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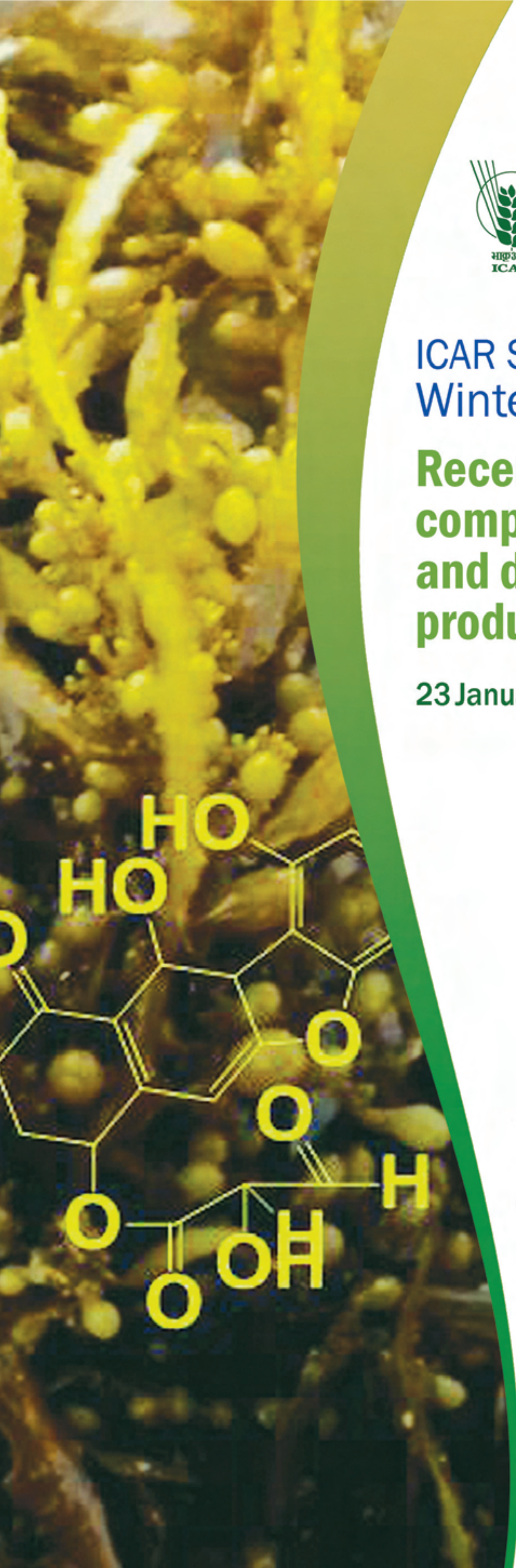
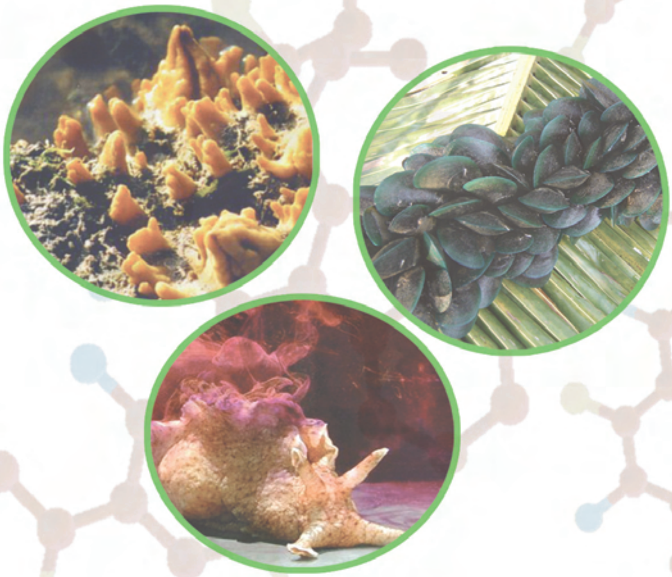


ICAR Sponsored
Winter School on

Recent advances in bioactive compounds from marine organisms and development of high value products for health management

23 January to 12 February 2018



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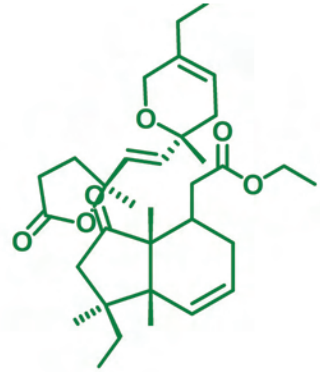

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Course Manual

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ICAR-Central Marine Fisheries Research Institute,
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FOREWORD

There has been a growing interest in the marine derived bioactive compounds in the recent years, and the functional foods, enriched with natural ingredients have been proved to provide beneficial action for human health. Marine derived bioactive components and the functional food ingredients demonstrated to possess potential health benefits. High value secondary bioactive metabolites from the marine organisms are attracting attention because of the growing demand for new compounds of 'marine natural' origin, having potential applications in pharmaceutical fields, and concerns about the adverse effects by synthetic drugs and their derivatives. The pioneering R & D works at ICAR-Central Marine Fisheries Research Institute on marine bioprospecting envisaged a systematic approach involving chemical profiling of major species of marine organisms for bioactive pharmacophore leads for activity against various diseases, and a library of molecules with bioactive potential. The research work in this institute developed protocols to prepare various pharmaceutical leads, nutraceuticals/ functional food supplements enriched with lead molecules with different properties against various drug targets for use against various life-threatening diseases.

