WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 4, 731-747. Research Article ISSN 2277– 7105

ISOLATION AND OPTIMIZATION OF AMYLASE PRODUCING BACTERIA AND ACTINOMYCETES FROM SOIL SAMPLE

CH. Sowjanya*, D. Rayalamma, D. Rani, Esenjungla, G. Sadhana

D. Rama Bramha Reddy, Nalanda Institute of Pharmaceutical Sciences.

Article Received on
25 Jan. 2019,

Revised on 15 Feb. 2019,
Accepted on 08 March 2019

DOI: 10.20959/wjpr20194-14500

***Corresponding Author**
CH. Sowjanya
D. Rama Bramha Reddy,
Nalanda Institute of
Pharmaceutical Sciences.

ABSTRACT

Amylases are amongst most widely used enzymes in industries such as food, fermentation, starch processing, textile and paper. The objective of the present study was to isolate, identify and optimize potential amylase producing bacteria and actinomycetes from soil samples. Soil is the ideal habitat for many extracellular enzyme producing bacteria. The soil samples were collected from Thaluru and Medikonduru of Guntur district. Isolation was done by serial dilution and spread plate method. Primary screening of amylolytic activity of the isolates was performed by starch agar plate method. The submerged state fermentation method followed for the production of amylase by the optimization of temperature, pH, fermentation time and substrate concentration. From the soil samples, 16 isolates were 3 identified and subjected to primary screening for amylolytic activity. Of which, four isolates were observed with maximum amylolytic activity during the primary screening. During the submerged state fermentation, maximum amylase activity was observed at 48 h and then declined. The optimum temperature observed for maximum amylase activity of Bacillus was 40°C and Streptomyces at 37°C. The highest amylase activity was observed at neutral pH and 4% of starch concentration.

KEYWORDS: amylase, actinomycetes, optimization, isolation.

INTRODUCTION

Amylase is an enzyme obtained from the microbes has been used by many industries as a source for production of foods and beverages. With the utilization of microorganisms it is possible to produce large scale and also easily manipulated for desired products (Sumrin et al., 2011). In general, enzymes produced from fungal and bacterial sources have many applications in industries (Aiyer, 2005). In addition, recent advancement in biotechnological

tool, utilization of amylase has widened in clinical research, medical chemistry and starch analytical chemistry. Earlier literatures highlighted that bacterial strains from the genus Bacillus, Pseudomonas and Clostridium and from the genus Streptomyces have been used to synthesize amylase (Kafilzadeh et al., 2012; Oyeleke et al., 2010). Multi-potential application and demand pave the way for increasing indigenous amylase production and searching for more efficient processes (Hmidet et al., 2009). In Ethiopia, there is no much work done in this area of research. The country has several undisturbed natural soil habitat. In this research work soil samples were collected from various locations of Thalluru and medikonduru since these areas are abundant in plant biodiversity and soil types. Therefore, to contribute new knowledge in scientific world amylase producing microorganisms were isolated, identified, optimized and reported for the first time from this study area.

Actinomycetes have gained prominence in recent years because of their potential for producing antibiotics (Kumar et al., 2005). Streptomycin, gentamicin, rifamycin are some of the antibiotics which are in use presently and erythromycin are the product of actinomycetes. The actinomycetes are important in field of pharmaceutical industries and also the agriculture. Previous study showed that actinomycetes isolated from Malaysia soil have the potential to inhibit the growth of several plant pathogens (Jeffrey et al., 2007). Oskay et al.(2004) also reported about the ability of actinomycetes isolated from Turkey's farming soil they have the ability to inhibit Erwinia amylovora a bacteria that cause fireblight to apple and agrobacterium tumefaciens a causual agent of Crown Gall disease(Jeffrey et al.,(2008).

1.1 Actinomycetes diversity and distribution in nature

Actinomycetes are a group of branching unicellular organisms, which reproduce either by fission or by means of special spores or conidia. They are closely related to the true bacteria frequently, they are considered as higher, filamentous bacteria. Classified in actinomycetes order, they are also belonged to Gram positive bacteria with a high Guanine-plus-cytosine(G+C) content in their DNA which is above 55%(Osada,2001).

