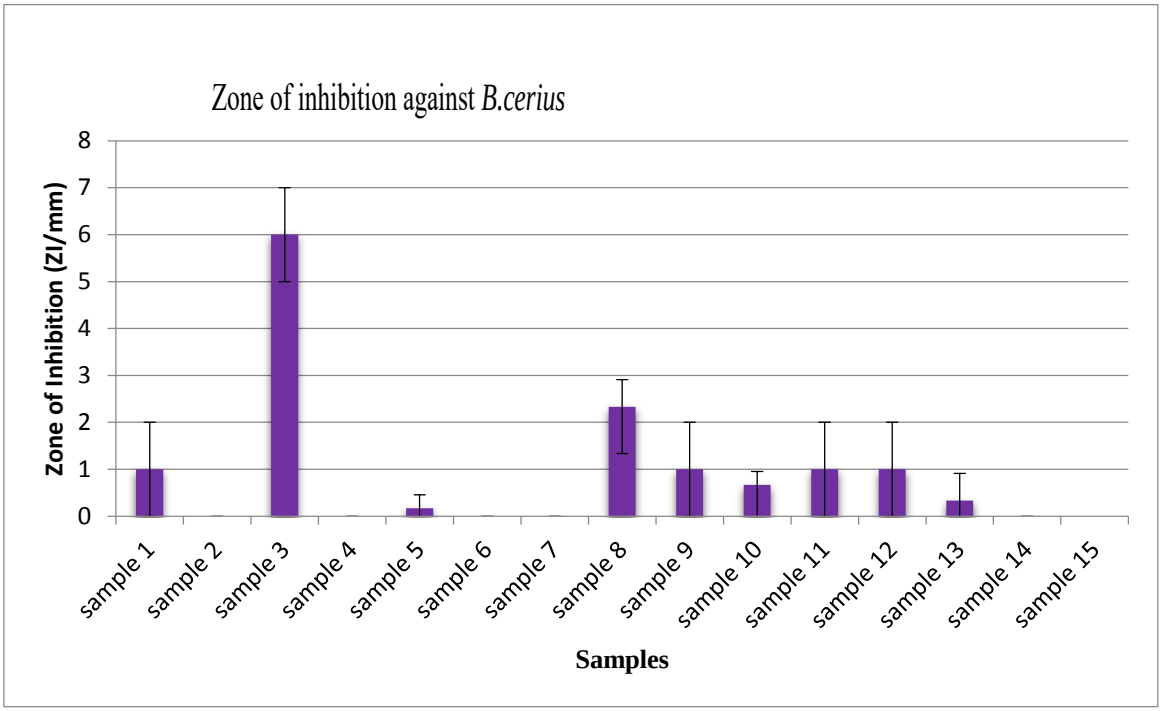


Fig 3.6. Zone of inhibition against *B.cerius*

Here we can see that our samples were not as effective as they were against E.coli. Though our sample number 3 showed very strong activity against B.cerius almost 5 mm and sample 2, sample 4 to sample 7 and sample number 14 and 15 was totally ineffective against this *B.cerius*. Average and standard deviation was calculated from 3 trials.

3.3. Discussion

Antibiotic resistance is increasing at an alarming rate throughout the world and the best chances that we have is the actinomycetes of unknown area or actinomycetes from less known or less explored area. If samples are collected from less known area the chances of getting new and effective compounds become very high. At present mankind's awareness and knowledge of the antibiotic potential of ocean related bacteria is still in low level and many of those bacteria is hard to understand. Here the purpose of the research was to find out the antifungal and antibacterial activity of actinomycetes collected from the mangrove forest of sundarbans.

