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Review on Antibiotic Potential Microbes

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ABSTRACT
The advent of biotechnology has prompted researchers to attempt the synthesis of pharmaceutical compounds. Some success has been achieved with synthetic combinatorial chemistry and biosynthetic gene cluster manipulations. However, natural product discovery still proves to be one of the best sources for new bioactive chemicals. Nature's superiority in bioactive chemical production and the unknown number of undiscovered compounds provide poignant motivation to protect the aquatic environment from human alteration. The alteration of the delicate aquatic environment due to human pollution has unknown consequences for the undiscovered life forms. The vast diversity of undiscovered life in the aquatic environment affords many opportunities for increasing the arsenal of therapeutic chemicals used to treat human disease. With each new finding we can continue to marvel at nature's ability to produce such complex and beneficial structures.

KEY WORDS: BIOACTIVE CHEMICALS, BIOTECHNOLOGY, THERAPEUTIC CHEMICALS.

INTRODUCTION

French bacteriologist Vuillemin used the term antibiosis for the first time that means "against the life," in the year 1877, later Louis Pasteur and Robert Koch observations came in to light and the word antibiosis renamed as antibiotics by an American microbiologist Selman Waksman, in the year 1942. The idea of microorganisms as a new source of novel pharmaceuticals spurred an extensive search for microbial metabolites of medicinal value. Early efforts led to the discovery and development of several diverse classes of antibiotics which are believed to have added over a decade to man's life span. A majority of the antimicrobials in clinical use today are microbial products, products of microbial origin or are their synthetic/semi-synthetic analogs. However the last three decades have been disappointing as new classes of microbial metabolites worthy of commercialization as antibiotics have not been found. The main focus has been on the semi-synthetic, "me-too" compounds and on synthetic classes the fluoroquinolones and oxazolidinones.

The widespread use of these various antimicrobial agents has resulted in the gradual emergence of multi-resistant pathogens. In response to the threat that these organisms now pose a search for novel agents lacking cross-resistance with the older compounds and perhaps possessing new modes-of-action has begun. Microorganisms from unique ecological niches are being explored, libraries of historical compounds are being reevaluated in the light of current needs and new sub-cellular targets discovered through bacterial genomics are being used for screening. These efforts may result to the discovery of new, more effective antibiotics that will meet current formidable challenges in the concerned field.

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The effect of microbes in the environment has been recognized for centuries but until the accidental discovery of penicillin by Alexander Fleming in 1928 their true beneficial potential was not recognized. This fortuitous event seemingly provided the first scientific clue that microorganisms could be an enormous source of novel pharmaceuticals.

Since then microbial products have been proven to be a rich source of novel compounds with diverse biological activities, Davies(1999); Demain(1998 and 1999), Imada and Hotta,(1992), Bernan et al.,(1997). Although synthetic compounds have continued to play important role in our fight against various diseases (Table.1) The antibacterial and anticancer therapeutic agents currently in clinical use are dominated by either microbial products or one of their analogs, Singh and Greenstein, 2000, Cragg et al.,(1997). The Physicians' Desk Reference of 1999 lists a total of 403 antimicrobial formulations of which 211 are listed as antibacterial (Physicians' desk reference, 1999). This list includes 19 penicillins, 26 cephalosporins, 1 monobactam, 2 carbapenems, 6 aminoglycosides, 5 tetracyclines, 7 macrolides, 6 fluoroquinolones, etc.

Most of these new antibiotics were discovered through extensive screening of microbial fermentations from the year 1940 - 1960 which is also referred to as the 'golden' period of antibiotics. There after conventional screening became less productive and an upsurge in semi-synthetic and synthetic chemistry efforts expanded the number and quality of antibiotics derived from the known classes Lawrence et al.,(1999); Lee et al.,(1999). In the year 1970's the discovery of the monobactams, carbapenems and clavulanic acid by three different microbial screening efforts re established the importance of microorganisms in the discovery of new antibacterial compounds Christopher et al.,(1991). Continued chemical and microbiological efforts provided several superior analogs of various antibiotic classes for the antibacterial market and semi-synthetic approaches are still being pursued to further improve the activities of old compounds.

