VISVESWARAIAH TECHNOLOGICAL UNIVERSITY

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"ISOLATION AND CHARACTERIZATION OF ACTINOMYCETES FROM SOIL TO PRODUCE VARIOUS BIOACTIVE COMPOUNDS WITH SPECIAL REFERENCE TO L-ASPARAGINASE"

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INTRODUCTION:

Actinomycetes are gram positive, filamentous bacteria having high g+c (>55%) content in their dna which shows marked chemical and morphological diversity which were originally considered to be an intermediate between bacteria and fungi but now are categorized as prokaryotic organisms. populations of actinomycetes in an ecosystem are determined by numerous physical, chemical and biological factors. actinomycetes are mostly free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. soil population varies with the soil type.

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century (Alanis 2005). *staphylococcus aureus*, for instance, a virulent pathogen that is responsible for a wide range of infections including pimples, pneumonia, osteomyelitis, endocarditis and bacteremia, has developed resistance to most classes of antibiotics (Enright 2003). for more than two decades, clinicians and public health officials have faced hospital acquired methicillin-resistant *s.aureus* (MRSA), which also bears resistance to many antibiotics. during much of this time, vancomycin has been the therapeutic answer to mrsa, but that paradigm has changed. vancomycin resistant strains have emerged clinically (Hiramatsu 1998, Bozdogan et al. 2003, Chang et al. 2003, Anonymous 2004). vancomycin-resistant *s. aureus* (vrsa) challenges clinicians, not only because of vancomycin and methicillin resistance, but also because of resistance to many other antibiotics, including aminoglycosides, macrolides, and fluoroquinolones. fortunately, newer therapeutic agents, daptomycin, linezolid, and a streptogramin combination (quinupristin/dalfopristin) have entered the clinical arena in the past few years (levy and Marshall 2004, Wenzel 2004). However, certain undesirable side effects and the spread of pathogens with this new antimicrobial drug resistance emphasize the need for the development of other newer antimicrobial agents with activity against such gram positive bacteria (Jevitt et al. 2003, Meka and gold 2004, Wenzel 2004, Nathwani 2005). another cause of great concern is the gram negative antibiotic-resistant opportunistic pathogens. gram negative environmental and enteric organisms currently threaten patients in hospitals and communities with multi-drug resistance, including broad resistance to first, second, and third generations of penicillin's and cephalosporin's (Urban et al. 2003, Obritsch et al. 2004, Paterson et al. 2004).

These bacteria, like *Pseudomonas aeruginosa*, are common environmental organisms, which act as opportunistic pathogens in clinical cases where the defense system of the patient is compromised (Lyczak et al. 2000). In addition, other intrinsically antibiotic resistant organisms such as *Stenotrophomonas maltophilia* (Saiman et al. 2002) are emerging as opportunistic pathogens. The end result of this phenomenon is that many strains of bacteria have become resistant, and in many cases multi-resistant to these therapeutic agents, thus rendering these drugs ineffective as treatments of