Here in the research sample were collected from Bay of Bengal and from the Sundarbans. In case of Bay of Bengal the samples were collected from 5 different locations and they were at channel mouth, 3.5 nautical mile, 18 nautical mile, 35 nautical mile and 38 nautical mile. In case of the soil samples they were taken from 3 different locations of Sundarbans and their coordinates is given in the initial part of methodology. Instructions were followed from peer-reviewed journals and articles for screening and isolation of samples and protocols were followed as much closely as possible in the research. Growth pattern of samples were observed in different medias like Potato Dextrose Agar (PDA), in Nutrient Agar (NA) and also in Yeast Malt Broth (YMB).Before observing the growth pattern samples were separated based on the colors of different colonies and new type of subcultures were made from those isolates. During the observation of growth in different medias and it was found that the samples grow very fast in yeast malt broth whereas the growth is very slower in nutrient agar almost opposite the growth of yeast malt broth. The speed of growth in potato dextrose agar is between the growth of potato dextrose agar and nutrient agar, which proves that the samples really belong to the group called actinomycetes because they grow very well and fast in yeast malt broth.

During the antifungal test only sample no. 3 showed extraordinary activity. It created inhibition zone which is almost in average 5.67 mm against *Magnaportthe oryzae* which is a wheatblast strain. Other samples showed absolutely no activity.

From the antibacterial test, against *Escherichia coli* we found several other samples beside sample no. 3 that showed good activity. For example sample no. 5 to sample no. 12 created

average zone of inhibition of 1.62 mm to 2mm. Sample no. 13 to sample no. 15 created average inhibition zone of 1mm. Sample no. 1 created average zone of inhibition of 2mm. Sample no. 4 showed no activity at all and sample no. 2 showed very weak activity by creating average inhibition zone of .5mm.

Another bacterial strain was *Bacillus cerius*, sample no. 3 showed way better activity against *B. cerius* than it did against *E. coli.* It created average inhibition zone of 6mm. other samples showed very weak activity or no activity at all. Sample no. 2, sample no. 4, sample no. 6, sample no. 7, sample no. 14 and 15 showed zero activity. The rest were able to create average inhibition zone of .5mm to 2.33 mm.

Chapter 4. Conclusion

4.1. Conclusion

From this study it is evident that, soil samples can still show good activity against both fungal and bacterial strains. That means if more research is conducted upon the unknown or unexplored terrestrial areas new and very powerful antibiotics could be found. With time the number of projects on actinomycetes is increasing especially the one that are in marine in origin. Marine actinobacteria are famous for not rediscovery of same materials and this reason alone is responsible for giving the spotlight to marine actinobacteria but it will not be right to completely give up on the potential of soil actinomycetes. For a better future research should be done equally on both marine and soil actinomycetes.

4.2. Further Direction

Followings will be done in the future:

- Biochemical characterization for genus identification
- 16s rDNA sequencing for identification for sample no. 3.

References

Antibiotics, O. (2018). Actinomycetes , an Inexhaustible Source of Naturally. https://doi.org/10.3390/antibiotics7020045

Betancur, L. A., Naranjo-gaybor, S. J., Vinchira-villarraga, D. M., Maldonado, L. A., Suarez-moreno, Z. R., Acosta-, A., … Ramos, F. A. (2017). Marine Actinobacteria as a source of compounds for phytopathogen control : An integrative metabolic-profiling / bioactivity and taxonomical approach, 1–25. https://doi.org/10.1371/journal.pone.0170148

Binda, E. (2018). Specificity of Induction of Glycopeptide Antibiotic Resistance in the Producing Actinomycetes, 1–10. https://doi.org/10.3390/antibiotics7020036

Braña, A. F., Fiedler, H. P., Nava, H., González, V., Sarmiento-Vizcaíno, A., Molina, A., … Blanco, G. (2015). Two Streptomyces Species Producing Antibiotic, Antitumor, and Anti-Inflammatory Compounds Are Widespread Among Intertidal Macroalgae and Deep-Sea Coral Reef Invertebrates from the Central Cantabrian Sea. *Microbial Ecology*, *69*(3), 512–524. https://doi.org/10.1007/s00248-014-0508-0

Brzezinski, R., & Beaulieu, C. (2018). Chitinolytic functions in actinobacteria : ecology , enzymes , and evolution.