Table1. List of common antibiotics and their origin.(M.P.Singh and M. Greenstein,Wyeth-Ayerst Research,Pearl River, New York, USA 2000)

	Antibiotics of microbial origin			(Majority of these are produced by *Streptomyces* sp.)
Year	Beta-lactams			
1928		Penicillins: Antibacterial activity of *Penicillium notatum* discovered.		
1940			Natural	Penicillin G, Penicillin V
1960's			Pen'ase resistant	Methicillin, Nafcillin, Oxacillin, Cloxacillin, Flucloxacillin
1960's			Aminopenicillins	Ampicillin, Amoxycillin, Bacampicillin
1970's			Antipseudomonal	Carbenicillin, Ticarcillin, Mezlocillin, Pipracillin
1945		Cephalosporins: Cephalosporin C was the first 6-membered b-lactam isolated from a *Cephalosporium* sp.		
1960's			First Generation	Cephalothin, Cefazolin, Cephapirin, Cephradine, Cephalexin, Cephadroxil
1960's			Second Generation	Cefachlor, Cefamandol, Cefuroxime, Cefonicid, Cefmetazole, Cefotetan, Cefprozil, Ceftibuten
1965			Cephamycin: first cephalosporin isolated from a *Streptomyces* sp., led to the development of Cefoxitin	
1970's			Third Generation	Cefetamet pivoxil, Cefperazone, Cefotaxime, Ceftizoxime, Ceftriaxone, Ceftazidime, Cefixime, Cefpodoxime proxetil
1980's			Fourth Generation	Cefpirome, Cefepime
1973		Monobactam		Aztreonam (synthetic version used)
1976		Carbapenems		Imipenem, Meropenem
1978		Oxacephem		Moxalactam
1980		Carbacephem		Loracarbef
		Lactamase inhibitors		
1973			Clavulanic acid	Amoxycillin+Clavulanate (Augnetin), Ticarcillin+Clavulanate (Timentin)
1980			Sulbactam	Ampicillin+Sulbactam (Unasyn)
1984			Tazobactam	Pipracillin+Tazobactam (Zosyn)
1939	Polypeptides			Polymyxin B (produced by *Bacillus polymyxa*)
1944	Aminoglycosides			Streptomycin, Amikacin, Gentamicin, Isepamicin, Kanamicin, Netilmicin, Sisomicin, Tobramycin, Neomycin

Table 1 Continue

1947	Phenylpropanoid	Chlorampohenicol produced by *Streptomyces venezuelae*
1948	Tetracyclins	Oxyteracycline, Doxycycline, Chlortetracycline, Minocycline
1950	Cyclic peptide	Bacitracin produced by *Bacillus licheniformis* and *Bacillus subtilis*
1950	Macrolides	Erythromycin (various esters derivatives), Azithromycin,
		Clarithromycin, Dirithromycin
1955	Glycopeptides	Vancomycin, Teicoplanin
1955	Lincosamide	Clindamycin, Limcomycin
1955	Lipopeptides/Glycolipopeptide	Daptomycin, Ramoplanin
1955	Streptogramins	Virginiamycin, Synercid (Quinupristin + Dalfopristin)
1959	Ansamycins	Rifampin, Rifabutin
1962	Steroidal antibiotic	Fusidic acid produced by *Fusidium coccineum*
1969	Phosphonate	Fosfomycin (the first C-phosphonate containing microbial metabolite)
1971	Mupirocin	Pseudomonic acid A from *Pseudomonas fluorescens*
	Synthetic antibacterial agents	
1932	Sulfonamides	Sulfadiazine, Sulfixazole, Sulfamethoxazole, Trimethoprim
1959	Nitroimidazoles	Metronidazole
1960's	Anti-TB drugs	Isoniazid, Ethambutol, Pyrazinamide, Thiacetazone, Dapsones
1962	Quinolone	Nalidixic acid, Cinoxacin, Oxolinic acid
1980's	Fluoroquinolones	Norfloxacin, Ciprofloxacin, Ofloxacin, Enoxacin, Lomefloxacin, Pefloxacin, Sparfloxacin, Trovafloxacin, Grepafloxacin
1980's	Oxazolidinones	Linezolid (approved in 2000)