ICAR-Central Marine Fisheries Research Institute is the pioneering marine research institute in India to work in the frontier area of bioactive molecule discovery from marine organisms as promising therapeutic agents against various diseases, aquatic food product technology, and development of high value products for health management. This prestigious research institute of Indian Council of Agricultural Research is working in the broad national interest of producing high value bioactive leads from the marine organisms, which would provide promising therapeutic agents against various diseases. This institute has developed and commercialized the nutraceutical products Cadalmin™ Green Algal extract (Cadalmin™ GAe) and Antidiabetic extract (Cadalmin™ ADe) as green alternatives to synthetic drugs to combat rheumatic arthritic pains and type-2 diabetes, respectively to a leading biopharmaceutical company in India. The anti-inflammatory nutraceutical Cadalmin™ Green Mussel extract (Cadalmin™ GMe) from Asian green mussel Perna viridis has been commercialized with Amalgam Group of Companies. Cadalmin™ Antihypercholesterolemic extract (Cadalmin™ ACe) has been developed from seaweeds to combat dyslipidemia leading to obesity, and the product was out-licensed to a leading Indian MNC in wellness and obesity management. Antimicrobial therapeutic product from marine bacteria as oral applicant has been developed and the product is in pipeline for commercialization. Seaweed-derived natural template inspired synthetic derivatives as potential pharmacophores were designed and developed. Several nutraceutical and cosmeceutical products from marine organisms are in pipeline, and are being commercialized.

The objective of the National level ICAR Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" is to provide up-to-date information and acquaint the participants with the latest technologies on isolation and characterization of marine natural products of pharmaceutical importance from marine organisms, general and advanced methods of isolation procedures by chromatography, classification of organic compounds and their characterization by advanced spectroscopic experiments. This program further aims to give exposure to the chemical perspectives of marine organisms, primary and secondary bioactive metabolites from fish and marine organisms to develop bioactive compounds and high-value functional food products. Theory and practical classes will be conducted in these areas to provide the participants a hands-on experience.

This ICAR Winter School is organized with the full funding support from ICAR, New Delhi, and the twenty-five participants from various parts of India who are attending this programme were selected after scrutiny of their applications based on their bio-data. They are serving as academicians, such as Professors/Scientists, and in similar posts. The faculties include the knowledgeable scientists and professors from various parts of India and abroad. This training will enable the participants to efficiently carry out their academic programmes, and to plan research on bioactive molecule discovery in their respective laboratories and institutes so that they can formulate the strategies for research.

The Winter School on "Recent advances in bioactive compounds from marine organisms and development of high value products for health management" is very ideal for the current scenario of increasing lifestyle diseases and human health. Understanding the importance of natural products in the health care system of India, ICAR-Central Marine Fisheries Research Institute has reasonably contributed in the various aspects. The Manual released on this occasion covers all aspects of marine natural products prepared by the experts in their respective fields. I congratulate the Course Director of this programme, Dr. Kajal Chakraborty and Head of the Marine Biotechnology Division, Dr. P. Vijayagopal, along with other staff members of Marine Biotechnology Division and Central Marine Fisheries Research Institute for their sincere efforts in bringing out the manual in time, and to arrange the programme in a befitting manner.

A. Gopalakrishnan
Director, ICAR-Central Marine Fisheries Research Institute
Kochi, Kerala

PREFACE

Marine-derived bioactive components and the functional food ingredients with potential health benefits are an emerging area of research. The rich diversity of flora and fauna in the marine and coastal habitats of the Indian subcontinent represent an untapped reservoir of bioactive compounds with valuable pharmaceutical and biomedical use. Considering the underutilization of these groups of marine organisms, exploring bioactive compounds and development of any biologically useful products have benefits as health products. Comprehensive analyses demonstrated that during the last decade the average proportion of bioactive compounds among the new compounds is declining, though there are a large number of marine natural products yet to be explored. This may indicate that the research level of bioactivity is not keeping up with the discovery of new compounds. Thus, the research tools and methods for finding bioactivity need to be improved. The first improvement is about methods of spectral and bioactivity-guided separation and purification of marine-derived secondary metabolites, which combine the discovery of new compounds. These improvements in technology are dependent upon the automation in spectroscopy, which also allows the study of the functions of new compounds extracted from the target marine organisms. Second, for the discovery of new lead compounds and artificial intelligence for drug development evolved to a more mechanistic approach that targets specific molecular lesions. Combined with high-throughput screening through a large number of drug targets, bioactivity research against various life-threatening diseases will be effective in revealing the potentially useful biological properties of marine natural products. Furthermore, the discovery of new bioactive compounds from marine metabolites will form the basis for new drug leads. Thus, the new compounds will absolutely compose an abundant resource for future bioactivity research and drug development. Various medicinal and biomedical products from marine flora and fauna provide a myriad of benefits for human health and multiple life-threatening diseases, and therefore, are the attractive options for the food and pharmaceutical industry. The increasing interest in marine-based functional food ingredients and nutraceutical formulations in the last decade along with increased number of patents filed/granted have appropriately demonstrated the possibilities of bioactive from marine organisms to maintain and improve human health and well-being.

