STUDIES ON BIOACTIVE COMPOUNDS FROM DIFFERENT MICROORGANISMS

Richa Shukla
z

Abstract— Natural products from microbial origin, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity and ease of simplicity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly by microbes has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. Bioactive compounds are synthesized by a number of microbial sources such as bacteria, fungi, actinomycetes, microscopic algae etc. Some of them are associated with antibacterial properties while some with antimicrobial properties. Apart from this some microbes showed broad range of action having properties of inhibiting bacteria, fungi, yeast etc. A few of the important sources of microbes for secretion of bioactive compounds are bacteria (*Bacillus sp., Pseudomonas sp.*), Actinomycetes (*Streptomyces sp.*), Fungi (*Penicillium*, *Yeasts*, *Slime moulds)*, Microscopic algae (Seaweeds, dinoflagellates, diatoms etc.). A number of Carbon sources, Nitrogen sources and various environmental conditions are responsible for maximum production of bioactive compounds using different isolates.

Index Terms— Anti bacterial Activity, Bioactive compounds, Bioactivity, Insecticidal Activity, Microbial Interactions, Natural Products, Stress conditions (Carbon, Nitrogen, pH, Bile salts).

1 INTRODUCTION

The broadest definition of bioactive compounds should include all microbial compounds exhibiting antimicrobial and/or antitumor and/or antiviral activities obtained either from microbes or from any other living thing. Microorganisms, including certain bacteria, fungi and algae, produce secondary metabolites which may have some degree of bioactivity against other microorganism. These metabolites, otherwise known as bioactive compounds.

1.1 Properties of Bio Active Compounds

The biological activity (antimicrobial, antitumor, antiviral, pharmacological, and similar activities) is the guiding line which connects the bioactive compounds, distinguishing them from the other "inactive" natural products. Several uncommon, specific chemical structures, structural elements, and unique chemical groups, (macro lactone, cyclopeptide skeleton, unusual functional groups, etc.), are more frequently occurring among bioactive compounds. It seems these unique structural features are rather belonging to the whole group of secondary microbial metabolites than specifically to the distinct groups of bioactive products. Interactions of microbes with other microbes and non-microbial systems higher plants, lower animals or mammalian systems, including humans and these interactions may be summarized as illustrated in Table 1 covering the whole area of known biological activities of microbial metabolites, at the same time representing their possible practical applications.

1.2 Biological Activities of Bioactive Compounds

Bioactive compounds exhibit a great numbers of diverse and versatile biological effects, first of all antimicrobial activities. Different pathogenic and other microbes (Gram-positive, Gram-negative bacteria, fungi, yeasts, etc.) are described as test organisms in the direct activity-based screenings. The most frequent test organisms were *Bacillus subtilis, Staphylococcus aureus, Micrococcus lutea, Escherichia coli, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Candida albicans* and others. Antiviral Tests, Inhibition Of Viral Enzymes, Activities connecting with Neoplastic diseases from simple Cytotoxicity Assay methods (P-388, KB, L-1210 cell lines) to Angiogenesis Inhibition, etc., are used most frequently for detection of other, non-antimicrobial activities of metabolites.

Table 1
Microbial Interactions

1.Microbe-Microbe	Antimicrobial antibiotics, microbial regulators, growth factors, signaling compounds, mating hormones, etc.
2.Microbe-Lower Animals (Invertebrates)	Insecticides, Miticides, Antiparasitic compounds, Algaecides, Antifeedants, (invertebrates) repellents, molluscicides,anti-worm agents,etc.
3.Microbe-Higher Plants	Herbicides, phytotoxins, plant growth regulators, chlorosis inducers, phytoalexins,etc
4.Microbe-Mammalians (Humans)	Antitumor antibiotics, pharmacologically active agents, enzyme inhibitors, (humans) immunoactive, CNS-active agents, feed additives,etc.

The list of non-antibiotic biological activities used in the new screening projects presently covers more than one thousand

different types of bioactivities; cell based receptor binding- or enzymatic assay methods and many other specific tests and targets. Most of the presently known non-antibiotic bioactivities may be classified as:

1) pharmacological-biochemical or medical activity,
2) agricultural activity,
3) regulatory, biophysical and other activities

2 Chemical Structures (Chemical Diversity)

2.1 Increasing the Biosynthetic Diversity

The wide range of specific and complex chemical structures, with fascinating array of diverse, unique functional groups occur, than in the group of antibiotics and other bioactive microbial secondary metabolites.

Compounds in this group cover all types of organic compounds from the simple acrylamidine (MW: 72) to the most complicated structures, such as the macrocyclic colubricidin (MW: 2154), which includes large glycosilated macrolactone ring, pyridine and pyrrole moieties. The polycyclic tetropetalone-A contains one of the most complicated heterocyclic ring systems with quinone and glycosidic functionalities or the macrocyclic versipelostatin also have fascinating new structures. The overwhelming majority of new compounds are 14 composed of new variations and arrangement of the old known structural elements. It is true that the structural variations, the unique combinations of rare moieties and skeletons of natural products due to the extremely versatile biosynthetic capacity (extensive branching, series of alternative reactions, isomerization, condensations, polymerization, oxidation, alkylation, etc.) of the microbes, first of all actinomycetes, are inexhaustible. This specific nature of the microbial biosynthesis, led to the isolation of a great number of analogs and a series of homologues, the so called minor components. A great part of "new" compounds discovered in the last decades (about 30%) were this kind of minor compounds. The reason of the declining effectivity to obtain new chemical types, in general may be the result of the

1) Exhaustion of the biological sources
2) Imperfect screening methodologies.

