Facets of Covid-19:

Issues, Approaches, Experiences and Consequences

Dr. Vinita Bhimrao Kekan

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Let us understand: An Online Counselling Initiative in COVID-19

*–Dr. Abhay M. Jadhav**

ABSTRACT

An online counseling is treated as the best way to offer counseling services during the COVID-19 outbreak. The main aim of this article is to explore the usage of online counseling during the covid-19 outbreak to solve psychological problems of covid-19 quarantine patients in solapur region, who were termed as Shubharthi. The author has examine the history and concepts, the therapeutic relationship, transference and counter transference of patients, the advantages along with the disadvantages, considerations, implications, and experiences gained in online counseling during covid-19 outbreak through

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the 'Let us understand' online counseling service program started by the wing of Rashtriya Swayamsevak Sangh.

KEYWORDS: Online Counseling, Services, Shubharthi, Relationship, Psychological Problems, Outbreak.

Introduction

According to Hindu culture, it's our tradition to share our joys and sorrows by meeting with each other in particular situations.The Sangh Parivar, "Family of the Rashtriya Swayamsevak Sangh" or the "RSS family" refers, as an umbrella term, to the collection of Hindu nationalist organizations spawned by the Rashtriya Swayamsevak Sangh (RSS), which remain affiliated to it (Hansen, Thomas Blom, 2014). The service programs of Sangh Pariwar, over the years, have led to the empowerment of the economically and socially underprivileged sections of the society.

Amidst India battling an unprecedented viral disaster, the Western Maharashtra unit of Rashtriya Swayamsevak Sangh (RSS) has joined efforts with different organizations and individuals to provide relief to COVID-19 positive patients i.e. Shubharthi, their relatives and frontline workers in the state of Maharashtra. The organization was providing hospital beds and oxygen concentrators to those infected along with wheelchairs to homebound patients. Apart from the physical aid, it also provides free of cost counseling and guidance related to ayurvedic, homoeopathic and allopathic medicines. In the present article, author shares the information and experiences received during the management of telephonic counseling program titled "Let us understand" ('Chala Samajun Gheu ya' in marathi) in Solapur district of Maharashtra state. In this volunteer program, we were calling counselee as a Shubharthi.

Telephonic Counselling

Online counseling has been characterized as the backbone of counseling services via the Internet, where the volunteer counselor or psychologist and counselee/client are not within the same physical area and they communicate utilizing computer-mediated communication innovations (Abney & Cleborne, 2004; Baker & Ray, 2011; Richards & Vigano, 2012).The benefits of online counseling incorporate more noteworthy availability, reaching people that would not immediately look for face-to-face counseling, reasonableness, and ease of record keeping (Leibert, T., Archer, J., Munson, J., York, G. 2006). Moreover, Suler (2002) and Partala (2011) expressed that clients taking part in online counseling are less likely to feel powerless for revealing their individual data and also feel less ashamed about their issues, due to the anonymity related to online counseling.

In April 2021, a group of Sangh Pariwar members identified the need of the COVID-19 victims to stay away from their families that leads to developed fear, anxiety in their minds, due to isolation and different agonies during the quarantine period.

Procedure and Method

The core team of four members was formed in association with RSS regional heads of Maharashtra State to plan the outline framework to initiate online activity in Solapur district. The formal permission was sought from the district authority to implement online counseling program for COVID-19 patients. The beneficiaries of this counseling program were called as 'Shubharthi' which means wishing the best wishes for their recovery from COVID-19 and allied disease. The interested volunteers were appeal to join this activity by filling up their basic information through Google from. The selected 75volunteer's i.e counselors and 04 core team members were oriented and trained into two online sessions. The online simulation training was imparted on 1st May 2021, by experts namely Dr. Omkar Joshi, MD, Psychiatrist, Pune, and Dr. Ghanshyam Khandekar, Ayurveda. The detailed procedure for having a dialogue with patients, pros and corns, coordination and reporting systems was explained to participants.

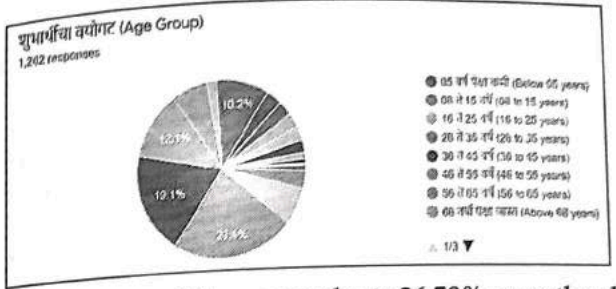
Counselors were asked to fill out an online call checklist recording demographics (age, gender, location of caller, reason for calling) and basic descriptive characteristics of the calls, including a brief qualitative written description of each call.

The author of this article worked as a core team members cum chief coordinator in collecting and distributing the names and contact number list of Covid-19 positive patients and it has been allocated to 10 team coordinators for distributing it to the counselors. The author coordinated and conducted close readings of the written descriptions of the calls, and each individually developed a set of categories, consisting of subcategories, of the issues presented by callers. Where ever the necessity felt, the referral and supportive services was provided with the help of Sangh parivar members. A final set of categories was decided upon through discussion and was as: psychological symptoms, mental illness, family/relational issues, managing quarantine, financial/livelihood concerns, health related concerns, and front-line worker concerns.

Demography and Results

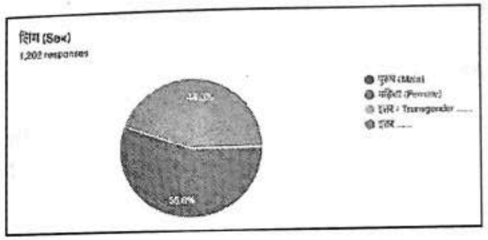
A total 1202 Shubharthi i.e respondents (COVID-19 positive patients) were covered in the period of 45 days in the month of May and June 2021. The details of it are as follow:

(A) Age Group:



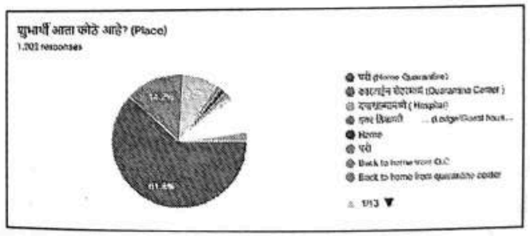
The majority of the respondents 26.50% were the above 60 years old. The 19.10% in the age group of 36 to 45 years. The 12.10% respondents were in the age group of 46 to 55 years old and the 25.00% respondents were in the below 25 years age group. The rest of the respondents were in other age group than mentioned above.

(B) Sex:



The majority of the respondents 55.65% were the male and the 44.35% respondents were female by gender.

(C) Place of Stay/ Isolation:



During the Covid-19 positive phase, at the time of online counseling, the majority i.e. 61.80% respondents were quarantined at their own home. The 14.20% respondents were quarantined in Covid-19 care centre and 08.30% respondents were hospitalized and the remaining respondents were either returned home after quarantined or staying at lodges or farmhouse or they refused to provide information about their place of isolation.

(D) ***Information related to other associated problems:*** When the respondents were asked about their associated problems along with covid-19, it has found that the majority i.e. 48.80% of the respondents were feeling that they have lost their immunity power, 38.60% respondents have felt that because of them other member in their family have become Covid-19 positive, 22.70% respondents have complexity feelings, and 05.60% respondents were become newly victims of blood pressure and diabetics.

Discussion

There were some common issues that have been shared during counseling dialogue by the respondents. Those issues can be summarized as psychological issues such as anxiety, sleeplessness, loneliness, depression, panic disorder etc.

It has been also found that family and relationship issue such as interpersonal problems due to lock down including fighting among family members, managing children in lockdown, and an increase burden in household chores and care taking were also common almost in all respondents.

The majority of the respondents faced difficulties in managing time in lockdown, boredom or restlessness, and shared about the problems raised due to working from home /balancing personal and professional life and few respondents forced towards unemployment, needing immediate financial support, or related livelihood concerns.

Conclusion

This article summarizes the issues and our understanding that raised during tele counseling to respond to the COVID 19 pandemic. The majority of Shubharthi display anxiety and sleeplessness related to the lockdown that indicating the prevalence of sub-syndrome of mental health issues due to COVID 19 (Rajkumar, 2020). This indicates the need to provide population level strategies for dealing with these symptoms, lest the mental health demands become a crisis in their own right. However, the additional issues raised also indicate the nature of people's experiences in present crisis.

These issues are an important starting point for future research and mental health interventions for persons/ families in lockdown in India. Financial and livelihood concerns also seem underrepresented that leads to the economic crisis due to lockdown. However, this may be explained by the existence of these sources of support for individuals. It also needs to note that these are the issues faced during the initial stages of the Corona, which may evolve depending on how long it continues.

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Impact of Covid-19 on Economy of Rural and Urban India

-Dr. Meghraj A. Kapurderiya*

Abstract

Since independence India has been a 'Mixed Economy'. India's large public sectors were responsible for rendering the country a 'mixed economy' feature. COVID-19 (corona virus disease 2019) is a disease caused by a virus named SARS-CoV-2 and was discovered in December 2019 in Wuhan, China. It is very contagious and has quickly spread around the world. Due to COVID-19 pandemic India has approach towards complete lockdown which came enforce on 22 March 2020. Due to which other than essential services and goods are closed down only food, medicine, electricity, banking and telecommunication services are active with minimum resources. The migrant labours due to lack of work they have started towards villages to get

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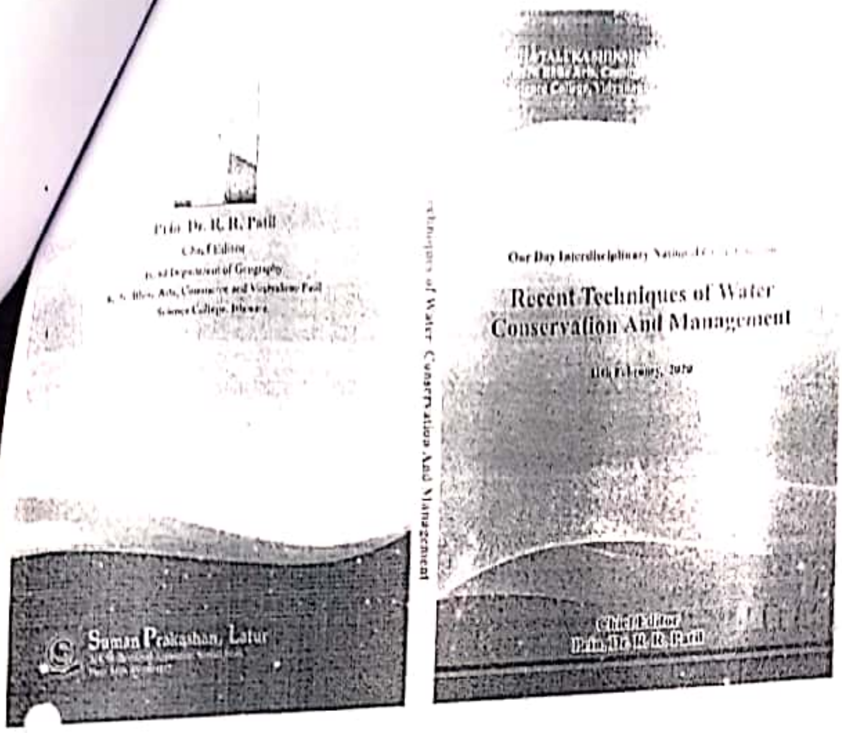

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...ader, Extension, Education and Learning Initiatives for ...of Water Conservation and Management. Four key ...ers were invited to discuss the theme and the thrust areas ...the conference. They were Prof. Dr. H. C. Vaidya, Dr. Pradeep Purandare, Prin. Dr. S. K. Wadkabalkar , Hon. Sampatrao Pawar. They all discussed recent theories and techniques of Water Conservations and Management and simultaneously gave the practical solutions to the problems by citing day today examples. The participants were enriched by the knowledge and discussion about the global need of Water Conservation and Management. I must thank all the advisors of the conference for the cooperation and helping us to make it success. Finally, without the support and the work of the organising committee of my college, the national conference and the present book would not have been realized.

Prin. Dr. R. R Patil
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7.

Water Conservation in India: Issues and challenges

Dr. Jitendra S. Gandhi
Assistant Professor, Department of Social Work,
Walchand college of Arts and Science, Solapur (Maharashtra)

Abstract

'Water' is a fundamental and important natural resource for human survival. Water has multiple usage and importance for the existence of human being. Water has been necessary ingredient from agriculture to the industrialization. Human kind will be miserable without the adequate amount of water on the earth. As water is been natural resources its management and equal distribution and usages is the responsibility to all. India is being developing country has to be very careful to avoid water crisis in the future which seems to be happen soon. The research article tries to discuss various issues and challenges which may encounter in the process of water conservation especially in the Indian context and has suggested proposed intervention in the same.

Key words: Water conservation, equal distribution, management, issues and challenges

Introduction:

Denying the importance of water and its mismanagement in India would be a great ignorance. India is being diverse country in all other aspects is as diverse as for its water resources and management too. Today water is not just been viewed as natural and important resource worldwide but also been viewed as a cause for conflict between men to men, state to state and nation to nation. Timely intervention only can delay the conflict which seems to be very obvious. There are many issues involved around

the water. Vast industrialization, urbanization, increased population, excess usages of water, lack of programme , planning, policies and implementation in the areas of water conservation, unequal distribution of water all such issues needs separate attention

Review Literature:

Gulati, Ashok & Banerjee, Pritha (2018) indicated rapid urbanization and industrialization increases water demand for competing uses is going to rise sharply. It further raises concern for issues related to water use in agriculture. The article has highlighted various issues related with water crisis specifically Indian context. KB, Ramappa & Reddy, Halappa & Patil, Savita (2014) highlighted the importance of efforts of the government and external aids along with creating awareness on water conservation are some of most important aspect in the process of water conservation. Dhiman J, Dhiman J.S, Aggarwal R., Dhiman M (2015) highlighted that sustainability of Indian agriculture, food demand, growing population and other important issues along with water management. The article has suggested various measure to overcome the issues. It also indicated that for water management collective action needed. Sandrasekaran, Manivannan & Thilagam, V. Kasthuri & Khola, Om Pal. (2017) highlighted the importance of soil and water resource management together. It indicated that soil and water management together would be holistic approach to deal the issue. It also highlighted various strategic areas for further intervention in this area. M, Dinesh & Ballabh, Vishwa. (2000) highlighted the rise and demand of water will grow in India. It further highlighted various gaps between the agencies and sectors working in the field of water management. Further it highlighted the importance of equatable allocation to resolve conflict. The above review literature indicates that water conservation has many aspects to intervene. We need to study the water conservation process in India more holistically and inclusive manner.

Issues and challenges in water conservation and proposed framework for the same

Following are some of most important issues and challenges which need to be encounter as early as possible to have holistic approach towards water conservation.

Issues and challenges	Proposed framework for the intervention
Explosion of Population	The increase numbers of the population of the country would be a greatest concern for the water conservation process. As the increase of population will add on the burden of existing water resources of the country. Controlling population explosion would indirectly would help water conservation in long run
Tradition resources	Traditional resources of the water conservation need to be upgraded along with new technological and technical up gradation
Lack of public participation	Water conservation, its protection and management has to become public movement. We need to increase public participation in all above aspect of water conservation.
Lack of NGOs/CBOs participation	NGOs and CBOs have greater reach and acceptance at local level in term of water conservation. The role of such organization need to be highlighted, enhanced and encouraged
Role of government machineries	The role of government machineries must be specified. The machinery must finalize and prioritize its role and responsibility in the process of water conservation.
Political willingness	Political willingness and it's support need to strengthen to uplift water conservation in the country.
Training and education	Variety and series of training and educational material need to be developed in local language and need to provide all public and private places to make educate and aware the common masses about the importance and practices in water conservation.
Policy planning and implementation	Water conservation policy must be compressive and must consider all opinion and voices of the stake holders working in the field of water conservation.
Encouragement and protection	Individual, organization, NGOs and other stake holders working for the Water conservation and protection must be safeguarded by the state.
Role of media	Media must keep water conservation as a national agenda and can do wonderful intervention in the process of water conservation.
Equal distribution	Agriculture, industry, household and other large usages of water must be distributed with consciousness. Water auditing and budgeting must be done carefully and must reflect its distribution. No sector should misuse and hamper the overall equal distribution of the water.

Conclusion:

Water as natural resources must be concern for all of us. Water crisis in the future will not have any alternatives to resolve it without water only. Timely intervention is the need of the hour when it comes to water conservation. The research article highlighted that controlling population explosion, up gradation of traditional resources of the water conservation, become public movement, public participation in water conservation, NGOs and CBOs have greater reach and acceptance at local level in term of water conservation, role of NGOs and CBOs need to be highlighted, enhanced and encouraged working in the field of water conservation, political willingness and it's support, training and educational material development, compressive water conservation policy, protecting and safeguarding Individual, organization, NGOs and other stake holders working for the Water conservation, media participation and equal distribution of the existing water resources would help water conservation process in Indian context.

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Issues, Approaches, Experiences and Consequences

Dr. Vinita Bhimrao Kekan

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Preface

The world experienced the pandemic, the whole world was put on alert and every nation was striving to save his citizens from the corona virus. Number of precautions were taken by the concerned government to prevent corona attack or treatment of infected. However, lockdown as a strategy to combat corona was almost followed by the majority of the nations. As far as India is concerned, lockdown was imposed when first wave started in India. suddenly impose of lockdown and guidelines to restrict the movement created chaos all over. This first lockdown was well-known for the difficulties and sorrows of the retuning migrant labour towards native place.