In nature all organisms need to compete in order to survive in their habitats and this Biological task can only be achieved by the development of competitive mechanisms such as the production of toxins, enzymes and antimicrobial agents like antibiotics. The search for antibiotics began in the late 18th century with the growing acceptance of the germ theory of disease, a theory which linked bacteria and other microbes to the causation of a variety of ailments as a result scientists started to devote more time to search for drugs that would kill these disease-causing bacteria without causing any side or adverse effect to the host. An antibiotic is a drug that kills or slows down the growth of bacteria. Antibiotics are one class of "antimicrobials" a larger group which also includes anti-viral, anti-fungal, and anti-parasitic drugs. They are relatively harmless to the host and therefore can be used to treat infection. The term originally described only those formulations derived from living organisms but now applied also to synthetic antimicrobials such as the sulfonamides.

Antibiotics are labeled (magic bullets) drugs which target disease without harming the host. Antibiotics are not effective in viral, fungal and other non-bacterial infections and individual antibiotics vary widely in their effectiveness on various types of bacteria. Some specific antibiotics target either gram-negative or gram-positive bacteria and others are more wide-spectrum antibiotics. The effectiveness of individual antibiotics varies with the location of the infection and the ability of the antibiotic to reach the site. Oral antibiotics are the simplest approach when effective with intravenous antibiotics reserved for more serious cases. Antibiotics may sometime be administered topically as with eye drops or ointments.

The first widely used antibiotic compounds used in modern medicine were produced and isolated from living organisms such as the penicillin class produced by fungi Penicillium, streptomycin from *Streptomyces* but the actinomycetes are the group of prokaryotic filamentous soil microorganims which are known as the top producers of antimicrobial agents especially *Streptomyces* Osborne et al.,(2000), Rondon M., et al(2000). Some of the antibiotics produced by *Streptomyces* are erythromycin, amphotericin, neomycin, streptomycin and rifamycin. Advances in organic chemistry led to contribute many synthetic antibiotics. Many antibiotics are relatively small molecules with a molecular weight less than 2000 Dalton, with different modes of action. Generally they interfere with biological processes such as replication, translation and cell wall synthesis. Some antibiotics like tetracycline interfere with protein synthesis by associating with the 30S ribosomal sub-unit penicillin, produced by Penicillium notatum prevent transpeptidation of N-acetyl-muramic acid resulting in a weakened peptidoglycan structure.

The search for new natural products from aquatic microorganisms has already shown promising results by discovering compounds with possibly useful as anticancer and cardiovascular agents Lei and Zhou (2002). With the worldwide sale of 45 billion dollars, anti-infective compounds represent the third largest therapeutics on commercial sale and is expected to increase by folds Bush(2004). During the last three decades pharmaceutical

companies have been searching for new antibiotics to counter the problem of increasing bacterial resistance. During this period use of new chemical derivatives of pre-existing antibiotics has been the only method available because no novel chemical class of antibiotics has been discovered Burgess(1999).The oceans covers over the 70% of the earth's surface and roughly half of the biodiversity found on this planet is by the aquatic environment with 34 of the 36 phyla of life as represented by Donia and Hamann(2003).

Over the last 40 years aquatic natural product research has become a multi-disciplinary field touching on subjects within biology, chemistry, chemical ecology and pharmacology. During this brief period bacterial secondary metabolites with pharmacodynamic properties encompassing such diverse biological activities as antibiotics, antivirals, antimitotic and antineoplastics have already been documented Kelecom(2002). Other aquatic organisms such as blue green algae, seaweeds, horse shoe crabs, marine fungi and sponge metabolites have also yielded substantial natural products such as steroids, cytotoxic and antimicrobial agents as well as novel and biologically active peptides Blunt et al., (2004).

The bacteria have long been the subject of scientific study due to their ability to cause disease in humans Lederberg(2000). One of the major advances in the health and well-being of human civilizations was the development of antibiotics. Although the introduction of antibiotics has had an enormous impact on the ability to treat bacterial infections bacteria continues to be the leading cause of deaths worldwide. Moreover the effectiveness of antibiotics has been eroded by the appearance of pathogenic strains that are resistant to nearly all classes of antibiotics coupled with the fact that only one new class of antibiotics has been introduced by the pharmaceutical industry since 1970 Binder et al.,(1999).