Certainly the second reason is the critical one. Nature as a source of new chemicals is supposed to be almost inexhaustible, but the classical screening methods used for long time do not meet the recent requirements of human therapy and agriculture. All chemotherapeutic agents used today belong to a limited number of chemical types (or close derivatives) discovered in the past by the classical methods.

2.2 Important Bioactive Compounds Examples

The first documented bioactive marine microbial metabolite was isolated by Burkholder and co-workers in 1966 from a marine Pseudomonas sp. (Burkholder 1966). The structure of this unique highly brominated pyrrole antibiotic.

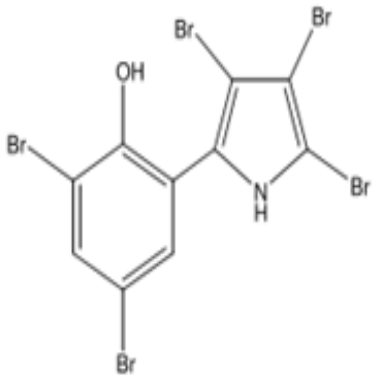
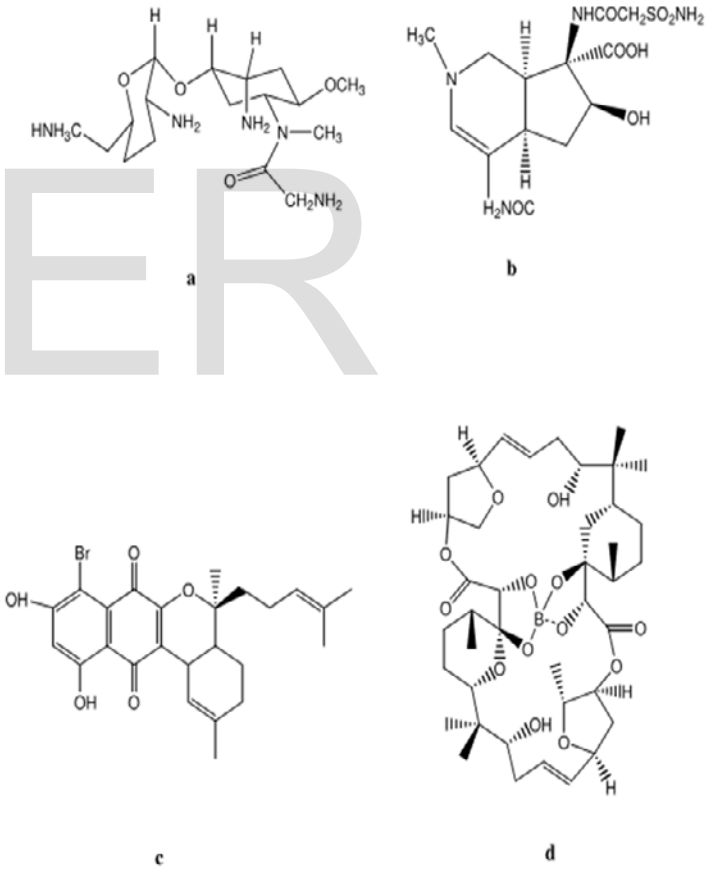


Fig 1 Brominated Pyrrole is an antibiotic

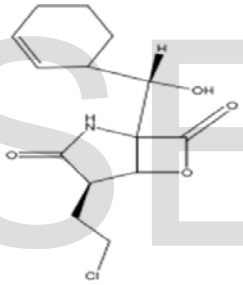
Fig 2 Bioactive compounds containing Chiral Amines



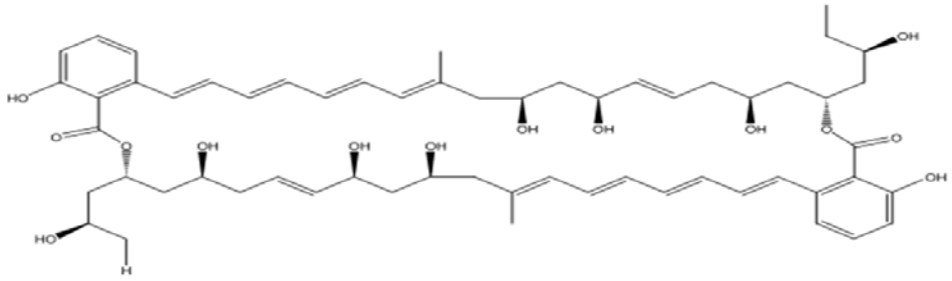
a) Amikacin
b) Sitagliptin
c) Oseltamivir
d) Chloroquine Intermediate