Terrestrial habitat

According to Hayakawa (2008), actinomycetes are distributed widely in various habitats but soil remains the most important with the largest population found in the surface layer. In natural soil habitat, streptomycetes exist as a major component of actinomycetes population. There are many studies on isolation of actinomycetes from soil have been reported (Iwami et al., 1986; Jain, 2007; Kavita &Vijaya Lakshmi, 2007). Streptomyces was encountered to be

the most abundant genus isolated in each of the studies, followed by micromonospora. To date, there is interest in assessing antimicrobial potentials among soil actinomycetes (Wijittra et al., 2006). Oskay et al. (2004) discovered farming soil – actinomycetes which were antagonistic against few bacteria had capability of being a producer of novel antibiotics. Recently, actinomycetes had been isolated from mangrove soil. Ismet et al. (2004) and Hong et al. (2009) reported isolation of Streptomyces and micromonospora from mangrove soil and plants which have potential in producing biologically active secondary metabolites. Through the actinomycetes population in anoxic mangrove rhizosphere was 1000 to 10000 times smaller than arable lands because of tidal influence, it was diverse and mainly represented by Streptomyces, Micromonospora, actinobacteria and nocardioform actiomycetes (Tan, 2007).

Recently, studies of mangrove actinomycetes were focusing on their antimicrobial activity against pathogenic bacteria and fungi (Vikineswary et al, 1997; Vikineswary et al., 2003). Besides, Schneider et al. (2007) reported a strain of actinomycete, belonged to genus Nocardia isolated from mangrove soil produced new cytotoxic metabolites that strongly inhibited human cell lines such as gastric adenocarcinoma.

Actinomycetes occur universally on the surface of plants and sometimes even in various parts of the plants themselves, especially streptomycetes spp. from the root which is in contact with soil. Tian et al. (2004) isolated 274 strains of actinomycetes from rice's sterilized root and leaves. Most of them belonged to Streptomyces spp., only a few fell into streptoverticillium. In other study, petrolini et al. (1991) reported the isolation of 1775 actinomycetes strain from surface-sterilized roots of 205 plants from 156 species. They identified eighty percent of the of the strains as Streptomyces spp. and the remaining as nocardioform, micromonospora, actinoplanes, streptosporangium, streptoverticillium and saccharomonospora. Isolation of endophytic actinomycetes from roots of healthy wheat plants was done by coombs and franco(2003). They found that the isolates were beloned to Streptomyces, spp.from other parts of grapevine berries, which exhibit widespread antagonistic activity against yeasts and fungi inhibiting the same environment.

Besides Streptomyces spp; matsumoto et al. (2003) used healthy plant tissues from leaves stems and roots to isolate endophytic actinomycetes which have antifungal potential particularly Streptomyces spp.

Aquatic Habitat

Freshwater Habitat

Waksman (1959) explained that actinomycetes are abundant in fresh water in lakes. An old report stated that thermophilic actinomycetes are found in river water. They were also found in sewage and grew well at 60℃. This statement was then proved when cross (1981) reported isolation of members of genera actinoplanes, micromonospora Rhodococcus, Streptomyces and the endospore- forming thermoactinomycetes from fresh water habitats. Good fellow &Haynes (1984) however explained that majority of these actinomycetes most probably are wash – in form land and accumulated in fresh water habitats. According to makkar &Cross (1982), sporangia of Actinoplanes could withstand prolonged desiccation and release motile spores when rehydrated. That is why they are common in soils, rivers and lakes. Actinoplanes is mainly discovered on allochthonous leaf litter washed to lake shore and twigs submerged in streams.

Waksman (1959) believes that members of the genus Micromonospora represents a truly indigenous group of microbial inhabitants of waters and bottom deposites of inland lakes. Therefore, a great number of Micromonospora also can be isolated from lake sediments. Complex organic compounds such as chitin, cellulose and lignin accumulated in lake mud were able to decompose by this genus of actinomycetes. Rowbotham & Cross (1977) elucidated that Micromonospora spores can survive as dormant propagules as they washed into streams, rivers and lakes. Comparable to Thermoactinomycetes endospores found in lake sediments, it was also reported remain dormant in cold waters as they were washed in from surrounding soils. On the other hand, the presence of Rhodococcus coprophilus (also has been isolated from water and sediments of rivers and lakes), a coprophilic species in lakes was believed due to wash in of contaminated herbivore dung.