choice for severe infections caused by these pathogens (Alanis 2005). Rising numbers of antibiotic un-responsive infectious disease agents confront patients worldwide (Levy 2002,Livermore 2003) and consensus has emerged that it is essential that novel antibiotic classes be developed as part of the strategy to control the emerging drug-resistant pathogens (Projan 2002, Abbanat et al. 2003, Barrett and Barrett 2003). In response, there is a renewed interest in discovering novel classes of antibiotics that have different mechanisms of action (Spizek and Tichy 1995, Barsby et al. 2001). Search for new antibiotics effective against multi-drug resistant pathogenic bacteria is presently an important area of antibiotic research. Natural products having novel structures have been observed to possess useful biological activities. Soil is a natural reservoir for microorganisms and their antimicrobial products (Dancer 2004). Filamentous soil bacteria belonging to the genus *Streptomyces* are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics (Williams et al. 1983a, Crandall and Hamil 1986, Williams et al. 1989, Korn-Wendisch and Kutzner 1992). Of all known drugs 70% have been isolated from Actinomycetes of which 75% and 60% are used in medicine and agriculture respectively (Miyadoh 1993, Tanaka and Mura 1993). The genus *Streptomyces* was proposed by Waksman and Henrici for aerobic and spore forming Actinomycetes (Williams et al. 1989). Indeed, different *Streptomyces* species produce about 75% of commercially and medically useful antibiotics. They have provided more than half of the naturally occurring antibiotics discovered to date and continue to be screened for useful compounds (Miyadoh 1993). In the course of screening for new antibiotics, several studies are oriented towards isolation of Streptomycetes from different habitats. The order *Actinomycetales* is composed of approximately 80 genera, nearly all from terrestrial soils, where they live primarily as saprophytes. All members of the order are characterized in part by their high G-C DNA content, and most exhibit a highly differentiated developmental life cycle. Several recent systematic reevaluations of the order reflected more closely the integration of molecular data (16S rRNA gene sequences). This reorganization resulted in the placement of the two most prolific producers of secondary metabolites, *Streptomyces* and *Micromonospora*, in the families *Streptomycetaceae* and *Micromonosporaceae*, respectively. The diversity of actinomycete secondary metabolites is unrivaled and unmatched in medical significance. Structurally and functionally diverse bioactive compounds have also been isolated from other prokaryotes, including members of the myxobacteria (e.g., *Sorangium*) and cyanobacteria (e.g., *Nostoc*), as antibiotics with antimicrobial, antiviral, and antitumor activities. Recently, the rate of discovery of new compounds from existing genera obtained from terrestrial sources has decreased, while the rate of re-isolation of known compounds has increased. Moreover, the rise in the number of drug-resistant pathogens and the limited success of strategies such as combinatorial chemistry in providing new agents indicate an uncertain forecast for future antimicrobial therapy. Thus, it is critical that new groups of microbes from unexplored habitats be pursued as sources of novel antibiotics and other small-molecule therapeutic agents.

Actinomycetes form a large and important segment of the micro flora of most natural environments. Soils, freshwater, lake and river bottoms, manures and compost contain an abundance of these organisms. They are of universal occurrence in nature, living and multiplying in both cold and tropical zones, and have been reported to occur even under the most extreme conditions of the desert. The temperate zones are, however, generally most favorable for their development. Actinomycetes

participate in many important biochemical processes in the soil. Most investigations of actinomycetes, and especially of their antibiotic activity, have dealt with isolates from soils of the temperate zones (Nakhimovskaia 1937S; chatz and Hazen 1948; Waksman *et al.* 1941; Strzelczyk and Strzelczyk 1958) and the tropics (Meredith 1944; Johnstone 1947). The antibiotic activity of actinomycetes derived from soils of northern Canada has been studied by Landerkin *et al.,* (1950) and Peterson (1954). However, actinomycetes of remote arctic soils have in general received less attention than bacteria (Boyd 1958; Boyd and Boyd 1964; McBee and McBee 1956; Brockman and Boyd 1963) or fungi (Cooke and Lawrence 1959; Cooke an Fdo urnelle 1960; DiMenna 1966).

Actinomycetes are noteworthy as antibiotic producers especially *Streptomyces* spp., making three quarters of all known products; *Streptomyces* are extremely prolific and can produce a great many antibiotics and other classes of biologically active secondary metabolites. They cover around 80% of total antibiotic product, with other genera trailing numerically; M*icromonospora* is the runner up with less than one tenth as many as *Streptomyces*. If we include secondary metabolites with biological activities other than antimicrobial actinomycetes are still out in front, over 60%, *Streptomyces* spp., accounting for 80% of these (Kieser et al., 2000).

IDENTIFICATION OF UNKNOWN MICROORGANISMS BASED ON 16s rDNA SEQUENCE ANALYSIS

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 sequences from distantly related organisms can be precisely aligned, making the true differences easy to measure. For this reason, genes that encode the rRNA (rDNA) have been been used extensively to determine taxonomy, phylogeny (evolutionary relationships), and to estimate rates of species divergence among bacteria. Thus the comparison of 16s rDNA sequence can show evolutionary relatedness among microorganisms. This work was pioneered by Carl Woese, who proposed the three Domain system of classification - Archaea, Bacteria, and Eucarya - based on such sequence information.