Soil microbial communities are among the most complex, diverse and important assemblages of organisms in the biosphere. They participate in various biological activities such as mineralization and decomposition of organic matter, biocontrol and antagonism Hackl et al., 2004. It is said that bacteria that are found colonizing soil are ubiquitous since the chemical, physical and biotic characteristics present in such medium vary immensely. Recently Horner et al., 2003 postulated that microbial population can be various in a particle of soil due to oxygen concentration. Sprusansky et al.,(2005) postulated that soil bacteria display amazing versatility in their ability to use relatively poor sources of carbon, nitrogen and thrive on a mixture of complex carbohydrates and proteins that result from the degradation of organic material which demonstrates that soil microorganisms are an important source for the search of novel antimicrobial agents and molecules with various biotechnological importance. Some of the cultivable microbes that most commonly are isolated from soil samples belong to the genera of *Bacillus, Streptomyces* and *Pseudomonas*, Belma et al .,(2002), Stabb et al .,(1994).*Streptomyces* are responsible for the production of over 70% of the antibiotics that have been isolated and reported Dairi (1999), Lo C.(2002). The genus *Pseudomonas* is comprised of a gram-negative bacteria and is vastly involved in biological control of many plant pathogens.

Soil is a diverse medium composed of many minerals and substrates essential for metabolic pathways of prokaryotic and eukaryotic inhabitants Dakora et al., 2002. The abiotic and biotic diversity present in this medium makes it difficult for the isolation of all the microbial community present therefore not even 1% of the entire soil microbial community has been identified Courtis et al.,(2003) , Hackl et al.,(2004), Rondon et al.,(2000). There is great opportunity for discovering new microorganisms of industrial and clinical importance in soil. It is not possible to recreate all of the specific requirements that every soil microorganism needs. That is why standard microbiological techniques and innovative molecular and genetic technologies are being designed Satoshi et al.,(2004), Sprusansky et al.,(2005), Zhou et al., (1996).

Applying these techniques to a given environment one can obtain large quantities of genomic material and study a vast part of a given microbial community. Scientists have developed a new molecular strategies in the field of functional genomics which involves the construction of soil metagenomic libraries for the better understanding of microbial diversity and its possible applications in medical research . Satoshi et al.,(2004). The irrational use of antibiotics has caused an increase in number of multiple drug resistant strains (MDR) of bacteria, fungi and many MDR strains are being reported from the genera *Pseudomonas, Streptococcus* and *Staphylococcus* Chitnis et al .,(2000). Some of these strains are resistant to most used antibiotics including methicillin, cephalosporins, and other beta-lactams that target peptidoglycan synthesis. Others have gained resistance toward neomycin and streptomycin which attack the bacterial ribosome.

Antibiotic resistance got lot of attention in many forms including the recent developments in which the superbug NDM made lot of noise in news at global level there fore the hunt for the novel antibiotics from nature is the need of the day. NDM-1 is a newly-identified enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics. United Kingdom Health Protection Agency has stated that "most isolates with NDM-1 enzyme are resistant to all standard intravenous antibiotics for treatment of severe infections NDM-1 is known as New Delhi Metallo-Beta-Lactamase. This bacterium which was found recently is called the Superbug because of its efficiency to resist almost all antibiotics invented. NDM 1 has high resistance to survive against antibiotics such as carbapenems and beta-lactams which is its dangerous side. Though the investigations are on it has already effected India's medical tourism. Infact US President Obama has issued an advisory to US nationals

not to go to the cheaper treatment in India to avoid the MDR. It is well understood that antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal; the underlying molecular mechanisms leading to antibiotic resistance can vary. Intrinsic resistance may naturally occur as a result of the bacterial genetic makeup.