TABLE 2
DIFFERENT METABOLITES PRODUCED FROM *STREPTOMYCES*

	Metabolite	Producing strain	Source	Activity	Class	Reference
a	Istamycin	*Streptomyces tenjimariensis*	Marine sediment	Antibiotic	Aminoglycoside	Okami et al 1979
b	Altemicidin	*Streptomyces sioyaensis*	Marine sediment	Anticancer	Alkaloid	Takahashi et al 1989
c	Marinone	*Streptomyces sp.*	Marine sediment	Antibiotic	Napthoquinone	Pathirana et al 1992
d	Asplasmomycin	*Streptomyces griseus*	Marine sediment	Antibiotic		Nakamura et al 1977



a



b

Fig 3 The Structures of Bioactive Metabolites from New Marine Actinomycete Genera a) Salinosporamide A b) Marinomycin A

3 MECHANISM OF ACTION OF BIOACTIVE COMPOUNDS

Various antibacterial targets exist, in which antibiotics interfere with the essential pathways of the bacterial metabolism. These targets are the interaction with the cytoplasmic membrane, the inhibition of cell wall biosynthesis, or the inhibition of replicational, transcriptional, and translational processes. Glycopeptide antibiotics also inhibit the cell wall biosynthesis. Glycopeptide antibiotics cannot cross the outer membrane of gram-negative bacteria because of their size and polarity, and thus, their antibiotic effects are restricted to gram-positive bacteria and so the most important bacterial strains, which are combated with glycopeptides, are gram-positive enterococci, staphylococci, and streptococci. The primary antibiotic effect of glycopeptide antibiotics is based on the binding to the D-Ala-D-Ala dipeptide motive of the bacterial cell wall biosynthesis. In contrast to penicillin, which covalently binds to an enzymatic target, glycopeptide antibiotics represent substrate binders that shield the substrate from transpeptidation but also from transglycosylation reactions. Varying antibiotic activities of these derivatives is based on the structural variations in the degree of glycosylation, N-terminal methylation, chlorination, and differences in the length of fatty acid side chains. Of some importance is the ability of most glycopeptides to form dimers (e.g., eremomycin) or to insert into bacterial membranes (e.g., teicoplanin). Dimer formation is strongly dependent on the nature of the carbohydrates attached to a glycon and on the attachment site of these residues. Chloroeremomycin, which contains the amino sugar 4-epi-vancosamine, forms dimers with six hydrogen bonds, whereas Vancomycin shows a weak dimerization tendency by the formation of only four hydrogen bonds. The dimerization behavior originally observed with NMR has also been confirmed by x-ray crystallography.

3.1 Insecticidal Activity

The insecticidal activity of bioactive compounds was first discovered by Hamill et al. from *B. bassiana* against *salina*, which was considered a model organism to study insecticidal activity. Bioactive compounds are not applied directly as a commercial insecticidal agent instead with the movement of insect, for example the entomopathogenic fungus could propagate in insect bodies and spread widely by insect movement. The entomopathogenic fungus would give rise to a good control efficiency of insects even if a small amount of the spores of the entomopathogenic fungus are used. The careful evaluation of bioactive compounds production should ensure that their does not increase above threshold limits

Antitumor Activity

The cytotoxicity of bioactive compounds (Beauvericin) to human leukemia cells has been frequently reported. Fig 4 shows the Antitumour activity of Beauvericin to human leukemia cells

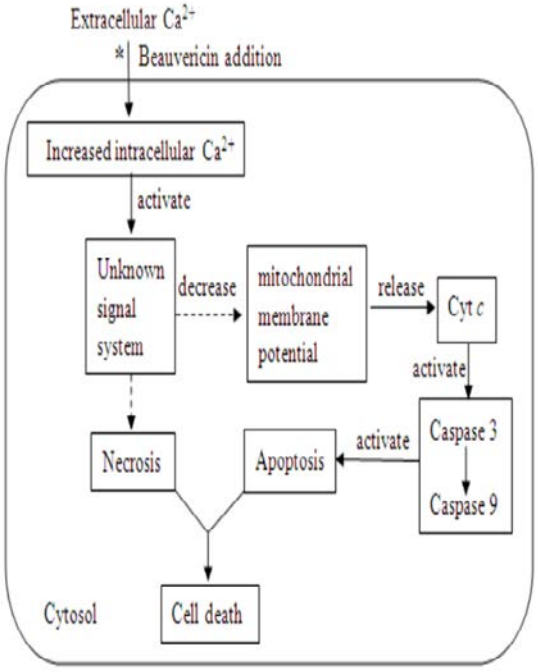


Fig 4 Mechanism of Beauvericin

Antibacterial Activity

A bioactive compound has a strong antibacterial activity against human, animal and plant pathogenic bacteria, with no selectively between Gram-positive and Gram-negative bacteria. It is possible that other cell organelles or enzyme systems are the targets of bioactive compounds. Despite its broad-spectrum antibacterial activity, the antifungal activity of bioactive compounds as a single agent is rarely reported. Therefore, the target of bioactive compounds is different for bacteria and fungi and could include targets such as the ribosome or the cell nucleus. The activity of bioactive compounds should be investigated against drug resistant bacteria. Based on the antibacterial activity against plant pathogens, bioactive compounds could be utilized in the control of non-food crop diseases, to solve the problems of drug resistance, deadly bacterial infections.