A study of freshwater actinomycetes isolation from sediments of lakes was carried out by Jiang & Xu (1996) was results the discovery of yielded predominantly Micromonospora followed by Streptomycetes. The presence of Streptomyces in freshwater habitat was because of their spores being continuously washed into rivers and lakes. This enables the Streptomyces spores could be found in foam of rapids at the water-air interface. Isolation of actinomycetes from lake water and sediments by Terkina et al. (2002) leads to an interesting conclusion that Streptomyces were dominant in water samples while great numbers of Micromonospora were found in sediments.

Marine habitat

Marine environment is a unique habitat that has exclusive characteristics which terrestrial areas do not have, like high hydrostatic pressure, high salts concentration and low concentration of organic matter. Thus, microorganisms surviving both in the marine and terrestrial environment are expected to be totally different. This makes a point of view that marine habitat would be an excellent sampling point as microbes in seawater and marine sediments are diverse.

Waksman (1959) enlightened that there were very few reports available concerning the occurrence of actinomycetes in sea and sea bottom until 1959. Early study reported that the existence of actinomycetes in this environment was believed because of soil contamination, or to their presence on algal material floating on the surface of the sea, or to the fact that the samples of water were obtained near the docks.

Isolation of actinomycetes from inshore marine sediments was done by Grein & Meyers (1958), based on absence of apparent morphological and biochemical difference between both marine and terrestrial isolates, they elucidated that the actinomycetes might be originated from terrestrial but adapted to salinity level of sea water. Okami & Okazaki (1974) studied the transportation of actinomycetes spores into shallow sea mud. The study explained that actinomycetes spores could be transferred from land to the sea by rain or river and survived. Spores were precipitated by the NaCI acceleration after reaching the sea into sediments. According to Okazaki & Okami (1975) later, on media formulated with sea water, terrestrial strains could grow well besides showed a wide range of halo-tolerance, whereas halo-tolerance of NaCI-sensitive strains could be induced by stepwise exposure to increasing NaCI concentrations.

However, the existence of indigineous marine actinomycetes had been reported by Weyland (1969) based on abundant actinomycetes isolated from deep sea sediments. Besides, Jensen et al. (1991) had also reported that maximum numbers of actinomycetes isolation from near-shore sediments in both shallow and deep sampling sites showed a bimodal distribution in relation to depth. This was characterized by an obvious decrease of streptomycetes and actinoplanetes increment with increasing depth Furthermore,98% of the streptomycetes were obtained from depth<3m and the percentage decreased radically with increasing depth. However, the actinoplanetes number was increased with up to 33m of depth increment. These discoveries indicated that the theory that marine-derived actinomycetes are

originated from terrestrial could be argued.In addition, they proved that near-shore marine sediments actinomycetes are and functional members of the marine microbial community.

Jensen et al. (1991) reported that actinomycetes isolated from marine environment are metabolically active and have adapted to life in the sea. Hakvag et al. (2008) isolated a total of 217 marine actinomycetes from sea surface microlayer. All these strains resembled the genus Streptomyces spp. Furthermore, studies on isolation of actinomycetes from marine sediments suggested that they were able to survive under marine conditions due to their salt tolerance. In fact they were able to endure for considerable periods of time under marine environment. According to Ghanem et al. (2000), marine actinomycetes isolated from sediments counts far exceeded those found in sea water. A total of 192 marine actinomycetes were isolated from sediments, these isolates were identified and resulted in the discovery of novel marine-derived actinomycetes within the family+Micromonosporaceae (Magarvey et al., 2004). Miller et al. (2006) also isolated marine Streptomyces spp. from the same source. All these studies proved that marine sedimentsare really a valuable source for isolating marine actinomycetes.

1.1.3 Extreme environments

Actinomycetes were also isolated from uncommon habitat. Alkalophilic actinomycetes (Strepiomyces and Nocardiopsis were the dominant gencra) had been 1993). isolated from alkaline soils (pH 10-12) surrounding mineral springs (Jiang el al.,1993).A new genus and species of alkalophilic actinomycetes from a soda lake soil (pH10) was also been described as Bogoriella caseilytica (Groth et al., 1997). In other study, Al-Zarban et al. (2002) isolated Saccharomonospora halophila, a halophilic actinomycete with optimum growth at 10% NaCl from marsh soil. Mevs et al. (2000) reported a study of Modestobacter multiseptatus, a psychrophilic strains with optimum growth at temperature 11-13 C was isolated from transantarctic mountain soils. An obligate psychrophilic actinomycetes, Cryobacterium psychrophilum, with optimum growth temperature 9-12'C and did not grow at temperature higher than 18'C was isolated from Antartica soil by Suzuki et al. (1997). Other than that, Zakalyukina et al. (2002) isolated acidophilic actinomycetes from acidic forest and peat soils, mainly Streptomyces and Micromonospora. Dey & Chaphalkar (1998) isolated 'thermophilic Streptomyces spp. from silt and water samples of meteoritic crater. This isolate grew on agar at 55'C, with only 18 hours sporulation time. It produced protease at 55 C and the enzyme was stable up