Cartuche, L., Cruz, D., Ramírez, M. I., Bailón, N., & Malagón, O. (2015). Antibacterial and cytotoxic activity from the extract and fractions of a marine derived bacterium from the *Streptomyces* genus. *Pharmaceutical Biology*, *209*(October 2017), 1–5. https://doi.org/10.3109/13880209.2015.1010739

Claverías, F. P., Undabarrena, A., González, M., Seeger, M., & Cámara, B. (2015). Culturable diversity and antimicrobial activity of Actinobacteria from marine sediments in Valparaíso bay , Chile, *6*(July), 1–11. https://doi.org/10.3389/fmicb.2015.00737

Cnq-, S., & Park, J. (2018). New Naphthoquinone Terpenoids from Marine, 1–9. https://doi.org/10.3390/md16030090

Ebel, R., & Jaspars, M. (2018). Natural product diversity of actinobacteria in the Atacama

Desert. *Antonie van Leeuwenhoek*, *111*(8), 1467–1477. https://doi.org/10.1007/s10482-018-1030-z

Fernández, E., Weißbach, U., Reillo, C. S., Braña, A. F., Méndez, C., Rohr, J., & Salas, J. A. (1998). Identification of two genes from Streptomyces argillaceus encoding glycosyltransferases involved in transfer of a disaccharide during biosynthesis of the antitumor drug mithramycin. *Journal of Bacteriology*, *180*(18), 4929–4937.

Goodfellow, M., Nouioui, I., Sanderson, R., Xie, F., & Bull, A. T. (2018). Rare taxa and dark microbial matter : novel bioactive actinobacteria abound in Atacama Desert soils. *Antonie van Leeuwenhoek*, *111*(8), 1315–1332. https://doi.org/10.1007/s10482-018-1088-7

Hug, J. J., & Müller, R. (2018). Concepts and Methods to Access Novel Antibiotics from Actinomycetes. https://doi.org/10.3390/antibiotics7020044

Hussain, A., Rather, M. A., Shah, A. M., Bhat, Z. S., Shah, A., Ahmad, Z., & Parvaiz Hassan, Q. (2017). Antituberculotic activity of actinobacteria isolated from the rare habitats. *Letters in Applied Microbiology*, *65*(3), 256–264. https://doi.org/10.1111/lam.12773

Joseph, T. A. H. Æ., Rajeetha, S. Æ. T., & Shanmughapriya, S. (2009). Production and characterization of lipopeptide biosurfactant by a sponge-associated marine actinomycetes Nocardiopsis alba, 825–835. https://doi.org/10.1007/s00449-009-0309-x

Karthik, L., Kumar, G., Keswani, T., Bhattacharyya, A., Chandar, S. S., & Bhaskara, K. V. (2014). Protease Inhibitors from Marine Actinobacteria as a Potential Source for Antimalarial Compound, *9*(3). https://doi.org/10.1371/journal.pone.0090972

Lasudee, K., Tokuyama, S., Lumyong, S., & Pathom-aree, W. (2018). Actinobacteria Associated With Arbuscular Mycorrhizal Funneliformis mosseae Spores , Taxonomic Characterization and Their Beneficial Traits to Plants : Evidence Obtained From Mung Bean (Vigna radiata) and Thai Jasmine Rice (Oryza sativa), *9*(June), 1–18. https://doi.org/10.3389/fmicb.2018.01247

Manivasagan, P., Venkatesan, J., Sivakumar, K., & Kim, S. K. (2014). Pharmaceutically active secondary metabolites of marine actinobacteria. *Microbiological Research*,

169(4), 262–278. https://doi.org/10.1016/j.micres.2013.07.014

Ng, Y. K., Hodson, M. P., Hewavitharana, A. K., Bose, U., Shaw, P. N., & Fuerst, J. A. (2014). Effects of salinity on antibiotic production in sponge-derived Salinispora actinobacteria. *Journal of Applied Microbiology*, *117*(1), 109–125. https://doi.org/10.1111/jam.12507

Pham, T. M., Wiese, J., Wenzel-Storjohann, A., & Imhoff, J. F. (2016). Diversity and antimicrobial potential of bacterial isolates associated with the soft coral Alcyonium digitatum from the Baltic Sea. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *109*(1), 105–119. https://doi.org/10.1007/s10482-015-0613-1