The present ICAR Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" is designed to acquaint the participants with the advances in marine bioactive compounds with emphasis on the latest technologies on isolation and characterization of marine natural products of pharmaceutical importance. The course is planned in such a way that it covers both theoretical and practical aspect of recent advances in bioactive compounds from marine organisms. This programme will strengthen the knowledge of participants with regard to the general and advanced methods of isolation procedures by chromatography, and their characterization by advanced spectroscopic experiments aspects.

I wish to thank the Education Division of Indian Council of Agricultural Research for giving us an opportunity to organize this ICAR Winter School. We are grateful to Dr. A. Gopalakrishnan, Director, ICAR-Central Marine Fisheries Research Institute, for his guidance, continuous interest in the course and providing all necessary facilities. I am highly obliged to Dr. P. Vijayagopal, Head, Marine Biotechnology Division for his guidance and support for the programme. All the scientists of Marine Biotechnology Division, technical staff, supporting staff and research scholars supported us in organizing the ICAR Winter School. I recall with gratitude the marvellous effort and help in preparing this manual by Minju Joy, Research Scholar of Marine Biotechnology Division. I take this opportunity to thank all the faculty members who have devoted their valuable time and contributed material for the preparation of the manual. I am confident that the Course Manual would aid the participants to enhance their knowledge and competence in the area of marine bioactive compounds and their applications for the development of high-value products for health management.

Kajal Chakraborty
Course Director

January, 2018

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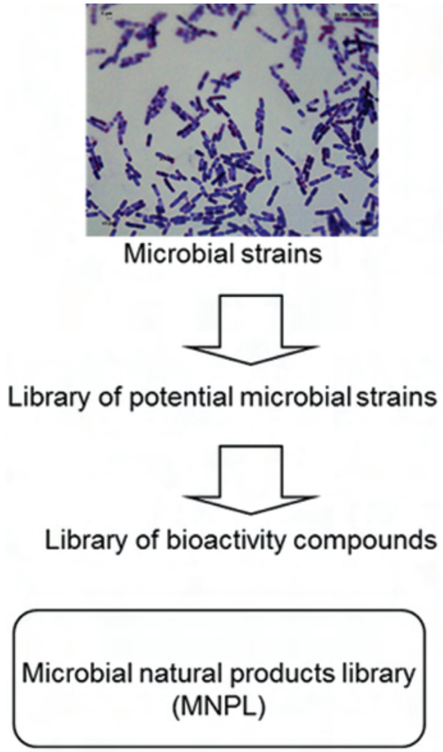
MARINE MICROBES AS A SOURCE OF ANTIMICROBIAL COMPOUNDS

Kajal Chakraborty, Vinaya K.K., Tima Antony, Minju Joy, Sreemol C.K.

Bioprospecting Section of Marine Biotechnology Division, ICAR-Central Marine Fisheries Research Institute, Kochi