Antifungal Activity

The lack of antifungal activity of bioactive compounds as a single agent arises because many of them are fungal product. However, Zhang et al. and Fukuda et al. reported the antifungal activity of bioactive compounds in combination with Ketoconazole or Miconazole. Bioactive compounds (0.5 mg/kg)

Combined with ketoconazole (0.5 mg/kg) had remarkable antifungal activity against *Candida parapsilosis*, which can quickly cause high mortality rates, particularly in neonates. Both bioactive compounds and ketoconazole alone have little to no effect on *C. parapsilosis*. If the antifungal mechanism of bioactive compounds is similar to the cytotoxic mechanism in leukemia cells, it would indicate that the fungus itself can inhibit the "unknown signal system" until another compound, such as ketoconazole, is added to unlock the signaling system. **The method of combining bioactive compounds with another compound offers a new way to develop and utilize the biological activity of bioactive compounds.**

Antiviral Activity

The antiviral activity of bioactive compounds has also been detected. According to Shank et al., bioactive compounds are the most effective inhibitor of the cyclic hexadepsipeptides that inhibit HIV-1 integrase. Enniatins have a comparatively weak activity despite having a similar structure, which implies that the activity of bioactive compounds could be due to the primary structural difference, N-methylation. Viral infections can result in fatal and epidemic diseases. Therefore, the antiviral activity of bioactive compounds should be investigated for its potential clinical effects and activity against other serious viruses, such as HBV, SARS, H1N1 and AIV.

4 PRODUCTION OF BIO-ACTIVE COMPOUNDS

Microorganisms as a whole are highly efficient in their ability to produce many kinds of antibiotics and other bioactive compound and appear to be very opportunistic wrt antibiotic production, respond differently to fermentation environments. Complexity is involved in the biosynthesis and biodegradation of these compounds. Effective suppression of diseases by many microorganisms is largely affected by environmental conditions and nutritional conditions (Hannusch & Boland, 1996; Guetsky *et al.*, 2001; Kurze & Bahl, 2001; Abdel-Gawad, 2002).

TABLE3
SALE VOLUMES OF FUNCTIONAL FOODS IN THE U.S DURING 2011 AND 2012

S.No.	Products	2011		2012		Change (2011-2012)
		$million	%	$million	%	%
1.	Dairy & Marine	1,459	71	1,959	74	34
2.	Cereal	410	20	434	16	6
3.	Bars & Snacks	92	5	197	7	113
4.	Bakery	79	4	56	2	-29
	Total	2,041	100	2,646	100	30

TABLE 5
CLASSIFICATION OF BIOACTIVE COMPOUNDS

S.No.	Source	Activity	Properties	Reference
1.	Phenolic Compounds			
	a)Essential Oils	Antimicrobial	Controlling Biofilm	Tyagi and Malik ,2010
	b)Tannins	Antimicrobial	Controlling Biofilm	Al-Shuneigat *et al.*, 2005
2.	Protobiotic Bacteria			
	a)Lactic Acid	Antagonistic	Inhibition of Pathogens	Bielecka et al., 1998
	b)Hydrogen peroxide	Antagonistic	Inhibition of Pathogens	Bielecka et al., 1998
	c)Acetic acids	Antagonistic	Inhibition of Pathogens	Bielecka et al., 1998
3.	Lactic acid bacteria			
	a)Bacteriocin	Antimicrobial	Anticarcinogenic	Fools et al., 1999
	b)Organic acids	Antimicrobial	Lactose tolerance	Hirayama & Rafter,2000
	c)Hydrogen peroxide	Antimicrobial	Reduces intestinal disorders	McNaught and MacFie, 2001
	d)Lacto Peroxidase	Antimicrobial	Anti Cholesterol	Zubillaga et al., 2001
	e)Diacetyl	Antimicrobial	Inhibition of Pathogens	Kamala, 1997
	f)Ethanol	Antimicrobial	Inhibition of Pathogens	Wilcox, 2003
	g) CO_2	Antimicrobial	Inhibition of Pathogens	Fools et al., 1999

TABLE 6
TOTAL U.S SALES AND FORECAST OF FUNCTIONAL FOODS AT CURRENT PRICE, 2002-2012

S. No.	Year	$million	%Change	Index 2002=100	Index 2012=100
1.	2002	1,620	-	100	61
2.	2003	1,666	2.8	103	63
3.	2004	1,776	6.6	110	67
4.	2005	2,041	14.9	126	77
5.	2006	2,385	16.9	147	90
6.	2007	2,646	10.9	163	100
7.	2008	2,879	8.8	178	109
8.	2009	3,117	8.3	192	118
9.	2010	3,359	7.8	207	127
10.	2011	3,611	7.5	223	136
11.	2012	3,874	7.3	239	146

Globally, digestive health claims are leading functiobal foods. A report also showed that functional foods and beverages providing digestive health benefits are growing, both in the traditional categories where the claim emerged for yoghurt and dairy based beverages are new and unique. New categories such as prepared meals and snack mixes (Prepared foods 2009). In coming years functional foods are expected to grow continuously. This trend stems from an ever growing number of products capitalizing on natural ingredients and providing health benefits. Table shows the top 10 U.S digestive health subcategories by number of new product introduction up to 2012.