Raveh, A., Delekta, P. C., Dobry, C. J., Peng, W., Schultz, P. J., Pennelope, K., … Miller, D. J. (2013). Discovery of Potent Broad Spectrum Antivirals Derived from Marine Actinobacteria, *8*(12), 1–19. https://doi.org/10.1371/journal.pone.0082318

Sun, Y., Shi, Y., Wang, H., Zhang, T., Yu, L., & Sun, H. (2018). Diversity of Bacteria and the Characteristics of Actinobacteria Community Structure in Badain Jaran Desert and Tengger Desert of China, *9*(May), 1–14. https://doi.org/10.3389/fmicb.2018.01068

Talukdar, M., Bordoloi, M., Dutta, P. P., Saikia, S., Kolita, B., Talukdar, S., … Bora, T. C. (2016). Structure elucidation and biological activity of antibacterial compound from Micromonospora auratinigra, a soil Actinomycetes. *Journal of Applied Microbiology*, *121*(4), 973–987. https://doi.org/10.1111/jam.13233

Tyurin, A. P. (2018). Chemical Elicitors of Antibiotic Biosynthesis in Actinomycetes, (December 2017), 19–21. https://doi.org/10.3390/microorganisms6020052

Undabarrena, A., Beltrametti, F., Claverías, F. P., & González, M. (2016). Exploring the Diversity and Antimicrobial Potential of Marine Actinobacteria from the Comau Fjord in Northern Patagonia , Chile, *7*(July), 1–16. https://doi.org/10.3389/fmicb.2016.01135

Undabarrena, A., Gonz, V., Claver, F., Rojas, C., & Beatriz, C. (2017). Biodiversity of Actinobacteria from the South Pacific and the Assessment of Streptomyces Chemical Diversity with Metabolic Profiling. https://doi.org/10.3390/md15090286

Voytsekhovskaya, I. V, Tokovenko, B. T., Shatilina, Z. M., Luzhetskyy, A. N., & Timofeyev,

M. A. (2017). The diversity and antibiotic properties of actinobacteria associated with endemic deepwater amphipods of Lake Baikal. *Antonie van Leeuwenhoek*, *110*(12), 1593–1611. https://doi.org/10.1007/s10482-017-0910-y

Wei, W., Zhou, Y., Chen, F., Yan, X., Lai, Y., & Wei, C. (2018). Isolation , Diversity , and Antimicrobial and Immunomodulatory Activities of Endophytic Actinobacteria From Tea Cultivars Zijuan and Yunkang-10 (Camellia sinensis var . assamica), *9*(June), 1–11. https://doi.org/10.3389/fmicb.2018.01304

Xu, D., Han, L., Li, C., Cao, Q., Zhu, D., Barrett, N. H., … Wang, G. (2018). Bioprospecting Deep-Sea Actinobacteria for Novel Anti-infective Natural Products, *9*(April), 1–9. https://doi.org/10.3389/fmicb.2018.00787

Yang, N., & Song, F. (2018). Bioprospecting of Novel and Bioactive Compounds from Marine Actinomycetes Isolated from South China Sea Sediments. *Current Microbiology*, *75*(2), 142–149. https://doi.org/10.1007/s00284-017-1358-z

Zhang, X. Y., He, F., Wang, G. H., Bao, J., Xu, X. Y., & Qi, S. H. (2013). Diversity and antibacterial activity of culturable actinobacteria isolated from five species of the South China Sea gorgonian corals. *World Journal of Microbiology and Biotechnology*, *29*(6), 1107–1116. https://doi.org/10.1007/s11274-013-1279-3

Zheng, Z., Zeng, W., Huang, Y., Yang, Z., Li, J., Cai, H., & Su, W. (2000). Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. *FEMS Microbiology Letters*, *188*(1), 87–91. https://doi.org/10.1016/S0378-1097(00)00215-9

Zhu, H., Sandiford, S. K., & Wezel, G. P. Van. (2014). Triggers and cues that activate antibiotic production by actinomycetes, 371–386. https://doi.org/10.1007/s10295-013-1309-z