Earth is a blue planet; oceans cover 70% of its surface, and in terms of phyla, the diversity of the oceans is about double that of the land. Environments such as the deep sea floor, once thought barren, are now known to be equally or more biologically diverse than tropical rainforests. It has been known for at least 40 years that microorganisms could be recovered from the sea. An impressive number of modern drugs have been isolated from microorganisms, mainly based on their use in traditional medicine. In the past century, however, an increasing role has been played by microorganisms in the production of antibiotics and other drugs (Fenical, 1993). The importance of terrestrial bacteria and fungi as sources of valuable bioactive metabolites is very well established for more than half a century. As a result, over 120 of the most important medicines (penicillins, cyclosporin A, adriamycine, etc.) in use today are obtained from terrestrial microorganisms (Alanis, 2005). For more than two decades, there has been an ongoing quest to discover new drugs from the sea. Most efforts have been directed towards chemical studies of marine invertebrates (Chin et al., 2006). Although these studies have indeed proven that marine invertebrates are an important source of new biomedical leads, a fact well demonstrated by the number of compounds currently in clinical trials, it has proven notoriously difficult to obtain adequate, reliable supplies of these compounds from nature. Because of these problems, a new avenue of study focusing on marine microorganisms has been gaining considerable attention (Faulkner, 2002). At first sight thus, the expectable enormous biodiversity of marine microorganisms might have been the reason for the interest in their study. Although marine microorganisms are not well defined taxonomically, preliminary studies indicate that the wealth of microbial diversity in the world's oceans, make this a promising frontier for the discovery of new medicines (Blunt et al., 2004). Marine bacteria are most generally defined by their requirements of seawater, or more specifically sodium for growth. In the case of marine fungi, which in general do not display specific ion requirements, obligate marine species are generally considered to be those that grow and sporulate exclusively in a marine habitat. Although such definitions can prove useful, they tend to select for a subset of the microorganisms that can be isolated from any one environment. This problem is compounded in the case of near - shore or estuarine samples where a large percentage of the resident microbes are adapted to varying degrees of marine exposure. For the purpose of microbial drug discovery, it seems only logical to study all microbes that can be isolated from the marine environment. Based on the species studied, most of the new compounds reported from marine microorganisms were obtained from species that can, in principle, be isolated from both land and sea. Although these facultative marine species are clearly a good source of novel metabolites, their ecological roles and degrees of adaptation to the marine environment is largely unknown. Screening of marine bacteria isolated from the surface of marine algae and invertebrates has shown that a high percentage produce antimicrobial metabolites. Marine microbial floras have an unrivalled capacity to synthesize bioactive secondary metabolites with a wide spectrum of bioactivities. Historically, microorganisms have provided the source for the majority of the drugs in use today. As new chemical entities are likely to be discovered from novel microbes, marine microorganisms are a likely target for improved technological platforms in the search and discovery of novel bioactive compounds. The first antibiotic from marine bacterium was identified and characterized in 1966 (Burkholder et al., 1966). In addition, bacteria in biofilms formed on the surface of marine organisms have been documented to contain a high proportion of antibiotic producing bacteria than some other marine environment (Lemos et al., 1985; Anand et al., 2006). Marine epiphytic bacteria, associated with nutrient rich algal surfaces and invertebrates, have also been shown to produce antibacterial secondary metabolites, which inhibit the settlement of potential competitors (Bernan et al., 1997). A number of surface associated marine bacteria have also been found to produce antibiotics (Hans et al., 2004). A *Bacillus sp* isolated from a marine worm in Papua New Guinea produced a novel cyclic decapeptide antibiotic, loloatin B, which inhibit growth of MRSA (methicillin resistant *Staphylococcus aureus*) and VRE (Vancomycin resistant *Enterococcus*) (Gerard et al., 1999). The marine bacterium *Alteromonas rava* was found to produce a new antibiotic thiomarinol (Shiozawa et al., 1993). Antibiotics from marine microorganisms have been reported, including loloatins from *Bacillus*. Agrochelin and sesbanimides from *Agrobacterium* (Acebal et al., 1999), pelagiomicins from *Pelagiobacter variabilis* (Imamura 1997), pyrones from *Pseudomonas* (Singh et al., 2003). Screening of seaweed and invertebrate-associated bacteria has shown their bioactivities (Chakraborty et al., 2010), and that over 25% of these isolates can produce compounds capable of killing methicillin resistant *Staphylococcus aureus*



Scehmatic representation of Biospectnig antibacterial molecules from microbial flora

(MRSA) and vancomycin resistant *Enterococcus* (VRE; Mearns-Spragg et al., 1997). This is a much higher proportion than found with free-living or soil-associated bacteria.

ANTIMICROBIALS FROM MICROBES, A BRIEF HISTORY

It has been argued that because of the high dilution effect of seawater, marine-derived bioactive compounds may have evolved great potency. This theory was supported in 2004 with the report of a first-in-class antimicrobial compound, was described from a marine isolate *Verrucosispora*. Renewed interest in marine microorganisms and their ability to produce antimicrobials has resulted in numerous reports of novel antimicrobial compounds. The period of antimicrobial drug discovery from the early 1940s to the 1960s is referred to as the Golden Age. During this time, the industrialization of penicillin production created the expertise and facilities to make significant quantities of antimicrobial compounds by fermentation. The clinical use of antibiotics heralded a health care miracle; deaths due to bacterial infections were significantly reduced, resulting in increases in life expectancy. The majority of compounds that were discovered during this period were isolated from soil bacteria, most notably the filamentous *Actinobacteria*. Microorganisms are a prolific source of structurally diverse bioactive metabolites and have yielded some of the most important products of the pharmaceutical industry. Microbial secondary metabolites are now being used for applications other than antibacterial, antifungal and antiviral infections. It was during 1928s when Alexander Fleming (Fleming, 1929) began the microbial drug era when he discovered in a Petri dish seeded with Staphylococcus aureus that a compound (penicillin) produced by a fungus/mold killed the bacteria. Later, penicillin was isolated as a yellow powder and used as a potent antibacterial compound during the Second World War. Following this extraordinary discovery be Flemming, the antibiotics chloramphenicol and streptomycin, were isolated. Naturally occurring antibiotics are produced by fermentation, an old technique that can be traced back almost 8000 years. Owing to technical improvements in screening programs, and separation and isolation techniques, the number of natural compounds discovered exceeds 1 million (Ecker et al., 2005). Among them, 50-60% are produced by plants (alkaloids, flavonoids, terpenoids, steroids, carbohydrates, etc.) and 5% have a microbial origin. Of all the reported natural products, approximately 20–25% show biological activity, and of these approximately 10% have been obtained from microbes. Furthermore, from the 22 500 biologically active compounds that have been obtained so far from microbes, 45% are produced by bacteria or bacteria-like microbes, 38% by fungi and 17% by others (Berdy, 2005). The increasing role of microorganisms in the production of antibiotics and other drugs for treatment of serious diseases has been dramatic. However, the development of resistance in microbes to various life-thretening diseases and in aquaculture has become a major problem and requires much research effort to combat it. The emergence of antibiotic resistance in 1970s coincided with high rediscovery rate of the major antimicrobial classes; the low-hanging fruit had apparently all been picked. Antimicrobial development after the Golden Age was characterized by semi-synthetic modifications of compounds that were already clinically proven. The poor antimicrobial discovery rate from microbes, coupled with the availability of chemically synthesized small molecule libraries, led to the abandonment of microbial screening programmes in the majority of pharmaceutical companies. To date, small chemical libraries have failed to deliver a new antimicrobial compound to the clinic, prompting many to speculate that the withdrawal of microbial screening was premature, exacerbating the threat of antibiotic resistant bacteria.