At the molecular level it is assumed that the bacterial chromosome may fail to encode a protein that the antibiotic targets or an acquired resistance may result from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. The spread of antibiotic resistance mechanisms occurs through vertical transmission of inherited mutations from previous generations and genetic recombination of DNA by horizontal genetic exchange.

Antagonism: Antagonism may occur for space or for a common resource. Nair and Simidu 1987 hypothesized that antagonistic properties are linked to the tropic status of the habitats. Studies by the authors Burgess et al.,(1991) show that in shallow areas such as Tokyo Bay, organic nutrients derived from metabolic processes or death and decay of massive phytoplankton population and constant nutritional inputs from external sources assuage the necessity for bacterial populations to produce antibacterial substances to survive competition. Auto-inhibition acts as a controlling factor in maintaining species diversity by allowing the population to partially limit itself and co-exist with competitors. Many marine free-living and sediment-inhabiting marine bacteria have been shown to produce secondary metabolites that display antibacterial properties Burgess et al., (1991).

Antibacterial activity has been widely exploited for the past 50 years and antibiotics have revolutionized Medical science by providing cure for formerly life-threatening diseases. Microbial populations have a resilient dynamic stability produced by biological buffering from competition. Microorganisms compete for nutrients, oxygen and favorable ecological niches and are selective for their tolerance towards ambient conditions i.e. pH, carbon dioxide, water and microbial toxins Baker(1980). Microorganisms secrete metabolites some of which inhibit other microorganisms (antibiotics) while others stimulate other microorganisms to form essential stages of their life cycle. A negative interaction can therefore directly inhibit a pathogen or inhibit a stimulatory microorganism there by indirectly inhibiting the pathogen. The aquatic environment harbors a wide range of microbes capable of exhibiting bacteriolytic and antibiotic activity. Bacteriolytic activities were found to be higher in the zooplankton than in sea water and the major group isolated were the gram negative bacteria in particularly the *Vibrio parahaemolyticus* followed by the gram positive strain, *Staphylococcus aureus* Nair et al., 1985. There are reports on such bacteria from nutrient-rich algal surfaces Jensen and Fenical, (1994); Bernan et al.,(1997).

The bacteriolytic bacteria are mainly inhabitants of the places where the organic matter is high and contribute to its decomposition Nair et al.,(1985).A number of surface-associated marine bacteria have been found to produce antibiotics. Trischman et al.(1994) isolated a species of *Streptomyces* from the surface of a jellyfish. Though the property of production of antimirobial compounds is constitutive, Patterson and Bolis(1997) observed that chemical signals received from potential competitor strains elicit an antagonistic response. However, this aspect still remains a little studied phenomenon Mearns-Spragg et al.,(1997); Mearns-Spragg et al.,(1998). In contrast to antibiotics which promote interspecies antagonism, bacteriocins are responsible for intra specific antagonism. Colicin produced by Eschereshia coli has been studied extensively and similarly there are brevicin, nisin, pediocin produced by various groups.

Bacteriocin produced by *Halobacterium mediterranei* ATCC 33500 has been shown active against many other halobacteria Meseguer et al., 1985. Bacteriocin -producing bacteria can change their strategy from anti- to pro-biotic depending on the environment. Therefore a brevicin producer could be skillfully used as probiotic to ward off unwanted microbes or to mitigate pathogenesis. A deep sea pigmented *Brevibacterium* sp has been shown to produce linocin-like compound that can be used as probiotic in aquaculture feeds. The extracts of this bacteria have not only been suggested to be useful in prolonging shelf-life of dairy products but the culture per se could be used as probiotics and also as feed additives in aquaculture Loka Bharathi et al., (2003).