In first wave, the government had instructed to all the villages to form *Dakshata Samittee* to observe the returned migrants. Before entering in the village, these returned migrant has to stay ten days or more at isolated place popularly known as "Quarantine Centre". In those days, the migrants experienced the pungent experiences, these experiences were about the discriminatory behaviour, ill treatment, a type of boycott etc. The book is intended to document issues, approaches and consequences aroused out of covid-19. It is also intended to document the experiences covid and post covid situation.

The editors of this volume are inviting original research articles on the following issues like 1.reverse migration and its impact on labour community2. case studies on experiences during quarantine at villages3. covid-19 and village level misconceptions4. community approach towards the covid positive patient and his family5. impact of lockdown on rural and urban economy6. problems and copping strategies of covid widow7. problems of families lost main earner8. domestic violence and mental health during covid-199. covid-19 and health services and 10. case studies on experiences of returning labourers to their home during 1st lock down.

On the basis of the theme provided, we have received the quality articles, chapters, field studies, case studies, personal experiences, articles based on secondary sources etc. this is an effort to document the experiences, observations during pandemic. I express my gratitude towards all the contributors for the valuable contribution for this book.

Yours

Dr. Vinita Bhimrao Kekan

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—मिलिंद शंकरराव दानव

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COVID-19: Issues and Challenges in Mental Health Services in Indian Context

-Dr.Jitendra S. Gandhi*

Abstract

The COVID-19 pandemic crisis has hit hard to all the globe. The entire globe has suffered through its economic, social, commercial, political parameter. Among the all the most affected phenomenon of this pandemic which is badly affected was health and mental health aspect. Though the globe has come up with massive vaccination programme, but still the health and especially mental health areas of the human being has been suffered a lot and will be suffering for many coming days. The research article is based upon secondary review data and field experiences and observations. The research article tried to put forward the overall issues and challenges of the health and mental health services through Indian context.

* Assistant Professor,Dept. of Social Work, Walchand College of Arts and Science, Solapur (MS)

Key words: COVID-19, health, mental health, services, crisis

Introduction

When we look at the entire COVID-19 pandemic crisis through Indian context; it has been observed that the health including mental health services has witnessed many issues and challenges. The world recently has accepted Indian medical professionals with lots of respect and welcome them to serve across the globe. Indian health care has succeeded with lots of barrier to provide basic and minimum health care services to the largest populated country like India. The limitation of our health care system is being minimized with the acceptance of multi-player health care model. As we are aware about that the health disparity which are still exist now need to be eradicated. The poor will be able to receive affordable health care services is still dream for all of us.

The COVID-19 pandemic crisis has made us to learn that the "Health must be for all of us" not for only someone. We have to rearrange our health care policy and practices which will be more inclusive rather than selective. The article tries to discuss various issues and challenges faced by our health and mental health care delivery system of our county during COVID-19 pandemic crisis.

Conclusion

The research article highlighted the overall mental health infrastructure, resources, manpower and level of awareness among the general population in our country. The article concludes that our country needs greater infrastructure and resources as far as mental health is concerned. The COVID-19 pandemic has indicated that

Sr.No	Issues and Challenges in the mental health care delivery system	Proposed recommendations to improve the mental health care delivery system
1	Lack of Mental health Professionals	The country has witnessed that paucity of the mental health professions which includes Psychiatrist, Psychologist, Psychiatric Nurses, Medical and Psychiatric Social workers, ECT technicians, rehabilitation expert and occupational therapist.
2	Lack of Mental health infrastructure/service provider	Our country has limited service provider as far as mental health services are concerned. The government machinery which deals with mental health services are only at district places. The private professionals and mental health service provider are beyond to afford by the majority of Indian population.
3	Lack of awareness about mental health issues/disorders	The majority of Indian population is not very much aware about various mental health issues/disorder. The common community religious and cultural healing practices are being adopted by larger section of our own country.
4	Lack of preparedness to face the crisis/disaster	As we all have understand the impact of COVID-19 pandemic on our mental health and well being; still we (community, governance mechanism, professionals and stakeholders) are not prepared to face the upcoming disaster pandemic crisis situation.
5	Lack of preventive and rehabilitative mental health services	Counselling, psychotherapy, after care and psycho-social rehabilitation, group therapy, family therapy, music therapy, day care, half way home and telephonic counseling are some of the neglected areas which deals with prevention, early identification and rehabilitative approach in mental health aspect. The COVID-19 pandemic has made us clear that we lack in all these areas which needs very low infrastructure and expenditure too

we lack and are far behind [illegible] resources. Mental health [illegible] integral part of our health care system [illegible] is ignored and neglected. The research article [illegible] the preparedness of mental health services etc. [illegible] should be considered as essential and integral [illegible] upcoming disaster management pandemic.

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डॉ. बाळासाहेब जी. जोगदंड

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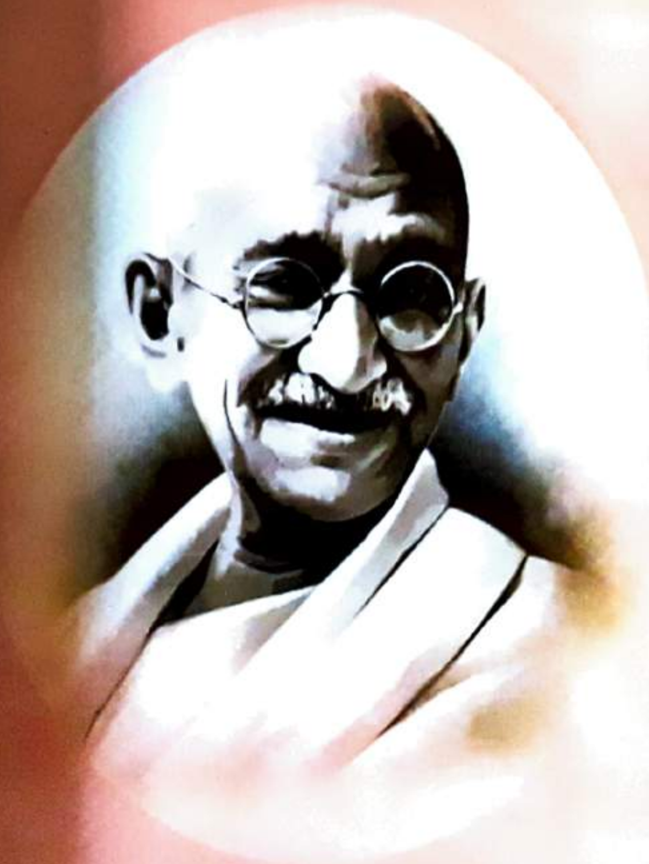

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संपादकीय

'गांधीवादी विचार' या प्रस्तुत पुस्तकाचे संपादन करताना मला अतिशय आनंद होत आहे. गांधीवादी विचारातील विविध पैलू प्राध्यापक व संशोधकांकडून लिखित स्वरुपात प्रतिपादित करण्याची संधी या संपादीत पुस्तकाद्वारे उपलब्ध करुन देण्याचा प्रयत्न करण्यात आला आहे.

भारतालाच नव्हे तर संपूर्ण मानवी समाजाला गांधीवाद अनुसरण्याशिवाय पर्याय नाही. रक्तपात, हिंसा, फसवणूक, भ्रष्टाचार, दशहतवाद, एकात्मता, भाषावाद, जातीयवाद यासारख्या समस्यांची मानवी समाजाला शिसारी येत आहे; यामधून बाहेर पडण्याचा मार्ग भारतच गांधीवादाच्या बळावर जगाला दाखवू शकेल. रक्तरंजीत मार्गाने मानवी समाजाला आपले कोणतेच ध्येय साध्य करता येणार नाही. आपल्या शासनाकडून आपणास हव्या त्या गोष्टी मिळविण्याचे अहिंसक मार्ग गांधीजींनी सांगितलेले आहेत. सत्याग्रह हा त्यातील प्रखर मार्ग आहे. सर्व घटनात्मक व सौम्य अहिंसक मार्ग निष्प्रभ झाल्यानंतरच त्याचा अवलंब व्हावा अशी गांधींची अपेक्षा होती, हा विवेक सुध्दा शस्त्रे वापरणाऱ्यांनी ठेवला पाहिजे. शासन आणि लोकांमध्ये नियमितपणे दुतर्फो संबंध राहावयाचा असेल तर तो एकाचवेळी संघर्षाचा व संवादाचा राहू शकला पाहिजे, तो हिंसक दहशतवादाच्या स्वरुपाचा असू शकत नाही, ही गोष्टही स्पष्ट आहे. गांधीच्या सत्याग्रहाचा मार्ग हा संघर्षात सुध्दा संवाद राखणारा मार्ग आहे.

महात्मा गांधीनी आपल्या विचारांना क्रमबध्द स्वरुपात मांडण्याचा प्रयत्न कधीच केला नाही. आपले विचार संकलित करुन त्यांनी कोणत्याही वादाला जन्म दिला नाही. गांधीवाद म्हणून कोणताही सिध्दांत नाही. सत्य व अहिंसा यांची ज्योत सतत तेवत राहावी एवढेच त्यांचे म्हणणे होते. एका निश्चित सिध्दांताचे ते समर्थक नव्हते. लॉर्ड बॉयड यांच्या मते, "माझ्या विचारानुसार गांधीजींनी प्रतिपादन केलेल्या सिध्दांतांना वैश्विक स्तरावर व्यावहारिक स्वरुपात अंमलात आणण्याची वेळ आली आहे. त्यांचा प्रयोग सातत्याने करण्यात आला. कारण लोकांना सुध्दा कळून चुकले की, गांधीवादाशिवाय वर्तमानकाळात दुसरा पर्याय मानवी व आंतरराष्ट्रीय समस्यांच्या सोडवणूकीसाठी उपलब्ध नाही"

Mahatma Gandhi as Peace Strategists

Dr. Sandeep Jagdale

Abstract

After the decade of liberalization, privatization and globalization the modern world is realizing the limitations of it (Pathy, 1995; Upadhyay, 2000) in terms of gaps between rich and poor, developed and undeveloped, technology vs. People, individual rights vs. social and cultural erosion. With some reservations we should accept that Gandhi's way of perceiving life has much deeper meanings and therefore fruitful repercussions for individual and society at large. What we see today, is nothing but political and economic mess which needed to be resolved. The proclaimed economic and political models of development yielded this vast disparities and agonies. The world if facing a big issue of violence of one or other type, and desperately needed some path to maintain world order. The author of this paper would like to pin the person of our home land for mitigate this violence and sharp disparities to have peaceful life for an individual and therefore a Nation.

Key words: Mahatma Gandhi, peace and development, strategist, modern world.

Introduction

The modern world, the new generation of this, after new economic policies of liberalization, privatization and globalization believed that people who don't follow the orders should be punished. Today, violence is not a new phenomenon, rather is became a pivotal part of human life. This new policies have made people believe that violence is necessary for just society, just peace (Pabst, 2007). People of today may respect Mahatma Gandhi as freedom fighter, but they may be not following him in their personal and professional life (Jagdale, 2017). This is broadly reflected in to the lives of human,

animal and in national scenario of poor, destitute, disadvantaged, handicapped, othered or cornered people.

In Indian context, the way violence is perceived before independence and after independence has significant difference. We can say that before independence the way violence is perceived before independence and after independence has significant difference. We can say that before independence, India as a nation has ruled by British and largely perceived as invaders therefore whatever violence incidences took place was blamed on them, but after independence, the scenario has changed, people got educated, raised by political philosophies, and individual aspirations made to perceive violence as tool to get success in their vested interests.

Ramachandran (2020) has tried to articulate the violence inflicted in India after current regime of government with solid evidences. It shows that how the communal violence incidents have raised prominently. In Indian parliament when the question of communal violence incidents raised, the Shri. H. G. Ahir responded with the answer that how these incidents have raised after 2014 (GOI, 2018) but stressed the law and order issue involved in it. Shastri (2020) also reflected on the communal violence in twenty first century in India well. What these authors are convinced about and try people to look at is the issue of 'peace building'. People know the reality that there is violence everywhere, whether personal life or social, cultural or political life. The problem is realized, but need to be resolved before blunders happen like wars – the most brutal part of human civilizations.

Violence is violence, there is no question of how much, who did it, why one does it, when it happened and at all. As a strong believer of 'Ahimsa', Gandhi visualized so cleverly and clearly that if generations wants to live healthy and happily, they have to have non violent way of thinking, behaving style of living.

Mahatma Gandhi as Peace Strategist

In today's world, the word 'strategist' become popular because of new avenues has come to conquer the perceived enemies in the field of academia, politics, corporate affairs, international relations etc. Cambridge dictionary defines it as a person with lot of skill and experience in planning, especially in military, politics or business matters. therefore political parties, corporate houses, CSR initiative even popular media tycoons appoint their chief strategists to ease or raise their interests.

Although, the strategists is someone who knows the past, present and can see immediate future; it seem ok with industry, media or some sectors of corporate but when we turn to human life, human sufferings, human agonies, human conflicts, issues where technology or traditional tools of conflict resolutions like Acts, Police, Courts yielding very little results then a vacuum becomes prominent; which makes people restless, reactionary and legitimating violence for their needs or greed's. Today we have everything we need -roads, electricity, communication, Internet, advanced technology, speed and what not. But what people are missing in their life is 'peace'.

When anyone thinks about peace, he has to encounter with Mahatma Gandhi. Not just because of Gandhi and non-violence became synonymous but because of his teachings, his life struggles and the evolution of relevant tools of conflict resolution and peace building. Based on following things, the author sees him as peace strategist;

Satyagruha

When in was in prison in 1930, his discourse mentioned that *"The word Satya (Truth) is derived from Sat which means being. And nothing is or exists in reality except Truth. That is why Satya or Truth is the most important name of God. In fact it is more correct to say Truth is God than to say God is Truth... Where there is no Truth there can be no true knowledge".*

Today the concept of truth has become so irrelevant and has lost its pragmatic value for human beings. In other way people may deny the whole proposition that truth posses reality. And to find a reality one must have faith in the human dialogical processes. Therefore it is inevitable to think that I may be imperfect so I must practice tolerance; here is the strategy of being and making people at peace. When no one is perfect then we all have to strive for the best. This philosophical frame of reference gives people a space for communication and has peaceful dialogue. And Gandhi believed that dialogue is the only way to have peaceful life. Dialogue has every facet that human need to survive. When there is no value to dialogue then human tends to become violent. Therefore satyagruhi must hold truth along with constant dialogue for making opponents realize the truth.

Fasting

For him *Satyagruha* or holding fast to the truth was not political activity but philosophical stand to demand right action from the both sides polity and the people of society. He said '*Satyagruha*' is not only premise but a quest for a *Satyagruhi*. One who is doing *Satyagruha*, his action may or may not be political but can remain non-ideological. It can also be the basis for challenging unjust laws - and this is a philosophical question, for it puts natural justice against the formal laws. So fasting, using personal pain to make people internalized the issue and its affect on society at large becomes a strategy. Strategy to hold an issue, to hold the reactionary behaviors, political decisions etc.

Nature Cure

According to Gandhiji, our health is cannot be a commodity to purchase, for him health is an amalgamation of various activities of human being which includes what he eats, his exercise, his cleanliness of surroundings, his education, his life style, housing, his work, everything he engages in have solid impact on his health

(Satyalakshmi, 2019). And therefore it becomes his sole responsibility to be self-reliant and not to depend on external control. He said India does not need imported drugs from west when she has an inexhaustible stock of a variety of drugs grown in its villages themselves. Being a poor country with rich heritage of medicine, he wanted people to adopt nature cure and to be self-sufficient.

Assertion of Seven Sins

Gandhi used his evening prayers as strategy to make people aware of his actions, thinking and teachings at one hand and on the other he use this as a platform to inculcate the human life free form seven deadly sins. If we look closer to each of the sin articulated by him we may resemble them to our today's life problems. Wealth without work, Pleasure without conscience, Knowledge without character, Commerce without morality, Science without humanity, Religion without sacrifice and Politics without principles (Grover, 2015); these are not just themes but it has a wide spectrum of understanding of human issues and also pathways to peaceful life and development. We just need to pick each lens and see through it and interpret human issues, conflicts and related violence.

Service, Character, *Swaraj* and Nation Building

Service is something Gandhi put higher in any learning or education. He said that an educated man is one who takes a broom in every day and clean his surroundings'. He had given highest value to the labor. Service should be out of heart, it should not be for any monetary benefit.

Gandhi emphasized over character, even he said that an education should have a prime motive to build a character in pupil. To build a character, one who build it, must have a character. Here Gandhi wants to say that a teacher should have purity in soul, idea and action. No extreme individualization included in character, one who thinks for all is a character man. This have seeds of nation building philosophy that this is my nation, these are my people, their suffering

have something to do with my sufferings and development, I alone can never be developed without development of all i.e. *Sarvodaya*. Here is the strategy to make people to develop character; they should serve the people of their land which in other way is can be seen as a strategy of nation building.

Moreover, *Swaraj*, or *independence* or the quest for self-recognition had to be pursued by pure, that is, non-violent means - to indulge the impulse to violence was to undermine the self. This is a strategy of planning the future of India which will be self-reliant, self-regulated, self-ruled and essentially 'Atmanirbhar Bharat'.

Conclusion

Though, many modern scholars of administration would not follow the traditional wisdom of India in different life problems. Still the philosophy, teaching, preaching, and life of Mahatma Gandhi may serve as an instrument of peace making, peace building, and peace communication. In this post-modern era of development, technology, his developed, practiced and advocated strategies of making people united, non-violent, truth and justice prone can be viewed as strategies of handling human conflicts. Therefore, Gandhi can be seen as peace strategist and further included in the syllabi's of conflict resolution strategies, managing international issues, man-animal conflicts, human right subjects, criminal justice etc.