MICROBIAL NATURAL PRODUCTS

Microbial natural products that have reached the market without any chemical modifications are a testimony to the remarkable ability of microorganisms to produce drug-like small molecules. Although still in clinical trails, a feature example of this is salinosporamide A (NPI-0052), a novel anticancer agent found in the exploration of new marine environments (Fenical et al., 2009). In 2008, over 1000 marine natural products were reported (Blunt et al., 2010). However, out of 19 microbial-derived drugs reported in 2008, no natural products from marine microbes were present, signifying the novelty of their systematic exploration (Ganesan, 2008). Currently, >30 compounds of marine microbial origin are in clinical or preclinical studies for the treatment of different types of cancer (Simmons et al., 2005) clearly demonstrating that marine microorganisms have become an essential resource in the discovery of new antibiotic leads. The evolution of marine microbial natural product collections and development of high-throughput screening methods have attracted researchers to the use of natural product libraries in drug discovery. These libraries include subsections of crude extracts, pre-fractionated extracts (automated HPLC-MS fractionation) and purified natural products. A research group in Ireland has developed a two-dimensional chromatographic strategy that includes a protocol to generate purified marine natural product libraries that are accurately characterized by mass during production to expedite dereplication of known compounds and identification of novel chemotypes. Although the biosynthetic and regulative crosstalk of secondary metabolite biosynthesis is complex within and between microorganisms, all levels can be influenced by imitating natural environmental changes. Development and testing of new culture media for the maximum expression of secondary metabolites is important as chemical diversity in the construction process of a marine natural products library.

An optimization of ‘one strain, many active compounds’ can be used together with ‘fingerprint’ methods (HPLC and nuclear magnetic resonance) including tandem analytical techniques such as MS/MS, GC-EI/MS, HPLC-SPE-NMR, LC-MS-MS and LC-NMR for the optimization/selection of culture media for high-throughput fermentation of novel strains. Tormo et al., (2003) developed a method for the selection of production media for bacterial strains based on their metabolite HPLC profiles, that yielded the highest metabolite diversity and least overlapping HPLC profiles were selected for large-scale fermentation. Targeted high-throughput screening methods are important for the speed and accuracy of identification of novel antimicrobials. From these evaluation models, many crude extracts or purified compounds were obtained as positive hits. In addition for evaluation purposes, it is worthy to note that these screening assays also provide mode of action hypothesis from the crude extracts.

ANTIBACTERIAL MOLECULES FROM NATURAL SOURCES

Drugs of natural origin have been classified as (i) original natural products, (ii) products derived or chemically synthesized from natural products or (iii) synthetic products based on natural product structures. Evidence of the importance of natural products in the discovery of leads for the development of drugs for the treatment of human diseases and aquaculture are provided by the fact that close to half of the best selling pharmaceuticals and antibiotics in 1990-2000 were either natural products or their derivatives (Cragg et al., 1997). In this regard, of the 25 top-selling drugs reported in 1997, 42% were natural products or their derivatives and of these, 67% were antibiotics. Today, the structures of around 140 000 secondary metabolites have been elucidated. Applications of chemically synthesized natural metabolites include the use of a natural product derived from plant salicyclic acid derivatives present in wintergreen and meadowsweet to relieve pain and suffering. Synthetic salicylates were produced initially by Bayer in 1874, and later in 1897, Arthur Eichengrun at Bayer discovered that an acetyl derivative (aspirin), reduced acidity, bad taste and stomach irritation. These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization (WHO) that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care (Farnsworth et al., 1985). The alkaloid quinine, the active constituent of *Cinchona succirubra*, has been known for centuries by South American Indians to control malaria. During the twentieth century, massive programs to synthesize quinoline derivatives, based on the quinine prototype, were carried out. The first of the new quinolones to be used clinically as an antibacterial agent was nalidixic acid (Topliss et al., 2002). The compound 7-chloro-1,4-dihydro-1-ethyl-4-oxoquinolone-3-carboxylic acid was obtained as a side product during purification of chloroquine and found to have antibacterial activity against Gram-negative bacteria and was shown to be an inhibitor of DNA gyrase. Its discovery led to a whole series of synthetic quinolone and fluoroquinolone antibiotics (pefloxacin, norfloxacin, ciprofloxacin, levofloxacin, ofloxacin, lomefloxacin, sparfloxacin, etc), which have been very successful in medicine and have achieved major commercial success. Secondary metabolites have exerted a major impact on the control of infectious diseases and other medical conditions, and the development of pharmaceutical industry. Their use has contributed to an increase in the average life expectancy in the world. In 2000, the market for major antiinfectives from bacteria and other natural sources was US$55 billion and in 2007 it was US$66 billion.