TABLE 7

TOP U.S DIGESTIVE HEALTH SUBCATEGORIES (BY NUMBER OF NEW PRODUCT INSTRUCTIONS)

S. No.	Category	2012	2011	2010	2009
1.	Spoonable yoghurt	42	28	24	0
2.	Cheese	7	15	1	0
3.	Dairy products	7	1	0	0
4.	Snack & bars	5	9	2	0
5.	Soy yoghurt	5	0	0	0
6.	Juice	4	2	0	1
7.	Prepared meals	4	0	0	0
8.	Hot cereals	3	2	0	0
9.	Liquid cultured milk	2	6	1	1
10.	Cold cereals	2	3	1	0
11.	Snack mixes	2	0	0	0

Source: Mintel GNDP (through May 2012); Prepared FOODS (2008).

Although bioactive compounds in milk and dairy products have been extensively studied the last couple of decades, there are very few publications available for dairy animal species that provide valuable information especially in developing countries of Asia and Africa

4.1 Effect of Carbon Sources

Carbon compounds constitute the major requirement for growth as they enter in different metabolic process resulting in the production of bioactive compounds (Gebreel *et al.,* 2008). The quantity of carbon source in the basal liquid medium affects the production of antimicrobial substances against plants pathogenic fungi. Dikin *et al.* (2005) reported that the quantity of **lactose** in the basal liquid medium affected the production of antimicrobial substances by *Burkholderia cepacia* RB47 and *Microbacterium testaceum* RU7 against *Schizophyllum commune*. Wicklow *et al.* (1998) reported that optimal quality of antimicrobial substances very much affected by the quantity of **Maltose, Dextrose, Glucose, Starch** etc as these in turn effect the density of bacterial cells.

4.2 Effect of Metal Ions

Several metal ions affect antibiotic production including both promotion and inhibition of antibiotic biosynthesis, which are caused at steps of transmethylation (**Co^{2+}**), biodegradation (**Cu^{2+}** and **Mg^{2+}**), and others. **Zeolite, Kaoline, Celite,** and other natural minerals interact with microbial cells to suppress pellet formation which leads to enhancement of antibiotic production. Increased uptake of oxygen and nutrients, which if occurs along with suppressed pellet formation, favors secondary metabolite **biosynthesis**. Cyclodextrins form inclusion complexes with higher fatty acids, thereby rendering lipophilic compounds water soluble, volatile substances, non-volatile and unstable substances becomes more stable, and compounds that are susceptible to microbial attack become resistant (Oswada et al., 1987).

4.3 Effect of Temperature

The effect of temperature on antibiotic production is similar to that of pH. Cryophilic, Mesophilic, and Thermophilic microorganisms must be cultivated under conditions of low **(0˚- 15˚C),** physiological **(20˚- 40˚C),** and high **(>50˚C)** temperatures, respectively. Antibiotic production varies when growth temperature are varied within any one of the three temperature ranges. A cryophilic strain of *Streptomyces* produced an antibacterial antibiotic A-60 at 15˚C, but did not so at 28˚C (Ogata et al.,1977).

4.4 Effect of Aeration, Agitation and Pellet Formation

Oxygen, absolutely necessary for aerobic growth of microorganisms, is dissolved into culture fluids by shaking culture flasks on a rotary (or reciprocal) shaking machine or by aeration and agitation in a jar fermentor. **Carbon dioxide** is dissolved together with oxygen when air is used, and both of these gases affect antibiotic production. Dissolved oxygen tension decreases to its lowest level when growth rate is maximum. If the minimum level reaches zero, the antibiotic biosynthetic machinery appears to be damaged in some organisms, and antibiotic production does not start when the normal production phase is entered.

According to Arai et al., (1976), when a strain of *Streptomyces* sp. was grown in a jar fermentor with agitation at 500 rpm, the minimum oxygen tension was sufficiently high to allow later co production of mimosamycin and chlorocarcins A, B and C. When the agitation was reduced to 250 rpm, the minimum oxygen tension reached zero, and no production of these two antibiotics occurred, however, production of Streptothricin did begin. Tetracycline production by *S.aureofaciens* was susceptible to low oxygen tension but was not to CO_2.

5 CONCLUSION

Natural products are a very important resource for the elaboration of medicines. Although a big number of plants, microbes and marine resources have been evaluated in the search of new bioactive compounds, it turns out to be insufficient and it is necessary and important to continue with the search of new secondary metabolites, especially those that are associated with fruit salads and other edibles. Extensive exploration of antibiotics has lead to development of bacteria strains that are resistant to many of the known drugs apart from search for new antibiotic compounds. The treatment with chemotherapy for the diverse causes of different types of cancer that are present today, appears effective, so the investigation becomes necessary in the chemistry of the natural products. The methods of bacterial culture and identification have become very promising especially, those done through molecular techniques, by which it is possible to identify a strain up to species and sometimes at subspecies level. The diverse relationships that exist between microorganisms provoke that bacterial compounds can eventually be used for human well-being.