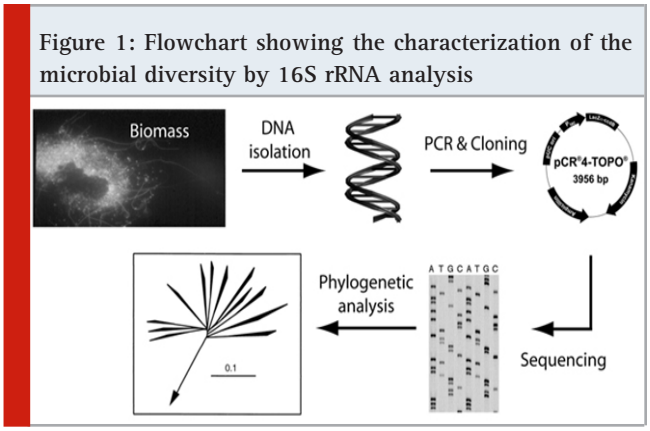
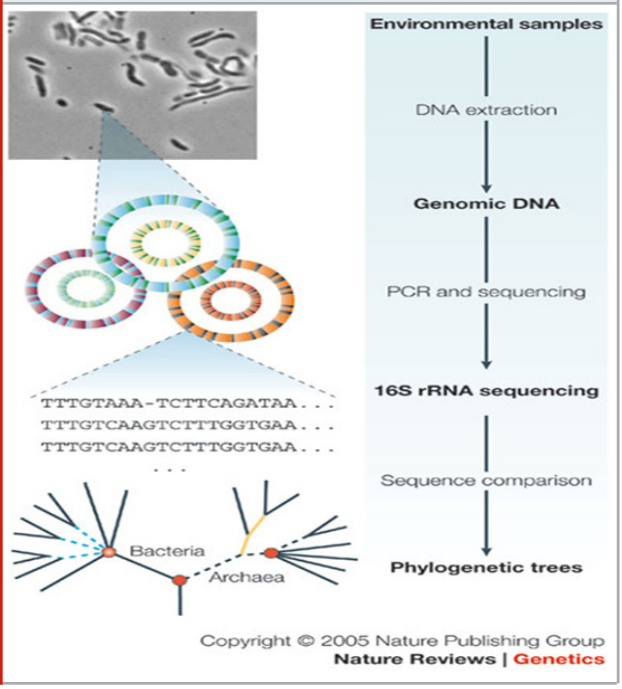


Figure 1: Flowchart showing the characterization of the microbial diversity by 16S rRNA analysis

16s rRNA sequencing & Metagenomics: There are various methods to identify, characterize and exploit the microbes and the most accepted and popular technique of bacterial identification and classification is 16s rRNA gene sequencing in bacterial systematics. The rRNA is the most conserved (least variable) gene in all cells. Portions of the rDNA sequence from distantly-related organisms are remarkably similar. This means that the sequences from the distantly related organisms can be precisely aligned making the true differences required for easy measurements, therefore the genes that encode the rRNA (rDNA) have been used extensively to determine taxonomy, phylogeny and to estimate rates of species divergence amongst the bacteria. The comparison of

16s rDNA sequence can show evolutionary relatedness amongst microorganisms. This work was pioneered by Carl Woese 1977 who proposed the three Domain system of classification - Archaea, Bacteria and Eucarya - based on such sequence information.

Figure 2



Prokaryotic microorganisms comprise the largest part of the earth's total biomass. This group contains a vast array of species, with enormous genetic, metabolic, physiological and behavioral diversity. However less than 1% of them have been cultured. Despite their ubiquity very little is known about their fundamental properties, range of diversity, interaction with the environment, evolution and the role they play in global biogeochemical cycles Rodrìguez-Valera,(2004). It is believed that progress towards filling these knowledge gaps will advance significantly when more whole genome sequences become available for the investigations.

The current availability of bacterial genome information originated from molecular biology accomplishments and made available hundreds of protein-protein interactions based solely on sequence comparisons. Moreover genome sequence information can now be coupled with other experimental data (structures, domain shuffling, expression patterns, and gene adjacency in genomes) to allow new approaches to determine gene function. Nowadays genomics and especially metagenomic approaches contributing advancement in knowledge and understanding of microbiology, since it is not possible to transform a bacterial strain, delete gene information or manipulate any level of protein expression of non-culturable bacteria using traditional classical genetics techniques. The information derived from whole-genome sequences following their comparative analysis can be used to study the novel aspects of biochemistry, physiology and metabolism of these organisms to investigate the role of microorganisms played in complex ecosystems and in global geochemical cycles, study their diversity and to predict the impact of microorganisms on the productivity and sustainability of agriculture, forestry and safety and quality of food supply. Simultaneously new genome sequences can be used to infer phylogenetic relationships among prokaryotes that deal with the organization and evolution of microbial genomes, mechanisms of transmission, exchange and reshuffling of genetic information Koonin(1997).