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COVID-19 and Gandhian Principles

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Preface:

Outbreak of COVID 19 pandemic has created an unprecedented situation throughout the world and spread threat to humanity at large during last 6 months but this is not the first time that pandemic has embraced the whole world. History had witnessed the severe effects on social, political religious and economical sphere very often like Swine flu, plague, H1N1, Ebola, AIDS, TB pandemic etc. and endangered the humanity. Now in 2020, due to spread of Corona virus (assumed to be biological war), China has been targeted by the world. The worst epidemic and pandemic about 3000 years BC wiped out the whole village "Hamin Mangha" in china where the whole community, irrespective of age, gender, was affected and dead bodies were collectively disposed after which the whole village was set to fire. Around 430 BC, an epidemic in Athens witnessed the sudden deaths of 100000 people due to brain fever. Plague and Spanish flu (1343 to 1920) wiped out over half of European population and bodies were buried in mass graves. It resulted in the course of Europe history by witnessing the emergence of technological innovations.

At present the devastation aroused out of corona epidemic proved that the notion of human beings to considered themselves as suprimo in this world rendered severe blow. Technology and religion seem to be helpless to fight with this virus. Till date, a proper medicine is not invented and still there are faint chances to get vaccine for lifelong protection. Earlier it was said that it would affect the children and the aged people, but fact is that no one is left unaffected. Hence now, it is clear that one must increase the immunity power to

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Chapter 7
Diversity and Classification of Rare Actinomycetes

Anil Kumar S Katti, Shilpa AK, and Sulochana B Mudgulkar

Abstract Rare groups of actinobacterial species are widely distributed in soil and water habitats. Even though soil consists of enormous actinobacteria, they can also be isolated from water, plants, sediments, limestone quarry, and animals. In any environment, various factors like physicochemical and biochemical reactions define the diversity and distribution. Environmental parameters such as soil type, soil conductivity, humus content, and characteristics of the humic acid content also affect the soil microbial community. One of the significant ways to explore rare actinomycetes lies in sampling the underexplored or unexplored environments, and these habitats provide unparalleled chemical diversity and potential novel communities. Several environments are yet to be explored to determine the productive types of rare actinobacteria. Recognition of unusual environments is crucial in isolating different groups of rare actinobacteria, and understanding the complex ecological interactions among these microbes is to be defined. There has been a significant advancement in isolation, identification, and characterization of the bioactive producing rare *Actinomycetes* gaining more importance.

Keywords Rare actinobacteria · Diversity · Distribution · Classification · Ecological study · Identification

7.1 Diversity of Rare *Actinomycetes*

Rare actinobacteria are generally classified as strains other than *Streptomyces* (Berdy 2005). The frequency of isolation of actinobacterial strains under normal parameters is significantly less (Baltz 2006). Compared to *Streptomyces,* the growth of

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non-*Streptomyces is* usually slow and requires very complicated procedures for isolation, cultivation, and preservation in some genera (Lazzarini et al. 2000). Terrestrial and aquatic ecosystems harbor a wide variety of rare *Actinomycetes,* but the primary habitat of rare *Actinomycetes is* soil. These organisms were also isolated from various niches such as sediments, stones, water, plants, and animals (Groth et al. 1999). The rare *Actinomycetes* diversity and distribution in individual habitat are affected by several physicochemical parameters that are soil type pH, humus type, and humus content (Tiwari and Gupta 2013). The rare *Actinobacteria* genera isolated by the research team from Egypt include *Micromonospora*, *Actinoplanes*, and *Actinomadura* from Egypt soil samples (Abd-allah et al. 2012). Another report confirmed the rare *Actinobacteria* isolated from Trondheim Fjord [Norway] of shallow-water sediments including *Actinocorallia, Actinomadura, Micromonospora*, *Glycomyces*, *Nocardia*, *Nocardiopsis, Pseudonocardia*, *Streptosporangium, Nonomuraea,* and genera of *Rhodococcus* (Bredholdt et al. 2007). Rare *Actinobacteria* biodiversity belongs to the genera *Micromonospora* reported in Lake Baikal's water (Terkina et al. 2002). Rare *Actinobacteria* can sustain their lives in extreme ecological habitats, such as caves with high relative humidity, low amounts of organic nutrients, high mineral concentrations, and low temperatures. The genera of *Nocardia* and *Micromonospora* were isolated from El Gola cave, Sinai, Egypt (Mansour 2003). Besides, the Altamira Cave, Cantabria, Spain, was the source of *Nocardia altamirensis* (Jurado et al. 2008). The extreme drought condition of hyper-arid deserts is often associated with lower water activity, excessive radiation, and high-temperature conditions (Horikoshi et al. 2011). The isolation of *Micromonospora and Kribbella* genera from the Sinai Desert, Egypt, was reported by Amin et al. (Tolba et al. 2013). The rare *Actinomycetes* physiology and genetics were poorly understood, while the discovery of these microorganisms may lead to the isolation of novel chemicals (Tiwari and Gupta 2012b). According to the previous reports, the actinobacteria were also isolated from very soil layers, but it decreases gradually with an increase of depth (Takahashi and Omura 2003).

7.2 Rare *Actinomycetes* from the Soil

Actinomycetes can be seen abundantly in all soil types around the globe, such as desert alkaline soil, salt pans, and snowcaps (Agarwal and Mathur 2016).

The rhizosphere soil samples from Madhya Pradesh, India, can be considered important sources for the bioactive pigment-producing *Actinomycetes* (Parmar and Singh 2018). An excellent producer of extracellular xylanases by a moderately thermotolerant *Streptomyces atrovirens* subspecies [strain WJ-2] was isolated from Jeju Island, Korea soil sample (Kim et al. 2016).

The total number of *Actinomycetes* of 1191 was isolated selectively from 10 different soil samples obtained from five regions of Egyptian Governorates, including Qalubiya, Giza, Alexandria, Asuit, and Sinai. The types of soil samples collected from various places in Egypt were sandy, clay, cultivated, and uncultivated soils.

The soil samples numbered 2, 3, 6, 8, 9, and 10 were clay cultivated soil, while those numbered 4, 5, and 7 were sandy soil. The collected soil samples represented that the diversity of rare *Actinomycetes* genera was distributed throughout the study area. The site number 1 recorded highest value of genera diversity, which was followed by sites numbered 2, 3, 5, and 9. The less diversity in genera has occurred on site 7. To enhance the non-*streptomycete Actinomycetes* isolation from the soil sample, increase their relative number on the agar plates, and inhibit the fungal and bacterial competition, different types of selective pretreatments and antibiotics are used which act as a selective condition (Hayakawa 2008). The clay uncultivated soil [170,000 CFU/g soil] found in site number 1 showed highest count of rare *Actinomycetes* followed by sites numbered 9, 8, 3, 6, and 2, while the lowest population of 35,000 CFU/g soil was recorded in sites 10 and 5. The soil sample obtained from site 7 [Barmine cave, sandy soil] showed no rare *Actinomycetes*. The high concentration of salts and ions may be the reason for this (Abd-allah et al. 2012). Hozzein et al. (2008) studies found that with the increase in the concentration of salts and ions in the soil, the rare *Actinomycetes* colony count decreases. The sandy soil has a lower population of rare *Actinomycetes* than clay soil. This can be due to environmental factors such as dryness, higher temperature, root exudates, physical parameters of soil particles, and the absence or presence of root exudates in the rhizosphere (Xu et al. 1996). The high diversity of rare *Actinomycetes* was found in uncultivated soil than cultivated soil and hugely affected by soil properties. According to Tolba et al. (2002), in uncultivated soil the diversity increases more than in the current orchards and apple soil. The most diverse group of microorganisms are found in equilibrated, stabilized biotopes as stated by Burges and Raw (Burges and Raw 1967). This view showed that the organisms of the rhizosphere should be less differentiated than that of root-free soil because the rhizosphere is subjected to root secretions effect and the antagonistic type interactions among microorganisms which lead to dominancy of the selected group of microorganisms. From the sandy, cultivated, and uncultivated clay soil, the members of the genus *Micromonospora* and *Actinomadura* were isolated. In contrast, from cultivated and wild clay soil, organisms of genus *Actinoplanes* were isolated. As the desert temperature reaches 70 °C during the day, *Actinoplanes* sporangia cannot show resistance to desiccation, and so only genera *Nocardioides* of high temperature were isolated from sandy soil. From uncultivated clay soil, the genus *Saccharomonospora* was finally isolated.

7.3 Rare *Actinomycetes* from Aquatic Environments

As physicochemical parameters such as pH, temperature, salinity, and nutrient loads vary in aquatic environments, the distribution of inhabiting microbial communities also vary (El-Gayar et al. 2017). Actinomycetes are predominant in habitats like lakes, rivers, and marine (Subramani and Aalbersberg 2013).

7.3.1 Freshwater Environments

The water and mud from freshwater lakes are the natural sources for a large number of indigenous *Micromonospora*; 10–50% can be isolated from lake sediments of the total population of microbial inhabitants in lake water. About 15% of *Micromonospora* out of 3300 bacteria per mL were from Nebish Lake, and 3600 bacteria per mL with 16% *Micromonospora* from Crystal Lake were reported. The aquatic environments are the indigenous inhabitant of the representatives belonging to *Thermoactinomyces*, *Streptomyces,* and *Rhodococcus* (Cross 1981). Xu and Jiang contemplated populations of *Actinomycete* from 12 lakes. They found that the *Micromonospora* was the dominant genus at the central plateau of Yunnan, China, that revealed 39–89% of *Actinomycetes* in the sediments of the above lakes (Xu and Jiang 1996). Moreover, the second most abundantly found genus in the sediments of the lake was *Streptomyces*. Lake sediments likewise have been accounted for members of rare genera such as *Actinomadura, Micropolyspora, Actinoplanes, Microbispora, Nocardia, Microtetraspora, Rhodococcus, Saccharomonospora, Mycobacterium, Nocardiopsis, Promicromonospora, Streptosporangium, Thermoactinomyces, Thermomonospora, Saccharopolyspora,* and *Thermopolyspora* (Xu and Jiang 1996). Many researchers declared the occurrence of *Micromonospora* in rivers and lake sediments. *Micromonospora* plays a vital role in the turnover of lignin, cellulose, and chitin (Chavan et al. 2013). Ten *Actinomycetes were* isolated from an estuary in India; out of which five were chosen for secondary metabolite screening and reported important antibacterial activity against *Proteus mirabilis* and *Enterobacter aerogenes*. The selected *Streptomyces* sp. ES2 demonstrated potent activity against elected microbes (Al-Ansaria et al. 2019). The rare aquatic *Actinomycetes* were good candidates for exploring new bioactive molecules isolated from Fetzara Lake (Benhadj et al. 2018). The sediments from shrimp ponds are an excellent resource for the isolation of promising *Actinomycetes* (Aly et al. 2019).

7.3.2 Marine Environments

The marine environments have several distinct habitats, including seagrass beds, numerous fish species, mangroves, salt pans, coral reefs, salt marshes, and various communities of microbes (Abdelfattah et al. 2016). Many natural habitats that are underexplored can be considered as an important source for the isolation of rare *Actinomycetes* (Tiwari and Gupta 2012a). Recently unexplored marine environments have currently become a prevalent research area due to the presence of enormous resources. The latest report (Stach and Bull 2005) of the deep-sea sediments microbial diversity has shown that they might possess greater than 1300 diverse actinobacterial taxonomic units and are expected to represent a high percentage of novel genera and species. As compared to terrestrial soils, sea sediments consist of a lesser amount of easily available organic material, with more chitin and cellulose as carbon sources occurring in intricate form. On the other hand, the culture-independent studies revealed that the sea sediment ecosystem contains *Actinomycetes* of broad diversity and various distinctive taxa, which highly differ from their terrestrial counterparts (Stach et al. 2003). Besides, according to culture-dependent studies, marine *Actinomycetes* are found to be ubiquitous in marine sediments (Jensen et al. 2005). In 2005, seawater-obligate marine *Actinomycete* species was isolated, which belongs to genera *Salinispora* (Maldonado et al. 2005); and which was further led by the finding of following genera such as *Solwaraspora, Demequina, Marinispora, Marinactinospora, Lamerjespora, Aeromicrobium, Salinibacterium, Serinicoccus, Sciscionella,* and *Williamsia*. In marine habitats, rare *Actinomycetes* are extensively present (Subramani and Aalbersberg 2012). In addition, until now, very few marine obligate species were isolated (Goodfellow 2010). The habitats such as seawater, marine sediments, symbiotic and mangrooves deep sea sediments (Emery 1969) covered 63.5% of the earth's surface and denotes under explored marine habitat (Butman and Calton 1995). The very first obligatory marine *Actinomycetes* belonged to the novel genus *Salinispora* (Maldonado et al. 2005) was described and then documented due to its strict prerequisite of seawater for growth and development. Another marine actinobacterial genus *Sciscionella* that can withstand growth in up to 13% of high salt concentrations was described by Tian et al. (2009). To date, marine milieu has been used for the identification of more than 14 new actinobacterial genera (Goodfellow and Fiedler 2010; Kurahashi et al. 2010; Chang et al. 2011). Marine ecosystems have become an obvious essential indigenous microflora for *Actinomycetes*.

From 2007 to 2013, from sea sediments, overall 38 new rare *Actinomycete* taxa were identified, belonging to 15 varied actinomycete families. Of these, nine unique genera, such as *Sciscionella, Actinotalea, Marisediminicola, Spinactinospora, Miniimonas,* and *Demequina,* persisted and were reported. In marine sediments, the reported families in that period were *Nocardioidaceae* [4 novel species], *Propionibacteriaceae* [3 novel species], *Streptosporangiaceae* [1 novel species], *Pseudonocardiaceae* [5 novel species], *Nocardiopsaceae* [2 novel species], *Promicromonosporaceae* [2 novel species], *Intrasporangiaceae* [2 novel species], *Micrococcineae* [suborder] [5 novel species], *Nocardiaceae* [2 novel species], *Cellulomonadaceae* [1 novel species], *Beutenbergiaceae* [1 novel species], *Micrococcaceae* [2 novel species], *Micromonosporaceae* [5 novel species], *Microbacteriaceae* [2 novel species], and *Geodermatophilaceae* [1 novel species]. The cultivable types of microbes from marine sediments [0.25%] are substantially greater than seawater [0.001–0.10%] (Amann et al. 1995). From 2007 to 2013, a total of 11 novel, uncommon *Actinomycete* spp. belonging to 6 varied *Actinomycete* families were described from marine water. Among them *Ornithinibacter, Marihabitans,* and *Oceanitalea* were the three new genera described in seawater. The families reported between 2007 and 2013 in seawater were *Nocardioidaceae* [4 novel species], *Intrasporangiaceae* [2 novel species], *Micrococcaceae* [2 novel species], *Propionibacteriaceae* [1 novel species], *Bogoriellaceae* [1 novel species], and *Micrococcineae* [suborder] [1 novel species]. More than two-thirds of the Earth's surface is covered with marine ecosystems. Therefore, the marine habitats are inexhaustible store for the under-used, uncommon, unique *Actinomycetes isolation.*

7.3.3 Symbionts as the Source of Rare Actinomycetes

Symbiotic microorganisms, especially *Actinomycetes* (Schneemann et al. 2010*;* Izumi et al. 2010) from aquatic invertebrates, animals, and plants, are progressively rising for application in the process of drug development (Ganachari et al. 2018; Piel 2009). The symbiotic microbial population is vastly diverse and novel which shows the sequential geographic variation in species composition (Webster and Hill 2001). As a result, very less information is available about the taxonomic relationship of marine symbiotic microorganisms (Friedrich et al. 1999). The widely occurred symbionts are still unculturable, even with significant advancements in cultivation-independent techniques used for studying these bacteria. These methods will have a huge impact on the upcoming chemical analysis of symbionts because many symbionts are still unidentified (Piel 2009). Interestingly, from the sea cucumber, *Holothuria edulis,* two novel families such as *Euzebyaceae* (Kurahashi et al. 2010) and *Iamiaceae* (Kurahashi et al. 2009) in actinobacteria were reported. Between 2007 and 2013, in plants and animals, 17 novel and rarely occurring *Actinomycete* species associated with 11 different families of *Actinomycete* have been reported, respectively. Of these, five new genera belonging to *Phycicola, Labedella, Iamia, Koreibacter,* and *Euzebya* have been reported in marine animals and alga. From 2007 to mid-2013, the families described in marine animals and plants are *Nocardioidaceae* [2 novel species], *Pseudonocardiaceae* [1 novel species], *Microbacteriaceae* [3 novel species], *Tsukamurellaceae* [1 novel species], *Euzebyaceae* [1 novel species], *Micrococcineae* [suborder] [3 novel species], *Micrococcaceae* [1 novel species], *Nocardiopsaceae* [2 novel species], *Alteromonadaceae* [1 novel species], *Micromonosporaceae* [1 novel species], and *Iamiaceae* [1 novel species].