6 FUTURE ASPECT

The strategies for drug discovery has been evolving constantly, today researchers do not conform with the finding of new and potent metabolites, now and for future days it has become important to do phylogenetic studies, structure elucidation of chemical compounds, bioinformatics approaches, genomics, proteomics, reverse pharmacology and so on. One fundamental field that has to be developed is the improvement of more efficient culture media, because we need to culture all the strains of potential microorganism to be able to evaluate and separate its bioactive compounds. At the same time we can identify the genes of cultivable and non-cultivable bacteria by molecular techniques to compare and try to demonstrate that the strains of bacteria with very similar DNA sequences have equally similar metabolites and culture requirements, if this is true, we can further work on the properties exhibited by different bioactive compounds. Methodological improvements studies based on the characterization of the bioactive compounds produced by microorganisms and a better understanding of host-microbe interactions should be used to provide further insight into the adaptive strategies against microbial pathogens that is how secondary metabolites regulate microbial interactions. The new soft ionization methods: Matrix-Assisted Laser Desorption Ionization (MALDI) and Electrospray Ionization (EI) are the recent approach used in a variety of new and innovative Mass Spectrometric (MS) applications. With them, is easier to analyze surfaces, they are tolerant to impurities and do not require extensive sample preparations. A sensitive and precise Mass Spectrometric approach like Desorption Electrospray Ionization (DESI) should be used to measure the physical location and quantities of natural products on biological tissue surfaces, cells or even complex mixed-species assemblages. These imaging techniques known as "molecular eyes" are very precise and represent the last technological advance used to locate natural products in biological tissues (Esquenazi et al., 2009) allowing the study of the interface between the confluence of natural products chemistry, biology and ecology. Bioinformatics is the part of molecular biology that involves working with biological data, typically using computers, with the goal of enabling and accelerating biological research. Bioinformatics comprises a wide range of activities: data capture, automated recording of experimental results; data storage and access, using a multitude of databases and query tools; data analysis; and visualization of raw data and analytical results (Pollock & Safer, 2001).Today, many recently developed bioactive compunds with antibacterial and anticancer activity fail in clinical trials because of inefficiency for the anticipated indication or unexpected toxicity (Kola & Landis, 2004). Apparently, it remains hard to establish a clear link between antagonism or organism of a specific target and its influence in human illness and its target associated toxicity. A significant cause for these high attrition rates is the often misjudged complexity of protein function in higher order organisms, in which, abundant protein-protein interactions, feedback loops and redundancies play a role. The collection of recognized pathways that can be found in public databases and commercial tools do not effectively address these issues because they are mainly a reflection of experimental data that are obtained from isolated cell lines and tissues. They address typically, the signaling events that lead to binding of transcription factors to the DNA, but do not detail the pleiotropic effects that arise downstream from the induced transcriptional program (Pollock & Safer, 2001). Most comparative genomics tools are intended at studying conservation of single genes or gene families, whereas computationally tools address orthologous biology, i.e. Conservation of the entire pathways in which the target is involved, are unusual. This truly obstructs the output and success of translational investigation from preclinical to clinical studies (Pollock & Safer, 2001). The developing of bioinformatics tools that addresses the above problems will allow for quicker and better experiments aimed at evaluating multiple targets and drugs for further clinical development. This will be a first step to reduce the high attrition rates associated with drug development (Pollock & Safer, 2001). Clinical events or phenomena not reported previously following the administration of a known or new drug can offer valuable perceptions for drug development. Natural products have provided many such unexpected bedside interpretations. Researches in genomics, proteomics and metabolomics have stimulated the discovery of many new molecules, which are yet to be tracked for their drug-like activities. A new discipline called Reverse Pharmacology (RP) has been designed to decrease costs, time and toxicity. The scope of reverse pharmacology is to understand the mechanisms of action at multiple levels of biology and to optimize safety, efficacy and acceptability of the leads in natural products based on relevant science in this approach, as the candidate travels a reverse path from 'clinics to laboratory' rather than classical "laboratory to clinics". Actual humans are used as the ultimate model and in-depth investigation of the effects of drugs and the nature of disease progression is becoming ever more feasible because of advances in clinical biomarkers and systems biology.

ACKNOWLEDGMENT

Richa Shukla duly acknowledges the support provided by the Department of Biotechnology, Birla institute of technology under the guidance of Dr V K Nigam as a part of her project work. The author also wishes to thanks Mrs Urmila Shukla Mr Y M Shukla and Amulya Gaurav for their contribution.

REFERENCES

[1] Abdel-Gawad, A.M. (2002). Biological Control of Some Tomato Diseases Caused by Fusarium spp. and Alternaria spp. Ph.D. Thesis, p: 106. Faculty of Science, Cairo University, Cairo, Egypt

[2] Al-Shuneigat J., Cox S.D. and Markham J.L.,(2005).Effects of a topical essential oilcontaining formulation on biofilm-forming coagulase-negative staphylococci. Lett. Appl. Microbiol., 41: 52-55.

[3] Arai, K., Rawlings, B.J., Yoshizawa, Y. and Vederas, C., (1989). Comparision of stereochemistry of polyketide and fatty acid enoyl thiol ester reductase. J. Am.Chem.Soc.111, 3391-3399

[4] Asha B. B., Nayaka S. C., Udaya shankar A. C., Srinivas C. and Niranjana S. R., (2011). Selection of effective bio-antagonistic bacteria for biological control of tomato wilt caused by Fusarium oxysporum f. Sp. Lycopersici. The Bioscan .6:239 - 244..