Figure 3: Rooted universal phylogenetic tree as determined by comparative analysis of ribosomal genes sequences. The data supports the discrimination of three domains, two of which contain prokaryotic representatives (Bacteria and Archaea). The root represents the position of a suspected universal ancestor of all cells. In dashed lines are indicated phylogenetic groups which are exclusively thermophilic or contain few thermophilic representatives (modified from Madigan et al 1997).

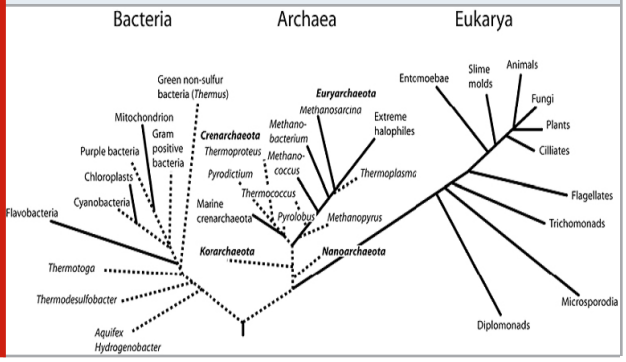
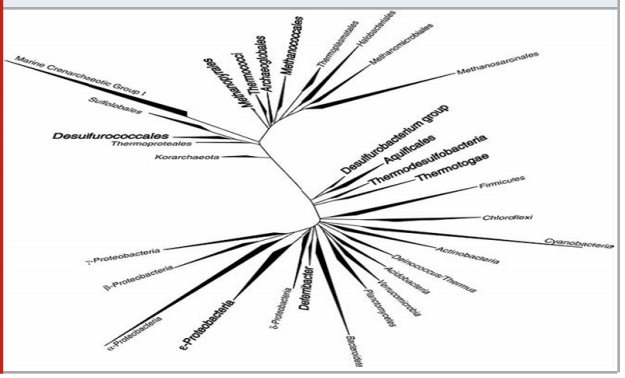


Figure 4: 16S rRNA-based tree showing the major groups of Archaea and Bacteria.



The ability to culture a microbe certainly assists the sequencing of genomes. Some laboratories have already developed techniques to sequence organisms without ever culturing them Kemmer and Fraser(2002). This technique is important for those organisms that are not well understood or those live in very complex environments like extreme habitats, deep sea, rocks etc. Nelson(2003). This technique allows scientists to discover new enzymes, antibiotics and other microbial products useful in various biotechnological applications

including medicine and industry. Another application of sequencing directly from environment gives better understanding of the soil metagenome, metagenome of a healthy vs. diseased individuals. The gene pools present in a prokaryotic species can be order of magnitude larger that that of the genome of a single strain. Contrasting with eukaryotic genomes the repertoire of genes present in a prokaryotic cell does not correlate stringently with its taxonomic identity. Therefore the gene catalogues from a particular environment may provide more meaningful information than the classical species catalogues.

The industrial sector and researchers have employed great efforts searching for novel antimicrobial agents. They have screened many types of soils in order to culture antimicrobial agent producing microbes. One of the biggest problem that groups encountered is the rediscovery of the same antimicrobial agents. Zachner and Fiedler(1995) stated that there is 99% of redundancy when searching for antimicrobial agents. The problem observed can be vastly related with the fact that 99% of the entire soil microbial population cannot be cultivated by conventional microbiological techniques.

Different approaches such as culture modification techniques in which culture media are prepared with ecological extracts and the use of density gradients for the separation of microbes based on cellular density are being used in order to culture the uncultivable, as mentioned above a limitation is that most of the time these methods render the same group of cultivable microbes resulting in the isolation of similar antimicrobial agents. In order to fulfill the need for novel antimicrobial screening methods significant contributions had been made by DeLong(2002), Gillespie et al.,(2002), Handelsman and Wackett (2004) using metagenomic tools and techniques leading to the construction of many metagenomic libraries.

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