Mangroves are woody plants that are a unique community in subtropical and tropical zones, situated between the transition of the sea and land region (Holguin et al. 2001; Kathiresan and Bingham 2001). The mangroves play a very vital role for many organisms in providing shelter, nourishment, breeding areas, and support a large food web, this is mainly based on the organic matter produced by the decomposition of organisms. The ecosystem of mangrove varies from others because of seasonal flooding and changes in environmental factors such as salinity and nutrient availability that result in metabolic pathway adjustment that could produce very uncommon biomolecules. This idea resulted in the increased exploitation of the resources from microorganisms thriving in the mangrove ecosystem (Long et al. 2005). Fourteen new rare Actinomycete species belonging to seven diverse families are reported in mangrove sediments during 2007 to mid-2013. From those families, *Ilumatobacter* and *Lysinimicrobium,* two novel genera, were reported from mangrove sediments. The reported seven families are Micrococcineae [suborder] [1 novel species], *Micromonosporaceae* [7 novel species], *Promicromonosporaceae* [1 novel species], *Streptosporangiaceae* [2 novel species], *Acidimicrobiaceae* [1 novel species], *Demequinaceae* [1 novel species], and *Thermomonosporaceae* [1 novel species]. A new family of *Actinomycetes* was reported from sediments of mangroves by Hamada et al. (2012). Therefore, sediments of mangrove are very rich resource for the *Actinomycetes* to produce various antimicrobial molecules and enzymes (Subramani and Narayanasamy 2009).

7.4 Rare Actinomycetes from Plants

Several rare *Actinomycetes* were isolated from various parts of the plant (Matsumoto et al. 1998; Shellikeri et al. 2018; Janso and Carter 2010), for the purpose of finding novel microbial resources for regular screening of novel bioactive molecules (Inahashi et al. 2011). For example, spoxazomicin, a new antitrypanosomal compound, was found in the culture broth of a novel endophytic actinomycete *Streptosporangium oxazolinicum* sp. nov. strain K07-0460T (Inahashi et al. 2011). This strain is phylogenetically related to the genus *Streptosporangium* which was isolated from the variety of orchid roots. *Actinophytocola oryzae* GMKU 367T and *Phytohabitans suffuscus* K07-0523T, two novel genera, were also discovered (Inahashi et al. 2010; Indananda et al. 2010). Therefore, plant roots are confirmed to be a potential source for the discovery of new *Actinomycetes*.

The inner tissues of higher plants are relatively an overlooked niche. Previous studies have shown that some actinobacteria form a close association with plants and inhabit their internal tissues. *Streptomyces scabies* and *Frankia* species can penetrate their hosts and establish either endophytic or pathogenic associations (Benson and Silvester 1993; Doumbou et al. 1998). The *Actinomycetes* that occur in the plant tissues and do not damage the plants are called as endophytic actinobacteria (Hallmann et al. 1997). These actinobacteria are comparatively least studied and are likely sources of new natural products for utilization in industry, agriculture, and medicine (Strobel et al. 2004). In recent years, endophytic actinobacteria have gained attention, with increasing reports of isolates from a variety of plant types, including crop plants (rice and wheat, as well as citrus, carrots, potatoes, and tomatoes) (Araujo et al. 2002; Coombs and Franco 2003; Sessitsch et al. 2004; Surette et al. 2003; Tian et al. 2007) and medicinal plants (Taechowisan et al. 2003; Zin et al. 2007). The endophytic culturable actinobacteria from these plant types fell within a narrow species distribution in that *Streptomyces* spp. were the major species, and common genera were *Micromonospora*, *Microbispora*, *Streptosporangium, Nocardioides,* and *Nocardia*.

Relatively, endophytic *Actinomycetes* are a new source for novel species and new bioactive molecules. By using special selective media and techniques, endophytic *Actinomycetes* were isolated and their diversity from medicinal plants in

Xishuangbanna, China, of the tropical rain forests studied (Qin et al. 2009). Thirty-two different genera have shown an unexpected level of diversity. It was the first report of *Saccharopolyspora, Dietzia, Blastococcus, Actinocorallia, Promicromonospora, Oerskovia, Jiangella,* and *Dactylosporangium* species isolation from endophytes (Tiwari and Gupta 2012a).

7.5 Extreme Environments

Actinomycetes, like other microorganisms, adapt and grow in different ecological niches such as deep sea, low temperatures in glaciers, alkaline pH, acidic in the industrial and mine wastewater effluents, extreme desiccation in deserts, high levels of radiation, the high salt concentration in lakes, thermal vents, and high temperatures in hot springs (Mahajan and Balachandran 2017). The microorganisms present in extreme environments have received tremendous interest because of their unique adaptation mechanisms to their harsh environments and also due to the production of unusual compounds (Meklat et al. 2011). Irrespective of the appeal, however, there has been little research carried out on *Actinomycetes* present in extreme habitats: An accidentally discovered pioneer was *Actinopolyspora halophila* (Gochnauer et al. 1975). In recent years, several new *Actinomycetes* were discovered from basic soils and salt in Qinghai and Xinjiang, the People's Republic of China, by research scholars from the Yunnan Institute of Microbiology at Yunnan University (Jiang and Xu 1996; Jiang et al. 2006). They reported a novel family *Yaniaceae*, many new genera of *Streptomonospora, Naxibacter, Jiangella, Myceligenerans,* and a vast number of novel species of the genera *Halomonas, Amycolatopsis, Isoptericola, Citricoccus, Massilia, Nocardia, Microbacterium, Prauserella, Jonesia, Kribbella, Nocardiopsis, Kocuria, Rhodococcus, Marinococcus, Saccharopolyspora, Virgibacillus, Liuella, Saccharomonospora, Nesterenkonia, Sphingomonas,* and *Thermobifida.* Recently, by use of a polyphasic approach, a wide range of halophilic *Actinomycetes* were evaluated and reported by Meklat et al., which revealed the occurrence of a new genus and many new species of the *Nocardiopsis, Actinopolyspora, Streptomonospora, Saccharopolyspora,* and *Saccharomonospora* genera. In addition, their discovery of *Nocardiopsis* strains which had a high number of NRPS genes could be an indicator of great potential *Actinomycetes* of halophilic nature for the production of enormous active biological molecules (Meklat et al. 2011). One new family, eight new genera, and more than 30 new species of alkalophilic and halophilic actinomycetes from alkaline and saline habitats, respectively, were isolated by Kavita Tiwari and Rajinder Gupta (2012a). *Actinomadura, Nocardiopsis,* and *Micromonospora* were isolated from soda salty soils of transient saline lakes in Buryatiya (Lubsanova et al. 2014).

Bacterial populations inhabiting Roopkund Glacier, Himalayan Mountain, were studied, and actinobacteria are the primary class, followed by β-proteobacteria (Rafiq et al. 2017). As these habitats being the rich diversity of culturable actinomycetes, the recent study revealed that the occurrence of novel *Streptomyces* spp.

from the Antarctic regions (Sivalingam et al. 2019). Two novel selected strains ZLN712T and ZLN81T belonging to actinomycetes were isolated from a frozen soil sample collected from the Arctic region (Kamjam et al. 2019). Some actinomycetes were isolated from rhizosphere soil from Lachung, Himalaya region, and exhibit antimicrobial activity (Singh et al. 2019). Bacterial diversity was explored and screened for several hydrolytic enzymes from soil samples of Dras, India, the coldest place after Siberia. Phylogenetic analysis showed that 40 different bacteria were grouped into three major phylum, *Firmicutes, Actinobacteria,* and *Proteobacteria,* differentiated into 17 diverse genera (Rafiq et al. 2017).

Some microbiologically specialized and diverse habitats for the isolation of thermophilic actinomycetes are hot springs, desert soil, thermal industrial wastes, and volcanic eruptions (Agarwal and Mathur 2016). In recent years, due to the economic potential of thermophilic actinomycetes, researchers have shown great interest in them, either in useful biological processes such as biodegradation or in the production of antibiotics and enzymes. Thermoactinomyces belong to the genus *Microbispora, Saccharopolyspora, Thermoactinomyces*, *Streptomyces,* and *Thermomonospora*. Among these, thermophilic actinomycetes of the genus *Thermoactinomyces* have clinical and industrial value. Few *Thermoactinomyces* strains are recognized as effective protease producers (Agarwal and Mathur 2016). Thermotolerant actinobacteria produce various enzymes of hydrolytic action like amylase, cellulase, and xylanase, which show their activity at elevated temperatures of 50–65 °C (Mohammadipanah and Wink 2016).

For the discovery of new actinomycetes and the bioactive compounds, the hot spring sediments are an excellent source (Thawai 2012). The strain YIM 78087T was isolated from a sediment sample collected from Hehua hot spring in Yunnan province, southwest China, during a study on thermophilic actinobacterial resources from hot springs. The isolate YIM 78087T represents a novel species of the genus *Streptomyces* named *Streptomyces calidiresistens* sp. nov. as indicated from the experimental data obtained (Duan et al. 2014). Actinomycetes were collected from the sediments of a hot spring pond located in Krabi and Trang province, Thailand. By studying the morphological properties and 16S rRNA gene sequence analysis, these actinomycetes strains were identified and classified. They belong to the member of genera *Planosporangium, Streptomyces, Micromonospora,* and *Microbispora* (Aly et al. 2019). Overall, 20 samples of hot spring sediment and soil samples from West Anatolia in Turkey were examined for the existence of thermophilic actinomycetes. Strains were grown at a temperature of 55 °C. Sixty-seven thermophilic actinomycete isolates are classified under *Thermoactinomyces sacchari* and *T. thalpophilus* species. The maximum isolates are found to be extracellular protease producers, among them (Agarwal and Mathur 2016). From hot water springs, actinomyces species that produce a remarkable amount of thermostable amylase and cellulose are active at acidic and alkaline pH (Chaudhary and Prabhu 2016).

In two actinomycetes strains, LC2T and LC11T, isolated from a filtration substrate made from Japanese volcanic soil, their taxonomic position was determined using a polyphasic approach (Agarwal and Mathur 2016). From a mud volcano in

India, two thermophilic *Streptosporangium* and *Rhodococcus* were isolated (Mohammadipanah and Wink 2016). It is apparent that volcanic spring is one of the extreme habitats on earth and harbors novel microbes as a source of potential drug leads. Although the knowledge of the *Streptomyces* population in volcanic habitat is sparse, there have been few noteworthy studies on the isolation of natural drugs from volcanic *Streptomyces* (Sivalingam et al. 2019).

7.5.1 Caves

Recently, numerous novel *Actinomycetes* species are isolated from caves, including those inhabited by bats in Spain, Reed Flute Cave in China, the Grotta Dei Cervi Cave in Italy, and a gold mine in Korea (Subramani and Aalbersberg 2013). From cave and cave-related habitats, 47 species in 30 genera of actinobacteria were reported (Rangseekaew and Athom-Aree 2019). From a soil sample collected from a karst cave in China, a novel actinobacterium was isolated. It was a novel species of the genus *Nocardioides* identified based on phenotypic, genotypic, and phylogenetic data (Zhang et al. 2018). From small stones collected from caves and agricultural fields, the novel rare actinomycete genera *Beutenbergia* and *Terrabacter*, respectively, have been reported (Subramani and Aalbersberg 2013). The rock walls of caves are often colonized by Actinobacteria. In a study on the biogeochemical role of actinobacteria, actinobacteria-coated spots on the cave walls in Altamira Cave [Spain] were found to uptake carbon dioxide gas, which exists in abundance in the cave. To dissolve rock and subsequently generate crystals of calcium carbonate, this gas is used by the bacteria (Fang et al. 2017).

In general, caves have high humidity, but they are short of nutrients, luminous intensity, and temperature (Schabereiter-Gurtner et al. 2002). The aforementioned factors may promote antagonism, which augments hydrolytic enzymes and antibiotics production, leading to growth inhibition of other microorganisms (Nakaew et al. 2009). Recently, numerous *Actinomycetes* species have been isolated from the caves including the Grotta Dei Cervi Cave in Italy (Jurado et al. 2005a), a gold mine in Korea (Lee et al. 2000; Lee 2006a, b), the Reed Flute Cave in China (Groth et al. 1999), and a bats-occupied cave in Spain (Jurado et al. 2005b). For the foremost time, the *Spirillospora* and *Nonomuraea* isolation from the soil of a cave was reported by Nakaew et al. (2009) and very rare genera such as *Nonomuraea, Catellatospora, Spirillospora,* and *Micromonospora*. From the caves were isolated members of genera *Actinomadura* and *Saccharopolyspora,* and other rare genera *Actinoplanes, Micromonospora, Microbispora, Nocardia, Gordonia, Nonomuraea,* along with principal genus *Streptomyces* by Niyomvong et al. (2012). Above studies validate that the caves may act as a wide source of novel *Actinomycetes* yielding new compounds.

7.5.2 Actinomycetes from Insects

For discovering novel and new microorganisms, the insect world is another important unexplored environment such as termites, ants, gall midges, and beetles (Kaltenpoth 2009) for practicing fungi culture. Ant workers also protect their fungal gardens through a combination of grooming and weeding (Little et al. 2006), producing their antimicrobials through metapleural gland secretions (Bot et al. 2002), and the use of weed killers. These weed killers produced by symbiotic Actinomycete bacteria (Haeder et al. 2009) are a natural producer of antimicrobials. However, latest evidence suggests that bacteria from the Actinomycete genera are also associated with attine ants; those genera are *Amycolatopsis* and *Streptomyces* (Mueller et al. 2008). Whether the attine ant associated with *Actinomycetes* produces antifungal compounds mainly remains unknown. Therefore, the world of insects is rapidly flourishing as the source for discovering unusual and novel biologically active molecules from *Actinomycetes*.

7.5.3 Other Habitats

From desert soil (Takahashi et al. 1996), *Actinomycetes* of rare genera such as *Nocardia, Saccharothrix, Microbispora, Microtetraspora, Amycolatopsis,* and *Actinomadura* have been isolated successfully. The novel rare *Actinomycetes* genera *Beutenbergia* (Groth et al. 1999) and Terrabacter (Lee et al. 2008) have been reported from small stones collected from caves and agriculture fields respectively. Recently, soils from the nests of solitary wasps and swallow birds (Kumar et al. 2012) and the rare *Actinomycetes* genera such as *Actinomadura, Nocardia, Saccharopolyspora, Thermoactinomyces,* and *Streptosporangium* were isolated.

7.6 Classification of Rare *Actinomycetes*

Among the 18 significant lineages presently documented in the domain *Bacteria, Actinobacteria* is one of the largest units of taxonomy, including five subclasses, six orders, and 14 suborders (Ludwig et al. 2012). The genera of this phylum show a wide diversity in their morphology, physiology, and metabolic capabilities. With the accumulation of knowledge over time, the taxonomy of *Actinobacteria* has evolved significantly. Buchanan (1917) established the order *Actinomycetales,* which belongs to this prokaryotic organisms group.

Based on its position of branching in gene trees of 16S rRNA, the phylum *Actinobacteria* was delineated. However, ambiguity occurs because sequences of rRNA cannot be well differentiated between closely related genera or species. For example, within the family *Streptomycetaceae* the status of taxonomy of

Kitasatospora genus (Omura et al. 1982) has been disputed for many years (Ludwig et al. 2012; Wellington et al. 1992; Zhang et al. 1997), while current details of genetic analysis provided strong confirmation that it should be considered as a separate genus (Girard et al. 2014). A similar type of close relationship does exist between *Salinispora, Micromonospora,* and *Verrucosispora*. For discrimination of closely related genera, rpoB and in recent times ssgB have been used as additional genetic markers (Girard et al. 2013).

In addition, detailed insights into genome evolution and identification of genes specific to organisms at the family and genera level have been provided by the recent massive increase in the availability of information of genome sequence (Kirby 2011). Based on 16S rRNA trees, for the phylum Actinobacteria, an updated status of taxonomy was recently reported (Ludwig et al. 2012). The ranks of the taxonomy of suborders and subclasses were eliminated, and former suborders and subclasses were elevated to levels of orders and classes, respectively, by that update (Gao and Gupta 2012). Actinomycetes are Gram-positive bacteria and have filamentous growth like fungi. They are aerobic and ubiquitous. The DNA of Actinomycetes is rich in G + C content with GC% of 57–75% (Lo et al. 2002). These Gram-positive bacteria have been placed within the phylum Actinobacteria, Class Actinobacteria, subclass Actinobacteridae, and order Actinomycetales, which at present consist of 10 suborders, more than 30 families, and over 160 genera (Chavan et al. 2013). They resemble morphologically with fungi and physiologically with bacteria (Sultan et al. 2002).

According to Bergey's Manual of Systematic Bacteriology, first edition, *Actinobacteria* belonged to the order *Actinomycetales* and was divided into four families *Actinoplanaceae, Mycobacteriacea, Streptomycetaceae,* and *Actinomycetaceae*. With the buildup of information over time, the taxonomy of *Actinobacteria* has considerably evolved. *Actinobacteria* were included separately in the fifth volume in the second edition of Bergey's Manual. The phylum *Actinobacteria* separated into six classes: *Rubrobacteria*, *Actinobacteria, Thermoleophilia, Nitriliruptoria, Acidimicrobiia,* and *Coriobacteriia*. The class *Actinobacteria* subdivided into 16 orders: *Frankiales*, *Actinopolysporales*, *Glycomycetales, Micromonosporales*, *Catenulisporales*, *Actinomycetales, Kineosporiales*, *Jiangellales*, *Bifidobacteriales, Streptosporangiales, Pseudonocardiales*, *Micrococcales*, *Corynebacteriales, Streptomycetales*, *Propionibacteriales*, and *Incertaesedis* (Zhi et al. 2009). The Actinomycetales order is currently limited to the family members of *Actinomycetaceae* (Gao and Gupta 2012).