THE RIBOSOMAL RNAS:

In Bacteria, Archaea, Mitochondria, and Chloroplasts the small ribosomal subunit contains the 16S rRNA (where the S in 16S represents Svedberg units). The large ribosomal subunit contains two rRNA species (the 5S and 23S rRNAs). Bacterial 16S, 23S, and 5S rRNA genes are typically organized as a co-transcribed operon. There may be one or more copies of the operon dispersed in the genome (for example, E coli has seven). The Archaea contains either a single rDNA operon or multiple copies of the operon. To infer relationships that span the diversity of known life, it is necessary to look at genes conserved through the billions of years of evolutionary divergence. An example of genes in this category are those that define the ribosomal RNAs (rRNAs). Most prokaryotes have three rRNAs, called the 5S, 16S and 23S rRNA.

RIBOSOMAL RNAS IN PROKARYOTES:

NAME	SIZE (NUCLEOTIDES)	LOCATION
5S	120	LARGE SUBUNIT OF RIBOSOME
16S	1500	SMALL SUBUNIT OF RIBOSOME
23S	2900	LARGE SUBUNIT OF RIBOSOME

The 5S has been extensively studied, but it is usually too small for reliable phylogenetic inference. The 16S and 23S rRNAs are sufficiently large to be useful.

The 16s rDNA sequence has hypervariable regions, where sequences have diverged over evolutionary time. These are often flanked by strongly-conserved regions. Primers are designed to bind to conserved regions and amplify variable regions. The DNA sequence of the16S rDNA gene has been determined for an extremely large number of species. In fact, there is no other gene that has been as well characterized in as many species. Sequences from tens of thousands of clinical and environmental isolates are available over the internet through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and the Ribosomal Database Project (www.cme.msu.edu/RDP/html/index.html). These sites also provide search algorithms to compare new sequences to their database.

MOLECULAR PHYLOGENIES CAN REFLECT GENEALOGY AND AMOUNT OF CHANGE

By comparing the inferred rRNA sequences (or those of any other appropriate molecule) it is possible to estimate the historical branching order of the species, and also the total amount of sequence change. An example of a 16S rRNA-based phylogenetic tree showing the three (identified) Domains of life - Bacteria, Archaea and Eucarya - is below. In this tree, lineages diverge from a common ancestral lineage on the far left. The lengths of the individual lines reflect the amount of sequence change (note that some lineages have modified the gene sequence substantially more than others, and thus have accumulated longer total branch lengths).

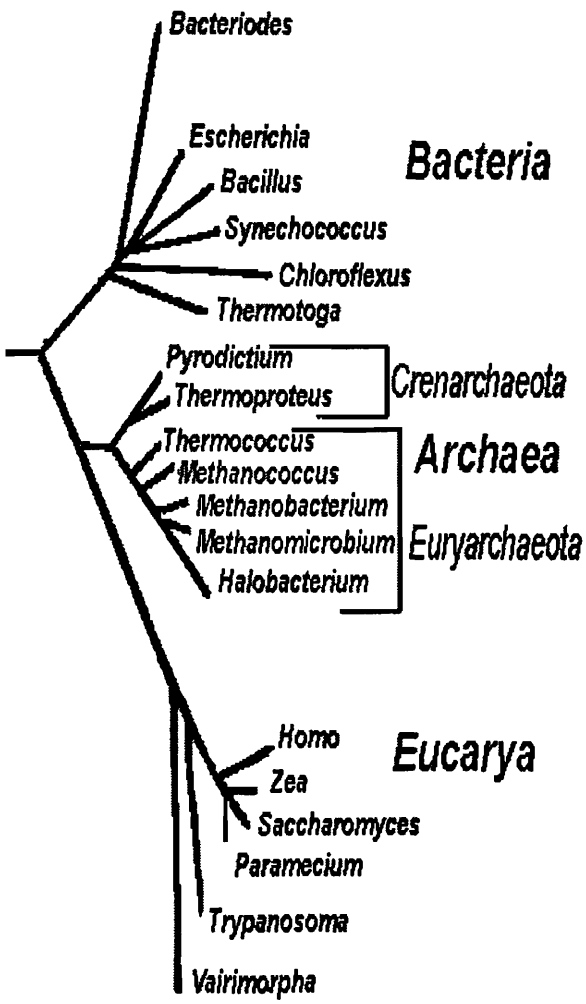

Figure 16s rDNA Phylogenetic Tree from: http://lecturer.ukdw.ac.id/dhira/ClassAndPhylo/molPhylogeny.html