to 85'C at 7.5-12 pH range. Few rare thermotolerant actinomycetes isolated from desert soils of Mojave Desert, California belonged to genera Microbispora, Nocardia. Microtetraspora, Amycolaptosis, Actinomadura and Saccharothrix were reported by Takahashi et al. (1996). These actinomycetes grew at temperature up to 50 C.

1.2 Role of actinomycetes in ecosystem

Actinomycetes are found in quite a significant number as a major component in most soils. In most ecological systems, they are saprophytic with a major role in soil organic matter decomposition (Arai, 1997). Referring to Goodfellow & Williams (1983), actinomycetes playing an important ecological role in biodegradation of plant litter, particularly in the recalcitrant lignocelluloses component. As they remain as dormant spores, sporangia or resting cocci, actinomycetes will automatically germinated in the occasional presence of exogenous nutrients (Mayfield et al., 1972). Apart from nutrient availability, there are other environmental factors that could affect the growth of actinomycetes such as soil temperature, pH and moisture tension (Goodfellow & Williams,1983).

In rhizosphere soil, actinomycetes are capable of producing antibiotics and other useful metabolites. Therefore, they have possibility in influencing the rhizospheric pathogens. Getha & Vikineswary (2002) reported the potential of Streptomyces spp. As biocontrol agent against fungal pathogens especially in commercial crops. Streptomyces spp. potential in biocontrol aspect also proved in other study by Vercesi et al. (1992) which reported that Streptomyces spp. isolated from grapes have antifungal activity against pathogenic yeast and fungi from the same habitat. On the contrary, there are certain species of Streptomyces considered as pathogen such as S. turgidiscabies which causes erumpent scab lesions on potatoes in Hokkaido, Japan. This specics was isolated from soil (Miyajima et al., 1998). Besides, Park et al. (2003) reported that the potato scab disease was caused by other species of Streptomyces (S. luridiscabiei, S. puniciscabiei and S.niveiscabiei.

1.3. Identification and characterisation of actinomycetes

In actinomycetes classification and characterisation, phenotypic and phylogenetic methods are involved as a polyphasic taxonomic approach (Tan, 2007). These include morphological, physiological and biochemical characterisation in phenotypic approach while molecular methods are in phylogenetic characterisation part. Some characterisation procedures for identification are outlined in this chapter

1.3.1 Cultural and morphological observation of actinomycetes

Based on Shirling & Gottlieb (1966) methods for Streptomyces spp. characterisation. the basic observation should involved cultivation of cultures on various medium; yeast extract - malt extract agar (ISP2), oatmeal agar (ISP3), inorganic salts-starch agear (ISP4) and glycerol-asparagine agar (ISP5). Growth and morphology of Strepiomyces spp. are observed when cultures turned matured with heavy spore mass in order to determine aerial spore mass colour, substrate mycelium and diffusible pigments colour (Williams et al., 1993). Preliminary differentiation could be done by colour grouping when dealing with large number of different isolates. However, this study only involved Streptomyces spp.; which proven by Williams et al. (1993) that this group of actinomycetes fell into the same group with similar morphological and physiological properties based on their colour grouping.