According to Bergey's Manual, *Archaea and Bacteria,* the phylum *Actinobacteria* includes five classes, 19 orders, 50 families, and 221 genera. However, as many novel taxa are continuously discovered, this listing is certainly unfinished. Based on the 16S rRNA gene, sequence-based groups, and taxon-specific 16S rRNA gene sequences, the class *Actinobacteria* and fundamental taxonomic ranks above the genus level were proposed. This classification showed an apparent change in the classification of *Actinobacteria* above the genus level as it represented that former classifications based on the form and function did not reflect natural relationships. The rank of a phylum has been assigned to *Actinobacteria* because the phylogenetic depth signified by the lineage resembles that of existing species based on its branching position in 16S rRNA gene trees (Barka et al. 2016). Among the 30 significant species currently recognized within the domain Bacteria, the phylum Actinobacteria represents one of the largest phyla. Until October 2016, 6 classes, 18 orders, 14 suborders, 63 families, and 374 genera have been recorded in this phylum.

7.7 Morphological Classification

The morphology and chemotaxonomy are the two main characteristic features considered to define the *Actinobacteria* taxonomy at the species and genus levels. The latter of the above characteristic features principally relates to whole-cell sugar distribution and composition of the cell wall. However, the composition of phospholipid and type of menaquinone might also be considered for enhancement purpose (Labeda 1987). In a special vegetative form of reproduction, mycelial fragmentation can be considered. However, reproduction by forming asexual spores is primarily the lifestyles of mycelial *Actinobacteria. Actinobacteria* show a broad diversity of morphology, differing mainly concerning the structure and appearance of their spores, the absence or presence of a substrate or aerial mycelium, the mycelium's color, and the ability to produce a diffusible form of pigments of melanoid.

7.7.1 Mycelial Morphology

Actinobacteria from a substrate mycelium in both solid-grown and submerged cultures, except for *Sporichthya* sp., produce an aerial form of hyphae that are uprightly initiated on the medium's surface by using holdfasts. However, many differences form aerial hyphae on solid surfaces, primarily for reproductive spores production (Flardh and Buttner 2009; van Dissel et al. 2014). From a germinating spores outgrowth, the substrate mycelium develops that usually is monopodial, which in few exceptional cases of *Actinobacteria* like *Thermoactinomyce* show branching of dichotomous nature (Kalakoutskii and Agre 1976). Alternatively, a large substrate mycelium with rudimentary or no aerial type mycelium is produced by members of the *Micromonosporaceae* family. *Actinobacteria* display various morphologies, including coccus [*Micrococcus*], coccobacillus [*Arthrobacter*], fragmenting hyphae [*Nocardia* spp.], and the ones with highly differentiated and permanent branching mycelia [e.g., *Streptomyces* spp., *Frankia*] (Atlas 1997). On the substrate, Corynebacteria do not produce mycelia at all, while Rhodococci produces filaments of elongated form but not a true mycelium (Locci and Schaal 1980). However, filaments develop at the apex rather than through the extension of the lateral wall in the case of other *Actinobacteria* (Flardh 2003*;* Letek et al. 2008). The development of branched hyphae on substrate breaks to form motile elements with flagella, which is the characteristic of *Actinobacteria* belonging to the genus *Oerskovia* (Prauser et al. 1970). *Rhodococcus* and *Mycobacteria* do not frequently form the hyphae of aerial type (Ochi 1995).

7.7.2 Spore Chain Morphology

In the taxonomy of Actinobacteria*,* spores are extremely important (Locci and Sharples 1984). The preliminary sporulation steps in many oligosporic *Actinobacteria* could be considered as a process of budding because they show property that satisfies the definition of budding in the other bacteria. The substrate and aerial mycelium form spores of single cells or chains of diverse lengths. Spores may occur in special flagellated vesicles [sporangia], in other cases. Therefore, the formation of spores occurs directly on substrate mycelium in genera *Micropolyspora*, *Micromonospora*, and *Thermoactinomycetes* (Cross and Goodfellow 1973), whereas spores develop out of the aerial mycelium in *Strepto-myces.* Motile spores are the characteristic feature of *Actinoplanes* and *Actinosynnema* groups, while unique heat-resistant endospores occur in *Thermoactinomyces* (Cross and Goodfellow 1973). Some other genera of *Actinobacteria* have sclerotia [*Chainia*], synnemas [*Actinosynnem*a], vesicles that contain spores [*Frankia*], or vesicles that are devoid of spores [*Intrasporangium*]. Based on their sporangial morphology, other genera are classified as *Actinoplanes*, *Ampulariella*, *Planomonospora*, *Planobispora*, *Dactylosporangium*, and *Streptosporangium.* The spores of diverse types are found in the Actinomycetes genera. Thus, to characterize the species, the morphology of spores can also be used: they might have spiny, smooth, hairy, rugose, or warty surfaces (Dietz and Mathews 1971).

7.7.3 Spore Chain Length

There exists wide variation from genus to genus in the spores number of every spore chain. The isolated spores are produced by genera *Salinispora*, *Micromonospora*, *Saccharomonospora*, *Thermomonospora*, and *Promicromonospora,* while spores of longitudinal pairs occur in *Microbispora.* Organisms of genera *Sporicthya*, *Saccharopolyspora*, *Actinomadura,* and some *Nocardia* spp. possess short length chain of spores, while the genera *Streptoverticillium, Nocardioides*, *Kitasatospora*, *Nocardia* spp., and *Streptomyces* produce a long length of chains up to 100 spores. Conversely, sporangia are spore-containing bags produced by *Frankia* species. The spore chains of *Streptomycetes* are classified as straight to flexuous [Rectus-

Flexibilis], open loops [Retinaculum-Apertum], open or closed spirals [spira], or verticillate (Pridham et al. 1958).

7.7.4 Based on Melanoid Pigment

Melanins are polymeric with varied molecular structures that are brown or black. They are formed by the oxidative polymerization reaction of phenolic and indolic compounds. Melanins are synthesized by a range of organisms, from humans to bacteria. For a long time, *Actinobacteria* are known for pigments production, depending on the strain, used medium, and culture age, which may be yellow, red-orange, brownish, pink, greenish-brown, distinct brown, black or blue (Lechevalier and Lechevalier 1965). These metabolic polymers are useful in taxonomic studies and are similar to humic substances in soil (Dastager et al. 2006; Manivasagan et al. 2013). In spite of melanins having a role in improving the survival and competitiveness of *Actinobacteria,* they are not indispensable for the growth and development of an organism.

7.8 Chemotaxonomic Classification

Chemotaxonomy is the grouping of organisms according to the similarity in their cellular chemistry based on the distribution of chemical components (Goodfellow and Minnikin 1985; O'Donnell 1988). In this chemotaxonomy, the constituents of cell wall lipids, amino acids, vitamin K2, muramate types, carbohydrates, proteins, and DNAs base composition are considered for grouping the organisms (Goodfellow and O'Donnell 1989; Williams et al. 1989) for gouping the organisms. Chemotaxonomic identification and classification are performed based on information resulting from techniques of chemical fingerprinting of whole organism. The valuable markers of chemotaxonomy that have been reported for the purpose of identification and classification of the *Actinomycetes* are discussed further (Ludwig et al. 2012). As the composition of cell walls differs between the suborders, this characteristic is valuable taxonomically for *Actinobacteria* analysis (Berd 1973).

Particularly, the information about the chemical structure of cell walls peptidoglycan is useful to classify actinomycetes because it promotes discrimination between *Actinobacteria* groups above the genus level. Several differentiating characteristics in relation to their composition and structure of peptidoglycans are identified (Willey et al. 2010). Non-proteinogenic amino acid 2,6-diaminopimelic acid [DAP] present in the cell wall of bacteria of Gram-positive nature is an important chemotaxonomical characteristic. Depending on the genus, the peptidoglycan may be DL or LL [*meso*]-DAP in *Actinobacteria.* By considering DAP isomerism, Lechevalier and Lechevalier had identified nine different chemotypes of the cell wall in *Actinobacteria* (Lechevalier and Lechevalier 1980). On the other hand, diverse *Actinobacteria* groups share the same profile of DAP. For instance, in spite of differences in families and morphologies of *Streptomyces*, *Arachnia*, *Streptoverticillium*, and *Nocardioides* genera, they all share identical chemotype, i.e., chemotype I. Therefore, for assessing diversity in the phenotype of *Actinobacteria,* profiling of DAP and other genotypic or phenotypic criteria should be used (Bouizgarne and Ait Ben Aoumar 2014). Thus, a system was proposed to classify *Actinobacteria* on the basis of both their chemical and morphological characteristics (Lechevalier and Lechevalier 1965).

For the identification of specific *Actinobacteria* genera, patterns of fatty acid in cell are useful indicators of chemotaxonomy (Kroppenstedt 1985). Generally, fatty acids in bacteria have carbon chain length of C2 to over C90, but only C10 to C24 have taxonomic value (Suzuki et al. 1993). In *Actinobacteria,* majorly 3 types of fatty acid profiles have been reported (Kroppenstedt 1985). In bacteria, various isoprenoid quinone types are characterized (Collins et al. 1985), of which menaquinones are found in cell envelopes of actinomycetes (Kroppenstedt 1985; Suzuki et al. 1993; Collins et al. 1985; Collins 1994). Menaquinone analysis has provided valuable information for taxonomical classification of *Streptomyces, Actinomadura* and *Microtetraspora* strains (Kroppenstedt 1985; Collins et al. 1988; Kroppenstedt et al. 1990; Yamada et al. 1982). Additionally, menaquinones of cyclic form occur in *Nocardia* genus members (Goodfellow 1992; Tindall et al. 2006), while cyclic menaquinones with full saturation occur in *Pyrobaculum organotrophum* (Tindall et al. 2006). In the *Actinomycetes'* cytoplasmic membranes, different types of phospholipids are unevenly distributed, which provide information for the identification and classification of genera of Actinomycete (Williams et al. 1989; Goodfellow 1989). On the basis of semi-quantitative analysis of important phospholipid markers present in extracts of whole organism, *Actinobacteria* are classified into five phospholipid groups (Lechevalier 1977; Lechevalier et al. 1977, 1981).

In the identification of *Aeromicrobium* (Yokota and Tamura 1994) and *Dietzia* (Rainey et al. 1995), this classification system was used. It has been reported that the same type of phospholipid occurs in a population of the same genus of *Actinobacteria.* For chemotaxonomy, analysis of the composition of sugar is vital. One of the major constituents of cell envelope of actinomycete is neutral sugars, which is a useful marker of taxonomy at the suprageneric level. *Actinomycetes* can be divided into five groups based on the discontinuous distribution of major diagnostic sugars. The group A species contain galactose and arabinose in the cell wall; group B cell wall has madurose [3-*O*-methyl-D-galactose]; species with no diagnostic sugars are clustered in group C; cell wall of group D species contains xylose and arabinose; rhamnose and galactose are present in the cell wall of group E species (Labeda 1987; Lechevalier and Lechevalier 1970). Additionally, for the classification of some actinomycete taxa, the occurrence of 3-*O*-methyl-rhamnose in *Catellatospora* (Asano et al. 1989) and tyvelose in *Agromyces* (Maltsev et al. 1992) has been reported.

7.9 Molecular Classification

More recently, by the rapid advancement of genome sequencing, the classification of actinomycetes becomes easy by molecular taxonomic data. Notably, based on molecular analysis, recently, some organisms have been reclassified as they were previously placed in inappropriate taxonomic groups (Zhi et al. 2009). Recently, genome sequencing gave the final classification of *Kitasatospora* as a distinct genus within *Streptomycetaceae* (Girard et al. 2013) which provided a solution to a debate of a long time about the relationship of this group with genus *Streptomyces* (Zhang et al. 1997; Girard et al. 2014; Ichikawa et al. 2010; Kim et al. 2004). At present, without genetic analysis based on sequencing the 16S rRNA gene and DNA-DNA hybridization, even genome sequencing a new species cannot be claimed. The criteria of chemical and molecular composition have been used to group the order *Actinomycetales* into 14 suborders: *Actinomycineae*, *Pseudonocardineae*, *Corynebacterineae*, *Propionibacterineae*, *Jiangellineae*, *Actinopolysporineae*, *Kineosporineae*, *Streptomycineae*, *Micromonosporineae*, *Frankineae*, *Glycomycineae*, *Catenulisporineae*, *Micrococcineae*, and *Streptosporangineae* (Euzeby 1997). Moreover, 16S rRNA gene sequencing led to the identification of 130 genera and 39 families. Based on these molecular and chemical criteria, all the groups that were previously assigned to the taxonomic rank of "order" have been recovered as strictly being monophyletic. Still, some paraphyletic groups are found within the rank "suborder."

Berdy (2005) reported that rare actinomycetes produce highly unique, diverse, and rarely complicated compounds with tremendous antibacterial activity and low toxicity. Currently, more than 50 rare actinomycete taxa are reported to produce 2500 bioactive compounds (Fig. 7.1). These bioactive compounds can be used for pharmaceutical and biotechnological applications (Kurtboke 2010). The investigation of secondary metabolites from rare actinomycetes has been less frequent than

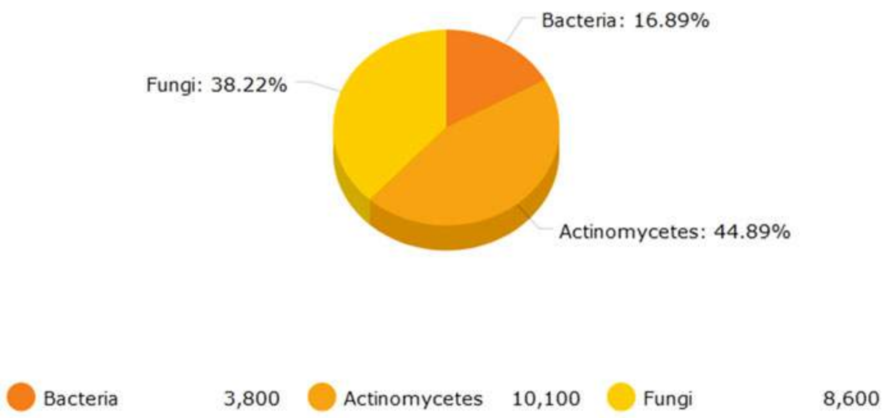

Fig. 7.1 Bioactive compounds of microbial origin

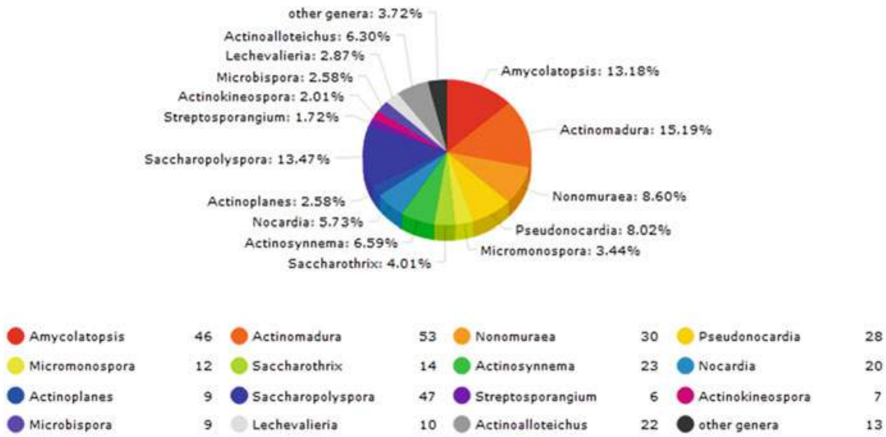

Fig. 7.2 The secondary metabolites of rare actinomycetes

Streptomyces. This has made rare actinomycetes a significant resource for finding new secondary metabolites with biological activity. A number of secondary metabolites discovered from 2008 to 2018 in 21 genera of rare actinomycetes isolated mainly from soil and insects were shown in Fig. 7.2 (Ding et al. 2019).

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Chapter 2
Actinobacteria in Marine Environments

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Abstract The marine environment is one of the significant habitats for exploring novel compounds from diverse microorganisms; among these organisms, marine actinobacteria are considered to be a leading contributor. Recently, imperative advancements have been made in the field of marine microbial ecology with particular emphasis on molecular studies, including 16S rRNA analysis and metagenomics libraries, which have indicated the predominance of actinobacterial diversity in the soil sample. Both culture-dependent and culture-independent approaches have revealed the importance of marine actinobacterial diversity in biomedical science and bioengineering applications.

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The major habitats for marine actinobacteria are the seashore, sea snow, hydrocarbon seeps, saturated brines, cold seeps, and hydrothermal vents. Many reports have shown the presence of epibiont and symbiotic actinobacteria in the marine micro as well as macrofauna. Actinobacteria are unevenly distributed in the marine environment in small but substantial quantities along with the significant levels of biodiversity. The presence of viruses within the marine actinobacteria forms the marine actinophages that have been recognized for their ubiquitous nature. However, the extent of marine actinobacterial biodiversity, distribution, and abundance is still undistinguishable due to fewer reports, intermittent research work, and inappropriate identification methods.

Keywords Actinobacteria · Marine environment · Metagenomics · Biodiversity · Habitats · Epibiont · Symbiotic association

2.1 Introduction

Gram-positive, aerobic, and nonmotile *Actinobacteria* can have a high DNA guanosine-cytosine (GC) base content of 70–80%. According to the 16S rRNA gene phylogeny, they are evolutionarily much more bacterial-like than fungal; although partly due to the filamentous morphology, they were originally considered to be intermediates between bacteria and fungi. Accordingly, members of the phylum *Actinobacteria* are classified as prokaryotes and belong to the order *Actinomycetales*, which have substrate hyphae and form aerial spores and mycelium. The aerial hyphae of actinomycetes tend to produce sporophores, and their structure varies widely. The spore-forming hyphae with aerial mycelium possess enormous lengths compared to substrate mycelium. An additional interesting characteristic feature of the spores is their resistance toward desiccation, and the spores can be viable for long periods. The life process gives resistance to harsh environmental circumstances, such as reduced water availability and nutrient deficiency. Such microorganisms are phenotypically and genetically unique and can be seen in most environments.