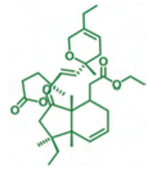
Various classes of antibiotics/drugs from microbial flora (upto 2000) (Barber, 2001).

Sl. No.	Antibiotics/drugs	Market share (US billion $)
1	Antiviral compounds	10.2
2	Penicillins	8.2
3	Cephalopsporins	9.9
4	Beta lactam antibiotics	1.5
5	Quinolines	6.4
6	Other antibacterials	6.0
7	Tetracyclines	1.5

Two antivirals that are chemically synthesized today were originally isolated from marine organisms. They are acyclovir (active against the herpes virus by inhibition and inactivation of DNA polymerase) and cytarabine (active against non-Hodgkin's lymphoma). Both compounds are nucleoside analog drugs, originally isolated from sponges (Rayl, 1999). Other antiviral applications of natural compounds are related to human immunodeficiency virus (HIV) treatment. Furthermore, reports have been published on natural product inhibitors of HIV integrase obtained from among the marine ascidian alkaloids; that is, the lamellarins (produced by the mollusk *Lamellaria* sp.), and from terrestrial plants (Baccharis genistelloides and Achyrocline satureioides). The most consistent anti-HIVactivity was observed with extracts prepared from several Baccharis species (Robinson et al., 1996).

ANTIBIOTICS FROM MARINE MICROBES

During recent decades, we have seen an increasing number of reports on the progressive development of bacterial resistance to almost all available antimicrobial agents. In the 1970s, the major problem was the multidrug resistance of Gram-negative bacteria, but later in the 1980s the Gram-positive bacteria became important, including methicillin-resistant staphylococci, penicillin-resistant pneumococci and vancomycin-resistant enterococci (Moellering, 1998). In the past, the solution to the problem has depended primarily on the development of novel antimicrobial agents. However, the number of new classes of antimicrobial agents being developed has decreased dramatically in recent years. The conventionally used antibiotics/drugs become resistant to most of the natural antimicrobial agents that have been developed over the past 50 years (Hancock, 2007) thereby limiting the effectiveness of current antimicrobial drugs. In 2004, more than 70% of pathogenic bacteria were estimated to be resistant to at least one of the currently available antibiotics (Katz et al 2006). The so-called 'superbugs' (organisms that are resistant to most of the



clinically used antibiotics) are emerging at a rapid rate. S. aureus, which is resistant to methicillin, is responsible for many cases of infections each year (Balaban et al., 2005). The incidence of multidrug-resistant pathogenic bacteria is increasing. The Infectious Disease Society of America (IDSA) reported in 2004 that in US hospitals alone, around 2 million people acquire bacterial infections per year (dedicated website: http://www.idsociety.org/Content.aspx). There are also other examples of Gram-positive (Enterococcus and Streptococcus) and Gram-negative pathogens (Klebsiella, Escherichia, Enterobacter, Serratia, Citrobacter, Salmonella and Pseudomonas) (Cragg and Newman, 2001). Among them, Pseudomonas aeruginosa accounts for almost 80% of these opportunistic infections. They represent a serious problem in patients hospitalized with cancer, cystic fibrosis and burns, causing death in 50% of cases. Other infections caused by Pseudomonas species include endocarditis, pneumonia and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin and musculoskeletal system (Levin and Bonten, 2004). In addition to the antibiotic-resistance problem, new families of anti-infective compounds are needed to enter the marketplace at regular intervals to tackle the new diseases caused by evolving pathogens. At least 30 new diseases emerged in the 1980-2000s and they are growing in incidence. Emerging infectious organisms often encounter hosts with no prior exposure to them and thus represent a novel challenge to the host's immune system. Several viruses responsible for human epidemics have made a transition from animal host to humans and are now transmitted from human to human. HIV, responsible for the acquired immunodeficiency syndrome (AIDS) epidemic, is one example. Although it has not been proven, it is suspected that severe acute respiratory syndrome (SARS), caused by the SARS coronavirus, and also evolved from a different species (Kremer et al., 2000). One additional reason for developing new antibiotics is related to their own toxicity. As with other therapeutic agents, the use of antibiotics may also cause side effects in patients. Some side effects are more severe and, depending on the antibiotic, may disrupt the hearing function (aminoglycosides), kidneys (aminoglycosides and polypeptides) or liver (rifampin).