[5] Bais Y.G., Nimbekar T.P., Wanjari B.E., Timande S.P., (2012). Isolation of antibacterial compound from marine soil Actinomycetes. International journal of Biomedical and Advance Research, 3:193-197

[6] Bhaskar J., Usman M., Smitha S., and Bhat G. K. (2004): Bacteriological profile of street foods in Mangalore. Indian J. Med. Microbiol. 22, 197-198

[7] Bhattacharyya P. N. and Jha D. K. (2011). Optimization of cultural conditions affecting growth and improved bioactive metabolite production by a sub surface Aspergillus strain TSF 146. International journal of Applied Biology and Pharmaceutical Technology . 11:23 - 29.

[8] Burkholder, P.R.; Pfister, R.M. & Leitz, F.H. (1966). Production of a Pyrrole Antibiotic by marine bacteria. Applied Microbiology. 14:649-653

[9] Collins J. K., Thornton G., Sullivan G. O., (1998). Selection of probiotic strains for human application. Int. Dairy J., 8, 487–490

[10] Dikin A., Sijam K.N., Kadir J. and Seman I.A. (2007). Effect of Different Carbon Sources and Peptones on the Production of Antimicrobial Substances from Bacteria against Schizophyllum commune FR. International Journal Of Agriculture & Biology. 14:113 - 129.

[11] Dikin. A., K. Sijam, J. Kadir and A.S. Idris, (2005). Extraction of Antimicrobial Substances from Antagonistic Bacteria against Schyzophyllum commune Fr. In: Proc. 27th Malaysian Microbiology Symposium. Innovation through Microbes. Grand Plaza Parkroyal Penang. Malaysia, 24-27 November 2005

[12] Duffy B.K. and G Defago, (1999). Environmental factors modulating antibiotic and siderophore biosynthesis by Pseudomonas fluorescens biocontrol strains. Appl. Environ. Microbiol., 65: 2429–2438

[13] Ekelund F., Ronn R. and Christensen S., (2001). Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. Soil Biol Biochem 33:475-48

[14] El-Banna N.M., (2006). Effect of carbon sources on the antimicrobial activity of Corynebacterium kutscheri and Corynebacterium xerosis.African J. Biotechnol., 5: 833–835

[15] Esquenazi E., Coates R.C., Grindberg R.V., Ishoey T., Brinda D., (2009) Single Cell genome amplification accelerates identification of the apratoxin biosynthetic pathway from a complex microbial assemblage. PLoS ONE 6: e 18565

[16] Gebreel, H.M., El-Mehalawy A.A., El-Kholy I.M., Rifaat H.M. and Humid A.A., (2008). Antimicrobial Activities of Certain Bacteria Isolated from Egyptian Soil Against Pathogenic Fungi. J. Agric. Biol. Sci., 4: 331–339

[17] Hannusch, D.J. and G.J. Boland, (1996). Interaction of air temperature, relative humidity and biological control agents on grey mould of bean. European J. Plant Pathol., 102: 133–142

[18] Heydari, A. and I.J. Misaghi, (1998). Biocontrol activity Burkholderia cepacia against Rhizoctonia solani in herbicide-treated soil. Plant Soil, 202: 109–116

[19] Hirayama K, Rafter J (2000). The role of probiotic bacteria in cancer prevention. Microbes Infect. 2: 681-686.

[20] Ismail Kola, Ph.D. (Med), is Senior Vice-President of Basic Research at Merck Research Labs, 126 East Lincoln Avenue, Rahway, New Jersey 07075, USA.

[21] John Landis, Ph.D., is Senior Vice-President Pharmaceutical Sciences and Compliance Clinical Sciences at Schering-Plough Research Institute, 2000 Galloping Hill Road, Kenilworth New Jersey 07033, USA.

[22] Khamna S., Yokota A., Peberdy J.F., Lumyong S., (2009). Antifungal activity of Streptomyces spp. isolated from rhizosphere of Thai medicinal plants. Int. J. Integr. Biol., 6(3): 143-147.

[23] Kobayashi M., Aoki. S., Sakai H., Kawazoe K., Kihara N., Sasaki T., Kitagawa I., Altohyrtin A., (2000). A potent anti-tumor macrolide from the Okinawan marine sponge Hyrtios altum. Tetrahedron Lett., 34, 2795–2798

[24] Kurze, S. and H. Bahl, (2001). Biological control of fungal strawberry diseases caused by Serratia plymuthica HRO-C48. Plant Dis., 85: 529–534

[25] Lee, Y. K., Senthilkumar, M., Kim, J. H., Swarnalakshmi K., Annapurna K., (2008). Purification and partial characterization of antifungal metabolite from Paenibacillus lentimorbus WJ5. World J. Microbio. and Biotechno. 24 (12), 3057- 3062.

[26] Nakamura, H.; Iitaka, Y.; Kitahara, T.; Okazaki, T.; Okami, Y. (1977) Structure of asplasmomycin. Journal of Antibiotics, 30, 714-719

[27] Newman D. J. and Cragg G. M. (2007), "Natural products as sources of new drugs over the last 25 years, "Journal of Natural Products, vol. 70, no. 3, pp. 461–477.