2.1.1 Basis and Distribution of Marine Actinomycetes

Actinobacteria or actinomycetes are typically discovered from oceanic sediments, and they occur abundantly within the soils. Their diversity and distribution in the aquatic system has mostly been unrevealed for several years. Many of the researchers have questioned the nativity of the marine actinomycetes due to resistant spores that may have migrated from the terrestrial environments to sea and other aquatic systems.

2.1.2 Actinobacteria in the Marine Environments

Marine microbiology is emerging globally with a discrete focus on secondary metabolite production. Blunt et al. (2007) reported that between 1965 and 2014, more than 25,000 new compounds were discovered in distinct marine organisms in 22 oceanic regions worldwide, including the Indian Ocean and islands; Atlantic Europe and Baltic Sea; South, North, and Central America; Australia; some African countries; and Arctic and Antarctica. Inspired by this, marine actinomycetes have also been explored for the possible ability to produce unique secondary metabolites, and, as the previous reports have revealed, they are the abundant sources for bioactive molecules. These microorganisms hold an exceptional position as significant targets for major screening processes, as their diversity provides support to anticipate that they also have the capability to synthesize various pharmaceutically important molecules and novel secondary metabolites (Ellaiah et al. 2004).

Following the discovery of actinomycin (Lechevalier 1982), bioactive molecules were screened from actinomycetes to produce antitumor agents, commercial bioactive molecules, and desired industrial enzymes (Tanaka and Omura 1990). As much as about two-thirds of the recovered natural metabolites have been derived from these microbes (Takaizawa et al. 1993), among which most of the bioactive compounds have been discovered from *Streptomyces* spp. (Goodfellow and O'Donnell 1993). The produced bioactive molecules have been found to be of major structural interest and essential in promoting the development of novel antibiotic derivatives from their molecular backbone (Sivakumar et al. 2007).

Although the microbial assortment in the terrestrial conditions is intrinsically remarkable, utmost diversity can also be seen in the oceanic environments (Donia and Hamann 2003). It is well-known that about 70% of the Earth exterior is the ocean, from which life was originated. Several research investigations have revealed that in the marine environments, like coral reefs and deep seafloor, the biological diversity is quite high compared to, for example, tropical rainforests (Haefner 2003). This is due to marine ecological circumstances, which are very unusual and different from the terrestrial environment; it can be inferred that actinobacteria from the oceans have possessed in evolution toward different characteristic features than the terrestrial ones (Yaradoddi et al. 2020a; Yaradoddi and Sulochana 2020). Consequently, they could have the potential to produce diverse classes of secondary metabolites. The adaptability of marine actinobacteria toward the extreme and harsh living conditions has resulted from the vast evolutionary range of extreme environments, covering high seabed pressure (upper limit about 1100 atmosphere), anaerobicity, sometimes extreme acidic conditions (pH low, about 2.8), and temperatures close to 0 °C or in the other extreme about 100 °C near the hydrothermal vents on ridges in the middle of the ocean.

The unique conditions are undoubtedly reflected in the metabolic and genetic multiplicity of the marine actinobacteria, which continues immensely to be unknown. Indeed, the marine conditions are almost untapped sources of novel types of actinobacterial diversity and, consequently, the novel metabolites (Stach et al. 2003a; Jensen et al. 2005a; Fiedler et al. 2005; Magarvey et al. 2004). The diversity and distribution of actinomycetes inside the sea have been hugely ignored, and many original marine actinobacteria remain uncharacterized. This gap is created because of limited research work conducted toward exploring marine actinobacteria, whereas the terrestrial actinobacteria are much more utilized for the investigation and production of novel bioactive molecules. Various computational approaches are promoting in understanding the actinobacteria at the gene level to explore novel natural products (Fig. 2.1).

2.2 Origin of Marine Actinobacteria

Marine actinobacteria have remained dormant for several years; actually, these bacteria have been estimated to be migrated as leached dormant spores from soil that are able to survive but not grow (Goodfellow and Williams 1983). Nowadays it is unambiguous that the explicit communities of marine inhabited actinomycetes not only occur in the marine environmental conditions but also contribute by adding diversity within a wide array of actinobacterial taxa (Mincer et al. 2002; Stach et al. 2004). Reports have also indicated that actinomycetes can be recovered from the coastal environments, deep-sea sediments, and mangrove swamps (Sivakumar 2001; Tae et al. 2005), despite the selective techniques used in the cultivation of actinobacteria aimed only at mycelium-producing strains, thus excluding the interesting marine populations such as mycolate actinobacteria (Colquhoun et al. 1998). It has been realized that the marine actinobacteria comprise of novel phenotypes and are undoubtedly different from those recognized to occur in soil. While the biological properties of marine and aquatic actinobacteria continue to be undefined, there is a scope in understanding their ecological roles as terrestrial ones. The terrestrial actinobacteria are involved in degradation of recalcitrant organic compounds, mainly chitin, a biopolymeric material abundant in the ocean. As long as actinobacteria are living inside the sea, which undergoes a significantly diverse ecological circumstances when compared with terrestrial populations, the occurrence of speciation in marine actinobacteria with several exceptional taxa is not surprising. Besides being a wide range of marine actinobacterial multiplicity, it has yet to be described. Researchers must understand the mechanisms of adaptation of the organisms in the ocean that lead to the production of bioactive molecules; there is a need for these interactions to be established.

Marine ecosystems have a substantial actinobacterial diversity, allowing for the extraction of new metabolites and their genes, thereby increasing global awareness to microorganisms in the oceans and their bioactive molecule products. Based on the potential associated with marine actinobacteria, several new molecules in previously unknown configurations have been uncovered (Subramani and Aalbersberg 2012). The intertidal or littoral zone regular changes between exposure to air during low tide and high tide flooding are a unique part of the sea shores and estuary. The zone is also a habitat for actinobacteria, though their communities, biological activity, and genetic capacity have been infinitely little studied, and the niche most likely is arousing curiosity for the discovery of novel genes of biological origin and potential antimicrobial producing strains. However, the biodiversity and bioactive molecule biosynthesis in intertidal sediments have been assessed using cultivation-based methods. The results using genomic fingerprints demonstrated the occurrence of

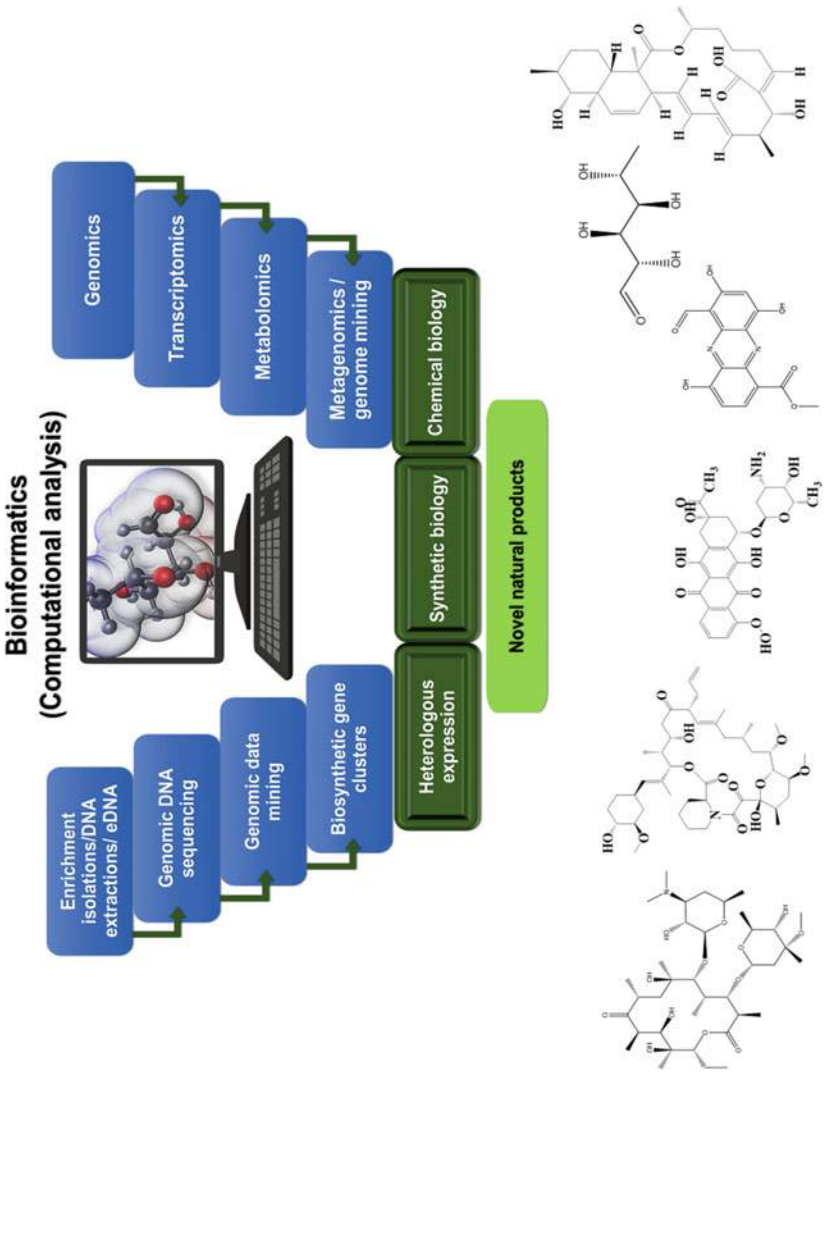

Fig. 2.1 Advanced computational and molecular approaches existing in the identification of novel natural products

high diversity and potential for multiple biosynthetic pathways (Jose and Jha 2017). Furthermore, the 39 km^2 Diu Island (20.71°N 70.98°E) near Saurashtra Peninsula (Gujrat, India) in the Arabian Sea is an area where unique, diverse microorganisms grow, and the diversity and biological capacity of actinobacteria have not been examined. These few examples reveal well that there are many areas in the marine environment that are poorly studied in terms of actinobacteria.

2.2.1 Different Niches of Marine Bacteria

Actinobacteria can be discovered in several free-swimming marine invertebrates and vertebrates, as well as in immobile organisms. These distinguished bacteria are found in marine living beings that produce bioactive molecules. For instance, the puffer fish was recognized as a producer for the potential neurotoxin, tetrodotoxin. However, several current marine/aquatic organisms are also found to be important producers of tetrodotoxin. The ability has often been associated with different prokaryotic bacteria, such as actinomycetes. Puffer fish usually has high levels of toxins in the liver and ovaries, where it also possesses tetrodotoxin-producing actinobacteria. The identified bacteria have been strongly associated with *Nocardiopsis dassonvillei*, which has also been recovered through puffer fish ovaries (Wu et al. 2005).

2.2.2 Actinobacteria in Marine Snow

Marine snow is mostly organic detritus that falls into deeper layers of the water column. Previous investigations (DeLong et al. 1993; Simon et al. 2002) revealed that actinomycetes have not been successfully detected in marine snow through molecular techniques, although cultivation-based methods have been flourishing (Grossart et al. 2004). A total of 10% fraction of actinobacteria was recovered from marine macroaggregates called marine snow. The actinobacteria in the aggregates were connected with competitive interactions, as 80% of the actinobacterial cultures indicated antagonistic effects on the growth of other bacterial cultures utilizing aggregates. The contradictory results of these investigations regarding the existence or lack of actinobacteria in the marine snow were emphasized to be based on the geographical conditions and environmental heterogeneity of marine snow.

2.2.3 Actinobacteria in Sediments

Since, as per the literature, actinomycetes are among the key phyla in marine sediments; several research advancements have been developed in isolation, identification, and classification of actinobacteria as part of the indigenous microbial flora. During the latest decades, various strategies have been successfully developed for isolation and screening for secondary metabolite production, with particular focus on marine actinomycetes (Ma et al. 2009). As a result, novel and unambiguously marine actinomycete genera, *Salinispora* and *Marinophilus*, were successfully defined, which later on led to a significant improvement in culture-dependent discovery of drugs (Jensen et al. 2005b; Newman 2016). The isolation of actinobacteria from samples collected from different sea areas, covering mud, (subtidal) sediments, sponge, ascidian, and different depth series has expanded the identified diversity. Detailed information is available on actinobacterial quantification and classification in different geographical locations and on their biological activity. This process has led to an increase in the number of isolated and classified actinobacteria and discovery of their new bioactive products (Blunt et al. 2011, 2016; Newman and Cragg 2012; Claverías et al. 2015; Stach et al. 2003b; Magarvey et al. 2004). As an example, hypersaline Hamelin Pool stromatolites in Shark Bay (Western Australia) are a structure of organo sedimentary material composed due to microbial metabolic activity. The morphology of living stromatolites is analogous to that of the fossil ones, which can be as old as 3.5 billion years. Examination of microorganisms in these exceptional mat communities showed significant differences between stromatolite structural types, with a cyanobacterial portion of about 5% being lower than expected, while an actinobacterial abundance was approximately 14% with the average sequence identity of 95.5% to the closest relatives in databases. Actinomycetes appeared to be ubiquitous in stromatolites under marine environmental conditions (Papineau et al. 2005).

2.2.4 Association with Fauna

Bioactive molecules from the sea can also be derived from fauna, sponges, and marine invertebrates, that is, from sessile organisms. Site-bound organisms require an effective mixture of chemical defense molecules. In particular, the sponges are considered to be abundant sources of new metabolites (Hill 2004). They are associated with sophisticated bacterial communities within tissues. Bioactive secondary metabolite-producing actinobacteria are widespread among these microbial communities, which comprise a wide variety of sponge-specific lineages that include actinobacteria from the genera *Theonella*, *Rhopaloeides*, *Aplysina*, *Xestospongia*, *Gordonia*, *Micrococcus*, *Brachybacterium*, *Salinospora*, *Micromonospora*, *Actinoplanes*, *Streptomyces*, and many inadequately characterized and uncultured bacterial clones (Hentschel et al. 2002; Montalvo et al. 2005; Kim et al. 2005).

2.2.5 Deep Subfloor Biosphere

The fascinating ecology of the seafloor and its sediments and the evolution of microbial communities reveal the abundance of bacteria, archaea, fungi, and viruses in extreme environments at sea depths down to 5500 m and even below (Orsi 2018; Walsh et al. 2016). The compiled data from 65 studies showed that bacteria and archaea in the subseafloor have almost the same abundances. Both microbial groups decreased with increasing depth, bacteria more than archaea (Lloyd et al. 2013). The species richness and genetic diversity of actinobacteria also varied, as sediment depth increased below the seafloor at 3814 m so that diversity shifted toward dominance, while each sediment section had distinct characteristic phylogeny, that is, the actinobacterial genetic relatedness in sediment sections collected 5–46 cm below the seafloor was different. Actinobacteria were most closely related to *Corynebacterineae*, *Frankineae*, and *Streptomycineae*, though only 9% of the operational taxonomic unit groups (OTUs) showed 99–100% homology to cultivated actinobacteria; the rest had 94–98% homology (Stach et al. 2003b). In the vertical microbial diversity profile from sea surface down to subseafloor sediments, the quantities of *Actinobacteria*, *Planctomycetes*, and *Firmicutes* OTUs were among the most abundant in water columns. OTUs, which were abundant in deep subseafloor sediments, were often common in shallow sediments and were also observed at low concentrations in the water column, suggesting that they are ultimately seeded from the water column (Walsh et al. 2016). Since only 7 actinobacterial strains could be isolated from subseafloor sediments out of 194 cultivated (southwestern Sea of Okhotsk) (Inagaki et al. 2003), while 16S rDNA sequencing has revealed a much higher diversity and abundance (Stach et al. 2003b), it can be concluded that the greatest part of the metabolic divergence and bioactivity of subfloor biosphere actinobacteria is yet to be discovered.

2.2.6 Methane-Hydrate-Associated Sediments

Actinomycetes contributed as much as 40% of all sequences present in methane-hydrate-associated sediment clones in Nankai Trough, indicating that actinobacteria may cover a remarkable portion of biodiversity in particular geographical extreme sites (Reed et al. 2002). Actinomycetes have spread widely to the marine ecosystem in a little but important portions of genetic multiplicity. Apart from actinomycetes, the oceans are also occupied by different groups of viruses (Suttle 2005), and the ubiquitous occurrence of the actinomycetes has also appeared in the existence of actinophages in the marine environment (Kurtböke 2005). The profusion and degree of actinomycete diversity in various biogeographical locations remains unclear; this is due to lower sampling rates. Further, the identification of actinomycetes by fragmented biased methods has not been clearly described (Suttle 2005; Kurtböke 2005).

2.3 Marine Actinobacteria in Phytopathogen Control

In recent decades, the major focus in the agricultural sector has been on pollution, which is usually released through widespread use of highly hazardous agrochemicals, mainly pesticides (Rai et al. 2011; Prévost et al. 2006). Meanwhile, in the 1970s, in addition to the hazardous effects on the public health conditions, over a period of time-continuous exposure toward the pesticides has led to progress in phytopathogen tolerance (Aktar et al. 2009). The occurrence of pathogenic infections in agricultural crops in the global economy position is relentless; both academies and industry have improved their studies in search of solutions to the present issue.