NEW MOLECULES AS A SOLUTION TOWARDS MULTIRESISTANT ANTIBIOTIC AND DRUG MOLECULES

In recent times, several scientific groups are making concerted efforts to find novel antimicrobial agents as a solution towards multiresistant antibiotic and drug molecules. Novel glycylcyclines (modified Tetracyclines) developed to treat tetracycline-resistant bacteria. These show potent activity against a broad spectrum of Gram-positive and Gram-negative bacteria, including strains that carry the two major tetracycline-resistance determinants, involving efflux and ribosomal protection. Two of the glycylcyline derivatives, DMG-MINO and DMG-DMDOT have been tested against a large number of clinical pathogens isolated from various sources. The spectrum of activity of these compounds includes organisms with resistance to antibiotics other than tetracyclines; for example, methicillin-resistant staphylococci, penicillin-resistant S. pneumoniae and vancomycin-resistant enterococci (Sum, 2006). Tigecycline was approved by the FDA in 2005 as an injectable antibiotic (Bacque et al., 2005). A new glycopeptide antibiotic, teicoplanin, was developed against infections with resistant Gram-positive bacteria, especially bacteria resistant to the glycopeptide vancomycin. In another instance, the approach involved the redesign of a mixture of two compounds, called streptogramin, into a new mixture, called pristinamycin, to allow administration of the drug parenterally and in higher doses than the earlier oral preparation (Bacque et al., 2005). The two components of streptogramin, quinupristin and dalfopristin, were chemically modified to allow intravenous administration. The new combination, pristinamycin, was approved by the FDA for use against infections caused by vancomycin-resistant Enterococcus faecium. Among the novel class of antimicrobial agents used in treating resistance to Gram-positive infections, we can also mention the cyclic lipopeptide antibiotic daptomycin produced by Streptomyces roseosporus. This compound was approved in 2003 by the FDA for skin infections resulting from complications following surgery, diabetic foot ulcers and burns (LaPlante et al., 2004). Telithromycin, a macrolide antibiotic, is the first orally active compound of a new family of antibacterials named the ketolides. It shows potent activity against pathogens implicated in community acquired respiratory tract infections, irrespective of their â-lactam, macrolide or fluoroquinolone susceptibility (Leclercq, 2001).

AQUACULTURE GRADE ANTIMICROBIAL CHEMICALS FROM MARINE MICROBES

Disease caused by bacterial pathogens has been widely recognized as a major cause of economic loss in many commercially cultured fish and shellfish species in India, with mortality of larval stages in hatcheries and the growing stages in different mariculture systems. Pathogenic vibrios are involved in significant mortalities in the larviculture and growout phases of famed finfish and shellfishes. In an attempt to control the proliferation of pathogenic vibrios, the prophylactic and therapeutic use of antibiotics has been practiced in commercial hatcheries, creating more serious problem of antibiotic resistance among the microflora in the environment. With safety concerns about synthetic antibiotics, considerable interest has arisen in finding alternative natural sources (Gomez-Gil et al., 2000). Screening and development of aquaculture-grade chemicals from bacterial flora could be a highly promising approach to produce these bioactive moleules. Members of the genus *Pseudomonas* and *Bacillus* either free living or associated with marine flora are common beneficial bacterial candidates, and are known to produce a wide range of secondary metabolites (Raaijmakers et al., 1997) inhibiting a wide range of pathogenic bacteria (Rengpipat et al., 1998). The metabolites 6-oxo-de-O-methyllasiodiplodin, (E)-9-etheno-lasiodiplodin, lasiodiplodin, de-O-methyllasiodiplodin, and 5-hydroxy-de-O-methyllasiodiplodin, were isolated from the mycelium extracts of a microbe obtained from

South China Sea (Yang et al., 2006). Bioactive compounds were isolated from a marine bacterium *Bacillus circulans* (Chakraborty *et al.*, 2010). Labda-14-ene-3a,8a-diol and labda-14-ene-8a-hydroxy-3-one were found to be inhibitory to the growth of *Vibrio parahaemolyticus* with minimum inhibitory concentrations of 30-40 μg/mL (Chakraborty et al., 2010), and their structures have been elucidated by ^{1}H NMR and ^{13}C NMR spectra, including 2D NMR. Several bacterial flora were isolated from marine ecosystem (*Bacillus subtilis*, *B. amyloliquifaciens*, *Pseudomonas putida* and *P. aeroginosa*) with potential activities (> 20 mm inhibition zone) against pathogenic Vibrios (Chakraborty et al., 2010). The antibacterial component in the $CHCl_3$ fraction of *P. aerogenosa* was found to be N-substituted methyl-octahydro-1-phenazinecarboxylate. The other important antibacterial molecules were found to be propyl 2-oxoacetate and phenethyl 2-oxoacetate.

About 4530 bacterial isolates were purified from seaweeds and sediments, and 23 isolates (*B. subtilis* MTCC 10402, 10403 and 10407, *B. amyloliquifaciens* 10456, *P. putida* MTCC 10458, *P. aeroginosa* MTCC 10610) were found to be potential against pathogenic *Vibrios*. *N*-substituted phenazinecarboxylate, propyl/phenethyl 2-oxoacetates were the major antibacterial molecules in bacteria.