[28] Ogata K,Osawda H,Tani Y (1977) J Ferment Technol 55:28

[29] Okami, Y. (1986). Marine microorganisms as a source of bioactive agents. MicrobialEcology. 12:65–78

[30] Pandey B, Ghimire P, Vishvanath P, Agrawal T.,(2004), Study on the antibacterial activity of the Actinomycetes isolated from the Khumbu Region of Nepal, Proceeding from, the great Himalays: Climate, Health, Ecology, Management and conservation Kathmandu; 12-15.

[31] Pollock, S. & Safer, H. M. (2001). Bioinformatics in the Drug Discovery Process. Annual Reports in Medicinal Chemistry

[32] Rajvanshi A., Kulshreshtha S., Nigam V. K., Gothwal R. K. and Pareek R. P., (2011). Antagonistic properties of different bacteria isolated from salads. Asian J. Exp. Science. 25:45 - 51.

[33] Rajvanshi A., Nigam V. K., Kulshreshtha S. and Pareek R.P., (2012).Characterization of bacteriocin production from a new lactic acid bacteria. World journal of pharmacy and pharmaceutical sciences.1:100 - 1112.

[34] Rajvanshi, A., (2010). Bacterial load on street vended salads in Jaipur city, India. Internet J food Safety., 12, 136-139.

[35] Shank, R. C. (1974). "Role of Aflatoxin in Human Disease". In Rodricks, J.V. (ed.), Mycotoxins and other fungal related food problems. American Chemical Society, Washington, D.C. pp. 51-58.

[36] Shiozawa H, Kagasak T, Kinoshita T and Haruyama H,Thiomarinol,(1993) a new hybrid antimicrobial antibiotic produced by a marine bacterium, J Antibiot, 46, 1834- 1841.

[37] Shoda, M. (2000). Review: Bacterial control of plant diseases. Journal of Bioscience and Bioengineering 89: 515-521.

[38] Silo-suh, L.A., Lethbridge, B.J., Raffel, S.J., He, H., Clardy, J. and Handelsman, J. (1994). Biological activites of two fungistatic antibiotics produced by Bacillus cereus UW85. Applied and Environmental Microbiology 60: 2023-2030

[39] Slininger, P. and M.A. Shea-Wilbur, (1995). Liquid culture pH, temperature and carbon (not nitrogen) source regulate phenazine productivity of teke-all biocontrol agent Pseudomonas fluorescens, p. 2-79. Appl. Microbiol. Biotechnol., 43: 794–800

[40] Strobel G. A. (2003).“Endophytes as sources of bioactive products,” Microbes and Infection, vol. 5, no. 6, pp. 535–544.

[41] Tayung, K. and Jha, D. K.(2007). Antimicrobial activity of a compound produced by Aspergillus sp.DEF 505, an endophyte on Taxus baccata. J Microbial World 9: 287-292.

[42] Thakur, D., Bora, T. C., Bordoloi, G. N. and Mazumdar, S. (2009). Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by Streptomyces sp.201. J Med Mycol 19:161-167.

[43] Wang H.M., J.L. Pan, C.Y. Chen, C.C. Chiu, M.H. Yang, H.W. Chang, et al. (2010). Identification of anti-lung cancer extract from Chlorella vulgaris C-C by antioxidant property using supercritical carbon dioxide extraction. Process Biochemistry 45: 1865–1872.

[44] Wang, J., Huang, Y., Fang, M., Zhang, Y., Zheng, Z., Zhao, Y. and Su, W. (2002). Brefeldin A, a cytotoxin produced by Paecilomyces sp. and Aspergillus clavatus isolated from Taxus mairei and Torreya grandis. FEMS Immunol Med Microbiol 34:51-57.

[45] Wang, S.L., Shih, I.L., Wang, C.H., Tseng, K.C., Chang, W.T., Twu, Y.K., Ro, J.J. and Wang, C.L. (2002). Production of antifungal compounds from chitin by Bacillus subtilis. Enzyme Microbial Technology 31:321-328..

[46] Wicklow, D.T., B.K. Joshi and W.R. Gamble, (1998). Antifungal metabolites (Monorden, Monocillin IV, and Cerebrosides) from Humicola fuscoatra Traaen NRRL 22980, a mycoparasite of Aspergillus flavus sclerotia. Appl. Environ. Microbiol., 64: 4482–4484

[47] Wilcox MH (2003). Clostridium difficile infection and pseudomembranous colitis. Best Practise and Research. 17(3): 475-493

[48] Yang, S. S.; Cragg, G. M.; Newman, D. J.; Bader, J. P. (2001), Natural product-based anti-HIV drug discovery and development facilitated by the NCI developmental therapeutics program. J. Nat. Prod., 64: 265–277.

[49] Zhang L., An R., Wang J. et al., (2005), “Exploring novel bioactive compounds from marine microbes,”Current Opinion in Microbiology, vol. 8, no. 3, pp. 276–281.

[50] Zhou, F., Guo, H., Liu, Y., Jiang, Y., (2007). Chemometric data analysis of marine water quality and source identification in Southern Hong Kong. Marine Pollution Bulletin 54, 745–756.