Bacterial cells of both beneficial and also pathogenic strains were identified as social populations, which are capable to control their gene expression in the density-based pathway, the mechanism called as quorum sensing (QS) (Helman and Chernin 2015). Quorum sensing controls the biological mechanisms associated with metabolism, growth, and virulence among bacterial cells by synthesizing signaling molecules, which intensify the concentration with respect to an increase in cell numbers (Grandclément et al. 2016). When the amount of the molecules attains a particular threshold, unlikely signal transduction cascades are stimulated as a result of changes in gene expression, which includes a pathogenic effect. The QS dictates the expression of various virulence characters, and several plant pathogens are dependent on this type of system to induce disease in its host plant (Andersen et al. 2010; Barnard et al. 2007). For instance, it is a well-known fact that quorum sensing system regulates toxoflavin biosynthesis in several members of *Burkholderia* species (specifically among the *Burkholderia glumae*) and, thus, phytotoxin can be recognized as a critical pathogenic factor in wilt disease affecting the plant vascular system and in rice rot disease causing black lesions (Kim et al. 2004). To control this problem, there are several antagonistic compounds, mainly antibiotics, which can be obtained from microorganisms. Thus, microbes are mainly recognized as a chief source of antimicrobial compounds that can be used against phytopathogens of agricultural crops.

To date, the most powerful source of such antibiotic-producing microorganisms has been the terrestrial environment (Sulochana et al. 2014a, b). However, microorganisms from seas have also been documented to be a vital basis for bioactive compounds in the future due to their ability to control these phytopathogens (Ma et al. 2009; Blunt et al. 2016). Furthermore, marine bacteria belonging to phylum *Actinobacteria* have been identified as one of the most imperative species cluster with immense biotechnological applications (Blunt et al. 2016; Shellikeri et al. 2018; Yaradoddi et al. 2020b), accordingly contributing by increasing the supply of novel bioactive compounds (Newman 2016). The metabolites from the marine origin have become a model for the advancements in putative antimicrobial and insecticidal compounds and, thus, they have turned to be an excellent candidate in agrochemical production (Blunt et al. 2011; Newman and Cragg 2012). For example, concerning to kasugamycin hydrochloride, it is a general antifungal agent used against the *Magnaporthe grisea* and a potential antibacterial agent against *Burkholderia glumae* (Yoshii et al. 2012). These secondary metabolites were initially recovered from the terrestrial actinobacterium *Streptomyces kasugaensis*, and afterward it was also extracted from the marine strain *Streptomyces rutgersensis* subsp. *gulangyunensis* (Betancur et al. 2017).

An approach of the therapeutic value of antibiotics can be ascribed toward in vivo bacterial growth inhibition once antibiotic concentrations surpass the minimum inhibitory concentration (MIC). Besides, although the concentration is lower than the MIC, it can still be able to reduce the growth activity and also the expression of different bacterial virulence factors, diminishing possible effects of the pathogenic organisms on causing the disease. The specific action of antibiotics known as sub-MIC effects, further compounds that are used in quorum quenching activities are called as quorum quenching compounds (QQC) (Helman and Chernin 2015).

The QQC have been applied for the inhibition of the expression of virulence factors of the phytopathogens. There are different mechanisms directed to the biosynthesis of enzymes, which lead to the interference with virulence factor signaling. Inhibiting enzymes can interfere with the signaling molecule biosynthesis at the transcriptional level, or the enzymes may inhibit receptor activation by producing quenching compounds (Helman and Chernin 2015). Numerous existing research outcomes demonstrate the ability of bacterial strains to inhibit QS systems of phytopathogenic strains. For example, several species from the genus *Streptomyces* encompass potential of inhibition against the various QS-controlled virulence factor expression in *Pectobacterium carotovorum*. The inhibition occurs by synthesizing several bioactive compounds that have been recognized as containing piericidin A and glucopiericidin A, indicating that the compounds have potential for biocontrol of plant pathogens (Kang et al. 2016). The molecules extracted from marine territory microorganisms could be valuable, when appropriately used as bioactive agents in quorum quenching to prevent pathogenic bacterial communication and to lower the injury to the host (Kalia 2013). Furthermore, *N*-amido–αproline and the linear dipeptide (proline, glycine) produced by actinobacterium in aquatic sponge presented preventing actions upon quorum sensing and facilitated the adverse influences of *Pseudomonas aeruginosa* (Naik et al. 2013).

2.4 Marine Bacterial Cultures

Several conventionally used cultivation media and their derivatives are available for cultivating actinobacteria from terrestrial environments, such as starch-casein-KNO_3 agar, actinomycete-isolation agar, glycerol-arginine agar, tryptone-yeast extract-glucose agar, tryptone-soy agar, glucose-yeast extract agar, and humic acid-vitamins agar (Suutari et al. 2002; Maldonado et al. 2005). Marine bacteria have typically been cultivated on marine agar (ZoBell 1946). As these media contain quite high concentrations of organic substrates and select microorganisms that grow rapidly to high densities under rich nutritional conditions, later especially in marine environments, the growth media development has focused more on low-nutrient substrates.

Media with low nutrient concentrations represent the composition of the marine environment. Among the first cultivation techniques developed were dilution cultures combined with flow cytometry, in which marine bacteria were diluted and then cultivated in seawater based-media (Button et al. 1993). The most probable number (MPN) cultivation on mineral media with different compositions was used to quantify Mediterranean sapropel bacteria (Süß et al. 2004). Further, high-throughput cultivation in small quantities (extinction culturing) under low substrate conditions on microtiter plates was developed to improve screening efficiency by mimicking nutrient concentrations in situ (Connon and Giovannoni 2002). End point dilution using microtiter plates and dilute growth medium, such as diluted nutrient broth, combined with automated cell array and imaging were used successfully to isolate novel marine bacteria (Janssen et al. 2002; Keller and Zengler 2004; Mincer et al. 2002; Rappe et al. 2002). Gel microcapsules were developed to encapsulate and cultivate individual cells under low nutrient conditions, and growth was monitored by flow cytometry until the microcolonies could be sorted individually into dishes with selective growth medium (Zengler et al. 2002, 2005; Toledo et al. 2006). Moreover, diffusion chambers were designed to simulate marine environmental conditions for bacterial cultivation (Kaeberlein et al. 2002). Incubation times were extended up to 6 weeks and even longer to allow growth of slow-growing microorganisms (Keller and Zengler 2004; Mincer et al. 2002; Toledo et al. 2006; Gontang et al. 2007).

Alongside media development, selective microbial isolation methods were improved. The practices include, for example, use of antibiotics with various carbon sources; $K_2Cr_2O_7$ to inhibit fungal growth; nalidixic acid to prevent the growth of fast-growing Gram-negative bacteria; and cAMP and acyl homoserine lactone supplements. Sample pretreatment was also developed, such as heat shock enrichment for spore-forming bacteria (Maldonado et al. 2005; Mincer et al. 2002; Gontang et al. 2007; Zhang et al. 2006; Bruns et al. 2002). Finally, the drying wet intertidal sediment overnight, followed by stamping onto various agar media, resulted in the isolation of 65.6% of actinobacterial strains, with the remainder of the isolates belonging to the class *Bacilli* (Gontang et al. 2007). The various approaches outlined above have significantly improved the cultivability of previously uncultivated marine bacteria.

2.4.1 Antimicrobial Actions of the Extracts

Various in vitro screening methods are available to examine antimicrobial susceptibility. Among the most commonly used bioassays are diffusion methods, including agar disk diffusion, agar well diffusion, and agar plug diffusion methods, as well as antimicrobial gradient, cross streak, and poisoned food methods. The agar disk diffusion method is simple to perform and allows large series of antimicrobials and microorganisms to be examined, and the results are easily interpreted. Thus by testing the antibiogram one can measure the susceptibility results to classify microorganisms to resitant, intermediate, and susceptible. However, the method does not distinguish microbicidal or microbiostatic effects and cannot really be used to evaluate the minimum inhibitory concentration (MIC). An antimicrobial gradient method is required for MIC determination (Balouiri et al. 2016). A modification of these methods, the direct confrontation assay, has been successfully used to evaluate the antibacterial activity of marine actinobacteria strains against *Burkholderia* species. The in vitro antagonism assay originally developed to test for fungal growth inhibition by soil actinomycetes has also been used successfully to measure the antifungal activity of marine bacteria (Betancur et al. 2017; Crawford et al. 1993).

Organic extracts of marine bacterial strains were used to survey their antibacterial activity against *Burkholderia* pathogens by the diffusion method. After cultivating the marine bacteria in 100 mL of tryptone-soy broth, the liquid phase was separated by centrifugation and sterile filtration (0.22μm), followed by liquid extraction with ethyl acetate. The antibacterial activity of the concentrated extract was determined using a diffusion test on a microtiter plate. *Burkholderia* sp. was cultivated in King B medium, followed by the dilution of 30μL in 200μL of the same medium using microtiter plate. The organic extract (500μg) and 5% DMSO (30μL) were added, and the plate was incubated for 24 h. In the absence of *Burkholderia* sp. growth, the extract was evaluated to be positive for antibacterial agents. *Burkholderia* sp. was not inoculated into the negative control, and gentamicin (0.2μg/mL) was added to the positive control as per the report (Balouiri et al. 2016; Betancur et al. 2017). Besides being potential producers of antibiotics, actinomycetes are susceptible to a few important antibiotics as listed (Table 2.1).

The activity of marine bacterial extracts against fungi was also examined. Fungal cultures on potato-dextrose agar plates were collected in 0.85% aqueous NaCl solution, and the suspension was inoculated into PDA (2 mL) in a well. The bacterial extract (500μg) dissolved in 5% DMSO (30μL) was added, and after 96-h cultivation, fungal growth was evaluated. Positive controls contained clotrimazole (5μL of 1% solution), and fungi was not inoculated to the negative controls (Betancur et al. 2017).

Table 2.1 Effective concentration of antibiotics on actinobacteria

Name of the antibiotics	Concentration per mL
Erythromycin	15 and 30μg
Aureomycin	30μg
Gentamicin sulfate	10μg
Kanamycin	15μg
Amikacin	30μg
Chloramphenicol	30μg
Novobiocin	5 and 30μg
Ciprofloxaci	10μg
Penicillin G	10 U
Tetracycline	10 and 30μg
Vancomycin	10μg

2.4.2 Marine Strain Quorum Quenching actions

A plate assay for disc diffusion to screen for antagonists of quorum sensing (QS) signals has been developed and proven to be suitable for marine bacteria as well (Betancur et al. 2017; McLean et al. 2004; Tello et al. 2012). The biosensor indicator *Chromobacterium violaceum* ATCC31532 synthesizing acylated homoserine lactones (AHLs) was inoculated on agar plates, where discs (diameter 5 mm) with the marine bacterial extract (300μg) were placed. The QS inhibition affects AHL-related signaling. After 24-h incubation, quenching molecules were assessed for the lack of production of purple violacein pigment in the discs surrounding, while the occurrence of growth inhibition was judged to be due to antibacterials. In positive controls, 4-hydroxybenzaldehyde (200μg) was added to the disc, and in negative controls, 300μL of DMSO (5%) was added.

2.5 Future Prospective

Microbes inherently possess unique biotechnologically important secondary metabolites (Jayachandra et al. 2013a, b; Anil Kumar et al. 2010). Extensive investigations have been carried out in screening the terrestrial ecosystem, and a large number of actinobacteria have already been explored for the production of interesting bioactive compounds (Mohan et al. 2015a, b). However, much research is still required to dig up coastal regions, slat pan, sponge, salt marshes, and other marine environments as sources of novel marine microorganisms, mainly marine actinobacteria. Marine actinobacteria have the ability to thrive well at a high concentration of salinity, pressure, and pH and thus improve the possibilities of using such microbes in industrial applications, as these industrial processes usually operate at relatively high temperatures, pH, and pressures. Most excitingly, these actinobacteria from marine origin have gained enormous potential for sustenance at adverse environments. Marine organisms could have a remarkable hidden genome with efficient novel genes that would only be expressed if specific substrates were provided.

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Chapter 4
Extremophilic Actinobacteria

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Abstract In nature, we can see many hostile or extreme environments, as these environments have made life more difficult to survive. Harsh environments can be designated as any considerably high change in the extent of chemical (pH, water content, organic solvents, and salt concentration) or physical variations (osmotic pressure, temperature, pressure, and radiation). Extremophilic organisms are rare

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organisms that can thrive well in these adverse physicochemical conditions. The discovery of novel actinobacterial species may lead to the recovery of new secondary metabolites. In another sense, the metabolites from the extremophilic actinobacteria have received immense value in harsh industrial applications. Extremophilic actinobacteria can be classified into thermophilic, psychrophilic, barophilic, acidophilic, alkaliphilic, halophilic, osmophilic, saprophytic, and xerophilic based upon their inherent properties. Apart from these extremophilic actinobacteria, there is a particular category of extreme tolerant actinobacteria in various environments. However, lots of research work needs to be carried out in the exploration of these groups of actinobacteria—both extremophilic and extreme tolerant actinobacterial communities' genomes inherently have novel potential bioactive compounds. However, the only fraction of the diversity of the extremophilic or extreme tolerant actinobacteria is known, but they have got enormous potential.

Keywords Extremophilic actinobacteria · Temperature · Pressure · Radiation · Acidophilic · Alkaliphilic actinobacteria

4.1 Introduction

4.1.1 Major Sources of Extremophilic Actinobacteria

Numerous environments could be known as extreme, moreover concerning chemical (salinity, pH, water content) or physical constraints (pressure, temperature, radiation) (Bull 2011). Organisms living in such an environment are known as extremophiles; these extremophilic organisms prefer to grow in the wide ranges of these physicochemical parameters (Yaradoddi et al. 2020a; Yaradoddi and Sulochana 2020). Despite these unique characteristic features, numerous microorganisms, denoted as extremotrophs, can able to grow; however, not basically adjusted despite the extreme environmental conditions such sas nutrient-depleted situations, those organisms can be considered as oligotrophs instead oligophile (Bull 2011). A number of *Actinobacteria* are isolated from a total range of extreme conditions.

The existence of alkaliphilic, acid-tolerant, thermotolerant, psychrotolerant, alkali-tolerant, halotolerant, xerophilous, and halo alkali-tolerant *Actinobacteria* has been described (Lubsanova et al. 2014). The novel chemodiversity is extra likely to be in rarely cultured strains. Consequently, the multiplicity among the extreme biosphere could help to address the challenges in rediscovering earlier known secondary metabolites to a significant period of time, because of this motive, exploration of the flourishing *Actinobacteria* in harsh conditions in recovering novel strains with immense industrial value. Though several widespread types of research were employed in the exploration of bacterial diversity, especially in the arid ecosystems, the multiplicity of *Actinobacteria* by such environmental conditions was not wholly investigated (Okoro et al. 2009).

4.2 Arid Niche and Subsistence of Biogeographical Barriers

Arid habitats encompass huge continental environment (which covers about 30% among the Earth area; within that, 7% is hyperarid) that is water proscribed. These arid or dry regions known as biomes by means of a ratio of average yearly rainfall denoted annual disappearance lower than 0.05 and slightly below 0.002 for hyperarid regions (Bull 2011). The extreme dried and aridity sorroundings in higher arid deserts is often acquired by higher temperatures, nutrient depletion, lower a_w (water activity), and prevailing radiation, whereas in some other ecosystems, lower temperature, pH, high salinity, higher metal concentration such as sulfate or nitrate and inorganic oxidant anions can be seen under arid regions (Koeberl et al. 2011).

Among all, the inaccessibility of aquatic resources and nutrients is the prime restraining factor for biological activity under arid and semiarid habitats (Saul-Tcherkas et al. 2013). Bacteria present in low water activity ecosystems inevitability of distribution higher energy to collect at a definite quantity of water and also the important robust bacteria typically arise, a condition of hydrobiosis as soon as a_w (water activity) is concentrated about 0.88 a_w, wherein the cells detained just before metabolize, though, persist workable (Connon et al. 2007; Yaradoddi et al. 2020b). Bacteria that thrive well in arid ecosystems can adjust to drought condition yet water is essential for their physiological requirements. Utmost occurs adjoining near mineral soils mainly halites, gypsum, or quartz; by spreading, a little water surrounds within mineral soils adequate for the bacterial growth and activity (Azua-Bustos et al. 2012).

Arid or dry zones are the interface alongside of the vegetated semi-arid regions, that also contains biologically infertile hyper or extreme arid desert ecosystems (Neilson et al. 2012). These regions harbor various untapped thermophilic, xerophilic, alkaliphilic, and halophilic *Actinobacteria* producing novel bioactive compounds. Adapting potential new techniques can lead toward the detection of culturable bacterial communities in deserts that were hypothetical to be infertile (Koeberl et al. 2011). The desert ecosystems are unique environments to tap the novel extremotrophs or extremophilic strains of *Actinomycetes,* they can be explored to yield new metabolites, *Actinobacteria* have possessed tolerance to desiccation, and solute stress among bacteria and these organisms were reported from the various antagonistic environment such as arid or hyper arids desert, which are supposed to be similar to habitats on Mars. However, high levels of propagation and that produce 0.5 a_w are described. *Actinobacteria* especially non-halophilic actinomycetes are generally improbable to be metabolically active beneath 0.8 a_w, but they might be ecologically active in water-suppressed microhabitats in soil that comprise water activity slightly higher than this value (Stevenson and Hallsworth 2014). In spite of the different geographical range of arid environments, a very minute is familiar in relation to the bacterial communities of these ecosystems with diverse metabolic activities. As for this concern, several reports are accessible in relation to the isolation, screening, and environmental diversity of rare actinomycetes inhabited in the desert habitat (Harwani 2013). In addition to this, habitats that alternate to the soils are besides deliberated as the novel basis in water-scarce conditions (Azua-Bustos et al. 2012).