CONCLUSIONS

The ability of marine microorganisms to produce novel antimicrobial compounds has been well demonstrated, and clearly they have a future role in the fight against antibiotic-resistant pathogens. Ongoing research efforts to isolate and screen new marine microorganism species should be accompanied by efforts to understand their ecology. Extensive culture-dependent and -independent surveys of marine microorganisms should be prioritized to determine the extent to which marine diversity differs, e.g. is the isolation of rare microorganisms' genera from the sea merely due to the fact that terrestrial-to-sea input skews the species distribution. The isolation of seawater-obligate microorganisms has proved that marine adaptation has occurred in this lineage, but so far this property has only been identified at the genus and species level, an indication that marine adaptation is a comparatively recent evolutionary event. If such adaptation is rare within the microorganisms, it is reasonable to expect that seawater-obligate strains will represent species that have no terrestrial counterparts, and thus they are unlikely to have been previously screened for antimicrobial compounds. This raises the intriguing possibility that there are antimicrobial compounds unique to marine species. Whole-genome analysis of the genus *Salinispora* indicates hat differences in secondary metabolite biosynthetic genes may be a driver of speciation, supporting the hypothesis that new species will produce new compounds. Further analysis is needed to determine whether this property will hold as more species are described. Finally, if antimicrobial compounds are to make it from the ocean to the clinic, big pharma must re-engage in drug discovery from microbes. Currently, small pharmaceutical and biotechnology companies have been, or are currently engaged in antimicrobial discovery from marine microorganisms

SUGGESTED READINGS

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Inaugration of winter school 2018 by Padma Bhushan Dr. Manju Sharma

Field visit to India Sea Foods

Photo with Dr. K. Gopakumar, Formerly DDG ICAR (Fy)

Field visit to Accelerated Freeze Drying Co. Ltd

Field visit to BOS Naturals

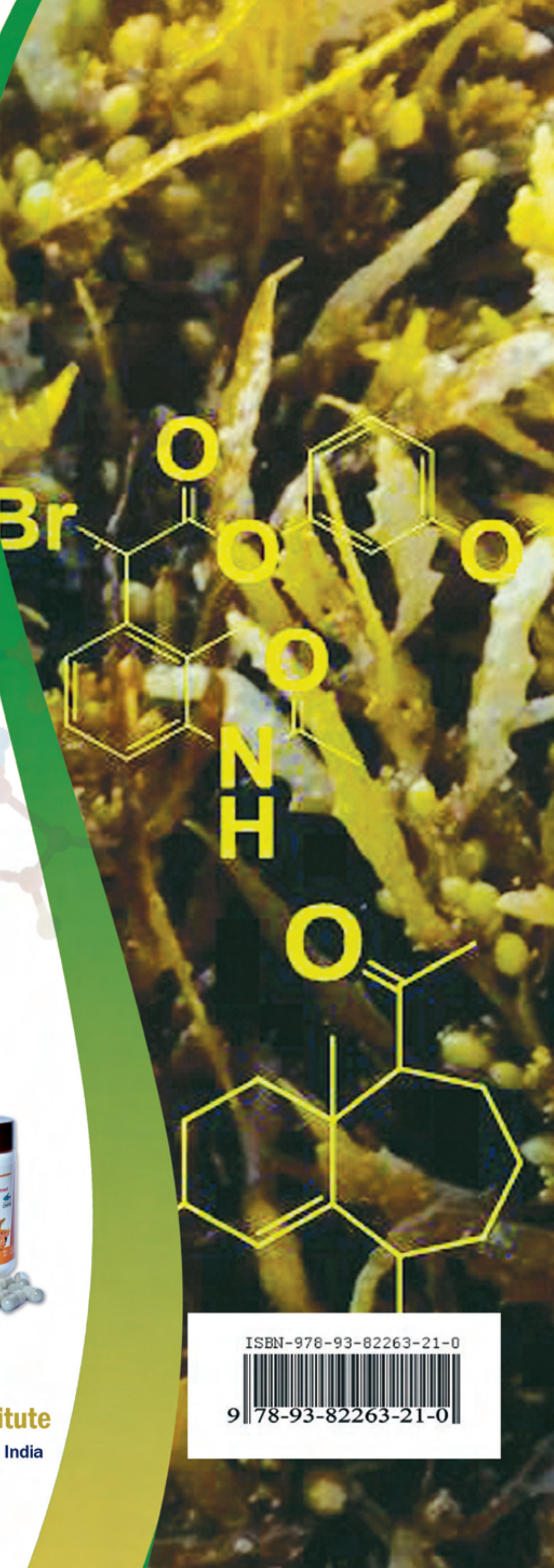
Photo with Dr. Meledath Govindan

Lectures and Interactive Sessions

Practical Sessions

Sitting (L to R) Minimol K.C., Grace Thomas, Kajal Chakraborty (Course Director), P. Vijayagopal (Head, Marine Biotechnology Division), A. Gopalakrishnan (Director), Paulson Mathew, Sathu T., Radhakrishnan E.K.

Standing (L to R) Aswathy Elizabeth Mani, Sreemol C.K., Prima Francis, Soumya Krishnan, Minju Joy, V. Rani, Seeja Thomachan Panjikkaran, Sheneya Festus, Drishya K., Anie Y., Suja Rani S., Sindhu Issac, Teena P. Varghese, Magna Thomas, Santwana Palai, Norma Xavier Chelat, Naheef K., Satya Narayan Sahoo, Jaimin Hareshbhai Bhatt, Ajoy Saha, Senthil Kuppusamy, Kedar Shashikant Damle, Shubhajit Dhara, Midhun Dominic C.D., Manukuttan K.S., Suji Chandru, Tima Antony, Soumya Salas



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