4.3 Xerophiles Recovered Under Arid Environments

The actinomycetes recovered under extreme warm or acidic environments using hyper radiation or aridity situations (like a desert and new arid ecosystems) are inclined toward characteristically deepest genera of actinomycetes (Rubrobacteridae, Acidimicrobidae). The higher dry state of deserts has been one of the most dynamic environments for the progression of DNA repair mechanisms, which has produced tolerance toward the ionizing radiation (Gamma and UV), distinguished by numerous desert-based *Actinobacteria* (Makarova et al. 2001). The most resilient genera from such environments are strains of *Geodermatophilus* and *Deinococcus* that can resist up to 30 Gy of radiation. Members of this genus are not so far isolated from the non-arid soil, even employing radiation treatments. The xerophilic *Actinobacteria*, *G. siccatus*, and *Geodermatophilus arenarius* were recovered from Sahara deserts in Chad (Montero-Calasanz et al. 2013). Another important member of the genera *Geodermatophilus* has been reported from Negev Desert soil, and *Actinoplanes* and *Streptomyces* strains were recovered from Mojave Desert soil and California-Nevada border, through selective chemoattractants (Kurapova et al. 2012). The *Geodermatophilaceae* comprises two other genera such as *Modestobacter* and *Blastococcus*, which thrive well in water and nutrient limiting conditions; *Geodermatophilus* chooses dry soils as usual environments among 15 species designated in this genus; at least nine species are recovered from the desert's region (Euzeby 2015). In contrast, *Modestobacter* and *Blastococcus* are occupied in rock surfaces. Apart from this actinobacterium which was discovered from the desert ecosystem in Egypt, *Citricoccus alkalitolerans* was designated as alkali tolerant, and maximum growth can be seen at pH 8.0–9.0 (Li et al. 2005a). New strains of the nonsporulating actinomycetes *Mycetocola manganoxydans* which have capability to bring oxidation of manganese ions were recovered within Takalime Desert (Luo et al. 2012). Associated with the *Terra bacteria* genera are also categorized by its adaptation to the radiation, high salinity, and desiccation. Concerning the members of the genera *Streptomyces*, mainly *Streptomyces deserti* initially reported under hyperarid Atacama Desert can be seen in arid habitats (Santhanam et al. 2013); *Streptomyces bullii* was from hyperarid Atacama Desert, and the moderate thermophilic *Streptomyces* sp. 315 are xerotolerant in Mongolia Desert soil (Kurapova et al. 2012).

Apart from the *Streptomyces*, strains belonging to *Saccharothrix*, *Strepto sporangium*, *Cellulomonas*, and *Micromonospora* were isolated from the Qinghai-Tibet Plateau (Ding et al. 2013a), whereas *Actinomadura*, *Nocardiopsis*, and *Micromonospora* were recovered from soda saline soils of ephemeral salt lakes in Buryatiya (Lubsanova et al. 2014). Thermophilic and thermotolerant actinomycetes can be seen much abundantly, sometime beyond that of the neutrophilic forms in Mongolia Desert soil. Other members of the *Actinomadura*, *Streptomyces*, *Streptosporangium*, and also *Micromonospora* are utmost extensively spread thermoresistant species in deserts soils. Numerous members of *Streptomyces*, which belonged to actinobacterial genera *Nocardia*, *Micromonospora*, *Saccharopolyspora*, *Nonomuraea*, and *Nocardiopsis*, were also reported from the Arabian Sea, solar salterns of the Bay of Bengal, and inland surrounding the Sambhar Salt Lake (Jose and Jebakumar 2012). However, surprisingly it has been revealed that *Actinobacteria* in desert soil land dominated 20.7%, whereas agricultural soil comprises 4.6% relatively in poorer quantity in farmland when compared with desert ecosystem (Ding et al. 2013b). Especially concerning to the genera *Rhodococcus*, an *Actinobacteria* has dominated in desert soil. More specifically, tolerant to the salinity (*Actinobacteria* obtained using saline soil of the infertile territories), higher temperature, alkaline situation, and drought have been practically proven. It was understood that all the halotolerant strains (strains can able to grow up to 5% NaCl), unlike non-halophilic isolates, have the potential to grow in medium with pH 10, whereas non-halophilic strains do not have such potentials. In this prospect, a moderate thermophilic strain of *Streptomyces sp.* which was recovered from desert soil was practically demonstrated as a xerotolerant. The halotolerant and alkaliphile *Streptomyces aburaviensis* reported from the salt arid region of Kutch in India have an antagonistic effect against Gram-positive bacteria. The strain was able to grow slowly at 15% NaCl and in neutral pH, whereas the maximum growth was observed in 5–10% NaCl and at pH 9 (Thumar et al. 2010). The mesophilic actinobacteria from the Mongolian desert soil habitat belonged to the genera *Streptomyces*, while thermotolerant organisms belonged to the genera *Actinomadura*, *Micromonospora*, and *Streptosporangium*. Plant associated with *Actinobacteria* from desert origin also exists. Concerning to drought-tolerant endophytic *Actinobacteria*, *S. olivaceus* DE10, *S. geysiriensis* DE27, and *Streptomyces coelicolor* DE07 were isolated from plants of arid and drought-affected areas. These strains demonstrated plant growth promotion (PGP) activity similar to other bacterial (Sulochana et al. 2014a, b) and inherent tolerance to water stress (−0.05 to −0.73 MPa) (Yandigeri et al. 2012). Roughly extremophile bacteria, mainly *Deinococcus-Thermus*, *Rubellimicrobium*, and *Acidimicrobium* intensely have below stated agricultural use.

In contrast to this, original desert bacteria can enhance plant health in desert agro-based ecosystems. *Actinobacteria* in lower water activity regions of Antarctica (comparable condition in desert habitat) were pronounced. The bacterial multiplicity of Lake Hodgson and the Antarctic Peninsula comprises 11.6% *Chloroflexi*, 20.2% *Plantomycetes*, 21% *Proteobacteria*, and 23% *Actinobacteria* (Pearce et al. 2013). Although from Dry Valley soil of Antarctic, the *Actinobacteria* (26%), *Acidobacteria* (16%), and *Cyanobacteria* (13%) belonged to the majority of the recognized as resident bacteria (Smith et al. 2006). The culture-independent evaluation of different domain bacterial variety in the cold desert of the McKelvey Valley established which is very specific communities to be colonized in discrete lithic habitats can be seen concurrently among this ecosystem. In spite of relatively barren soil, the maximum part of variety was found in chasmoliths and endoliths of sand stone. The complete phylum level structures of numerous arid regions are indicated to be dominated by the *Actinobacteria*. They were also disclosed to be most abundant phyla about 72–88% from areas of Atacama Desert (Crits-Christoph et al. 2013), whereas in other dry area, they are among the three predominant phyla (generally along with the *Proteobacteria* and *Firmicutes*) in the deserted soil of the Aridic Calcisols in Kazakhstan (Kutovaya et al. 2015), alkaline-saline (Keshri et al. 2013), deserts comprising shrub root zone (Steven et al. 2012), and elevated deserts (Lynch et al. 2014). The dominant genera of *Actinobacteria* are not yet described as per metagenomic studies in concern, except the studies focused on haloalkaline semiarid regions in India, wherein two-thirds of the *Actinobacteria* clones were identified among order *Rubrobacterales* (Keshri et al. 2013).

4.4 Mixed Extreme Environments

Among other extreme environments, brief structures of two ecosystems detailed here are water polluted sites and inland. The inland waters comprised of salt and soda lakes could be unseemly on primary encounter of freshwater lakes, nevertheless the circumstantial attention on freshwater lakes turns around their high radiation exposure and their oligotrophy. Recently, there have been distinctive freshwater bacteria identified (Zwart et al. 2002); mostly, the predominant category belonged to *Actinobacteria* (70%), and they have been considered as ultrasmall microorganisms (Hahn et al. 2003). Warnecke et al. in the year 2005 ensured that the bacteria of planktonic origin dominating in the high altitude and in ultraviolet (UV) transparent lakes were native actinobacteria; however, it has been cautioned that the adjustment to ultraviolet stress was relatively, not essentially, causal. At present, no such pure strains have recovered from these original actinobacteria concerning to the UV tolerance. The cocultures and phylotypes of these freshwater organisms are often associated with the representatives of *Micrococcineae*, and more recently, Hahn has identified the potential novel monophyletic and recently has described a novel monophyletic group among family *Microbacteriaceae* (Hahn 2009). Seven new species were recognized but again lonely as candidate species because pure and isolated cultures have not been accomplished; the helper bacteria mostly related proteobacteria are required to form quite close interaction to allow the development of the actinomycetes. The mechanism about this interaction remains unknown. Aside from freshwater, inland waters such as soda and salt lakes are also abundant sources of new actinobacteria; soda lake-derived organisms consist of *Nitriliruptor* (Sorokin et al. 2009), *Yonghaparkia* (Yoon et al. 2006), and *Microcella* (Tiago et al. 2005). However, the *Nutriliruptor alkaliphilus* is probably the exciting organism because it is the first identified member of a novel, extremely branched order within the *Actinobacteria*, and it is moderately halophilic, obligatory alkaliphilic and can able to grow in a range of nitriles. Also, thermophilic actinobacteria have been recovered from hot springs (*Rubrobacter* (Chen et al. 2004)), whereas the first culture-independent methods have revealed the foremost diversity of actinobacteria most commonly seen within the environments of higher temperature (81 °C) (Song et al. 2009); the significant phylotypes and associated members include the *Rubrobacteriales* and the actinobacteria suborder *Frankineae*. The salt lake is embedded with the presence of *Haloactinospora* (Tang et al. 2008), *Haloglycomyces*

Table 4.1 Bioactivity of compounds extracted from various actinomycetes

Compounds	Actinomycetes	Action
Sclerothricin	*Streptomyces sp.*	Antifungal activity
Lomofungin	*Streptomyces lomondensis*	Antifungal
Spoxamicin	*Streptosporangium oxazonolinicum*	Antitrypanosomal
Antimycin	*Streptomyces* sp.	Antifungal
Avermectin	*Streptomyces avermitilis*	Antiparasitic
Rosamicin	*Micromonospora rosaria*	Antibacterial
Roseoflavin	*Streptomyces dawavensis*	Antibacterial
Validamycin	*Streptomyces* sp.	Antifungal
Rifamycin	*Micromonospora rifamycinica*	Antibacterial

Fig. 4.1 Indicating potential strain of thermostable actinobacteria from compost sample

(Guan et al. 2009), and *Streptimonospora* (Cui et al. 2001). However, the culture-based methods can be more beneficial in isolation and maintenance of the potential microbial consortia for various industrial applications (Anil Kumar et al. 2010; Jayachandra et al. 2013a; Mohan Reddy et al. 2015a, b; Jayachandra et al. 2013b). There are numerous actinomycetes reported for their antimicrobial activities such as antifungal, antibacterial, antitrypanosomal, antiparasitic, etc. (Table 4.1). Recently we could be able to recover potential lignocellulose degrading actinobacteria from the compost samples in Finland (Fig. 4.1).

4.5 Actinobacteria in Alkaline Soils

In traditional terms, actinobacteria which are the organisms that are tolerant to the environmental conditions recognized as mycelia prokaryotes occurring under alkaline conditions have been investigated. The actinobacteria isolate grown well on alkaline media were initially described by Baldacci (1944). The alkaliphilic actinomycetes were recovered from a variety of soils by Taber (1960). These actinobacteria are reportedly recovered from soda lakes and saline soils. Mycelial-forming bacteria of the *Geodermatophilus* genera employ specific life cycle amount to the multiple part of the microbial consortium in desert ecosystems, plants of the Kyzylkum Desert, salt crust, desert, and solonchaks in the southern coastal regions of the Aral and Dead seas (Dobrovol'skaya 2002). On the other hand, these actinobacteria were not studies from the perspective of their resistance capacity to higher pH and a higher concentration of salinity. Before, a considerable amount of information on the isolation of alkalophilic actinobacteria from the soils and concerned substrates has been utilized. During one of the study, the amino acid composition of the cell wall of certain alkaliphilic actinobacteria (Mikami et al. 1982; Yoshida et al. 1979) reported the occurrence of a mesoisomers of DAPa (diaminopimelic acid) in some of them. The alkaliphilic types of bacteria not only were restricted to the *Streptomyces* genus but also common among other genera such as *Streptoverticillium*, *Elytrosporangium*, *Microellobosporia*, *Nocardioides*, *Chainia*, *Sporichthia*, *Saccharothrix*, *Micromonospora*, and *Nocardiopsis* (Prabahar 2004*;* Prauser 1976a, b). Thereafter, they were grouped under alkaliphilic actinobacteria to few of the abovementioned genera confirmed by using 16S rRNA gene sequence analysis (Antony-Babu et al. 2003). Previous reports also revealed these alkaliphilic actinobacteria could be resulted in the description of new taxa (Kroppenstedt and Evtushenko 2004; Nakajima et al. 1999) and biologically active substances synthesized by novel species, alkaline proteases, and new antibiotics (Sato et al. 1983; Song et al. 2001).

Several attempts able to divide the alkaliphilic isolates according to their requirement for acidity were mentioned in the literature. Few authors (Jiang and Xu 1993) distinguish the extremes as alkaliphilic actinobacteria with an optimum growth rate at pH 10–11 and not viable at pH 7.0; moderate alkaliphilic can be classified based on the pH 10 but weakly grows at pH ranging between 6.0 and 11.0. As per the report of Jiang et al., the alkaliphilic type can be classified into 2 groups: alkaliphilic with the optimum growth at pH ranges between 9.0 and 9.5 and growth stops at 8.0–11.5 and alkali tolerant with the optimum growth occurs at pH 7.0 and growth stops at pH 11.5. The range of the pH value optimum toward the growth of the isolates was analyzed, measuring the intensity of incorporated adenine into the cell wall. For alkaliphilic actinobacteria, this intensity is maximum at pH between 9.0 and 9.5. Till now, a great amount of information is available on the recovery of actinobacteria in unusual requirements for the acidity of the environment has accumulated. However, there is a lack of data on the regular distribution of population, and ecological persistence of the alkaliphilic actinobacteria is not yet

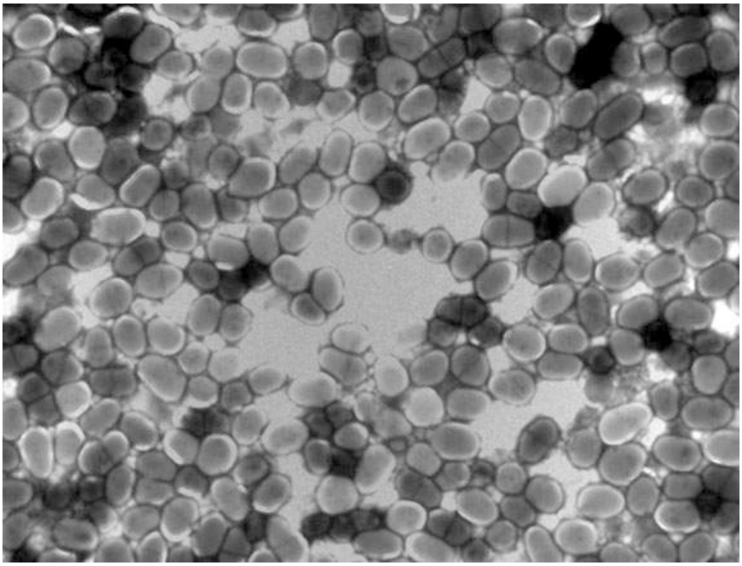

Fig. 4.2 TEM micrograph of actinobacteria: *Cellulomonas* sp.

widespread. There is also scarcity of detailed descriptions of taxa belonging to mycelial prokaryotes by unique pH requirements. As per the literature, the issues are associated with the specific property of secondary metabolism in alkaliphilic actinobacteria for the production of antibiotics (Mikami et al. 1986), and thus the synthesis of alkaline-stimulated enzymes (Sato et al. 1983) is most commonly considered, or novel taxa among the alkaliphilic and acidophilic actinobacteria were reported (Tsuchiya et al. 1997; Li et al. 2005b; Wael et al. 2004; Wang and Ruan 1994; Wang et al. 2001, 2004). The likely occurrence of the mycelia bacteria under alkaline medium is of no doubt. Applied methods like TEM can be used in understanding the structural morphology and behaviors of actinobacteria (Fig. 4.2).

4.6 Prospective

Several properties of these isolates have been investigated (Hozzein et al. 2004); however, the ecology of alkaliphilic actinobacteria is poorly understood. There are huge opportunities in exploration of the actinobacteria complexes in a broad range of soils and artificial substrates and the identification of the taxonomic structure and ecological specificities of alkaliphilic actinobacteria, corresponding to the places of these mycelia bacteria under the microbial consortia of alkaline and saline soils and which could significantly contribute toward the understanding of biological diversity. The soils by means of their pH values (saline chestnut, saline alluvial meadow, meadow solonchaks, semidesert brown, and crusty) were studied. With the high alkaline (pH >8) soils, numerous actinobacterial species were recovered by cultivating on medium with pH 9 which was quite high compared to neutral and, specifically, on the acidified media (Selyanin et al. 2005). This excess can be seen interestingly in the solonchak by about pH 9.5 produced within the underneath of the dried salt lake in Buryatia. On the substrate, numerous actinobacterial species can grow under alkaline medium conditions that surpass the density that was isolated under acidic pH. Reportedly in every soil that was investigated at above pH 7, numerous actinomycetes were recovered under the alkaline medium, which was relatively higher as compared to that cultivated under neutral conditions.

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