Morphological observation in this study is referring to sporophores morphology as it is considered stable and clearly defined feature for actinomycetes classification. However, this is only valid without the occurrence of strain degeneration due to subculture or improper maintenance (Pridham et al., 1958). Based on different spore chain morphology, the authors divided Streptomyces spp. into seven groups of "morphological sections" (Table 1). Whereas every section was further categorized into another six "series" according to the spore mass colour: white, olive-buff (buff to tan to olive-buff), yellow, blue (blue to blue-green to green), red (pink to red to lavender to lavender-grey) grey (light grey to mouse-grey to brown-grey to grey-brown). The spores then could be subdivided into distinct ornamentation: smooth, rugose, spiny, hairy, warty, knobby tuberculate or verrucose (Vobis, 1997)

1.3.2 Physiological characterisation of actinomycetes

Basically, any actinomycetes physiology consideration involves a study of their growth and nutrition, their metabolic processes and their reaction to environmental conditions (Waksman, 1959). According to Shirling & Gottlieb (1966), only melanoid production and carbon sources utilization were being characterized for Streptomyces spp. physiology. However, there are some other physiological characteristics that had been considered to categorize actinomycetes, especially Streptomyces spp. depending on number of isolates and objective of study. For instance; optimum temperature range, nitrate reduction test, NaCl tolerance, production of hydrogen sulfide (H2S), starch hydrolysis, liquefaction of gelatin, nitrogen source utilization, pH sensitivity and

sensitivity to some antibiotic are some of the characteristics that are being tested in characterizing any actinomycetes isolates physiologically (Gottlieb, 1960; Williams et al., 1989; Sakai et al., 2004).

1.3.3 16S rRNA gene sequence analysis of Streptomyces spp

Stackebrandt et al. (1992) explained that 16S rRNA gene sequences used in phylogenetic relationship inference among microorganisms besides to characterize unknown isolates has been commonly accepted. This is due to the fact that relationships measurement among bacteria can be done because of the nature of universality of this gene in bacteria (Clarridge, 2004). About 1,550 base pairs (bp) long, the 16S rRNA gene sequence is basically composed of both variable and conserved regions. Chen et al. (1989) elaborated that "The gene is large enough, with sufficient interspecific polymorphisms of 16S rRNA gene, to provide sufficient sequence information that permits statistically significant comparisons. Universal primers are usually chosen as complementary to the conserved regions at the beginning of the gene and at either the 540-bp region or at the end of the whole sequence (about the 1,550-bp region), and the sequence of the variable region in between is used for the comparative taxonomy. Although 500 and 1,500 bp are common lengths to sequence and compare, sequences in databases can be of diverse lengths."

Clarridge (2004) stressed that the 16S rRNA gene sequences comparisons allows organisms differentiation at the genus level. This including strains classification at species and subspecies level. A comparison of partial nucleotide sequences (120 bp) by Kataoka et al. (1997) spanning the variable a region of 16S rRNA in 89 Streptomyces strains demonstrated that these short nucleotide sequences are useful for Streptomyces species rapid identification. At the intra-species level, partial 16S rRNA gene sequence is enough to deduce the Streptomyces strains phylogenetic relationships. Similar approach was used by Thamchaipenet et al. (2001) to differentiate antifungal producing streptomycete isolates into different species by partial 16S rRNA sequencing. They found that phylogenetic analyses of the partial sequences indicated possible new species with those of known antifungal-producing streptomycetes. However, the 16S rRNA phylogenetic marker usage is often criticized because of its heterogeneity of the same genome (Acinas et al, 2004) or according to Pontes et al. (2007), due to its lack of resolution at the species level. Fortunately, it is still used as a unique and valuable standard for bacterial identification. Therefore, the implementation of next-generation sequencing technology

has impressively increased the size of 16S rRNA sequence databases (Armougom & Raoult, 2009),

1.41 Antimicrobials and antitumor potentials of actinomycetes

In a review article, Berty (2005) discussed about microbial metabolites production since year 2002, which filamentous actinomycetes produced over 10.000 bioactive compounds (45% of all microbial metabolites). 75% or 7600 of the valuable production were of Straumoes spp. origin and 2500 or 25% were from rare actinomycetes (Micromonospora, Actinomadura and Streptoverticillium). The approximately proportion of all acinomycetes products demonstrated antitumor activity was 70:30 respectvely. Production of a great number of important drugs by actinomycetes is well known. Okami & Hotta (1988) reported some significant dnugs provided by actinomycetes such as aminoglycosides, anthracyclines, chloramphenicol, β-lactams, macrolides and tetracyclines. Among the actinomycetes, Streptomyces contributed the greatest chemical diversity (Sanglier et al, 1993a). A study on obtaining antifungal compounds from marine Streptomyces spp. was carried out by Cho et al. (1999), These compounds displayed strong antifungal activity against C albicans E coli and P.aerugenosa. More recently. Ogunmwonyi et al. (2010) reported a wide range antimicrobial activity of ten most potent marine Streptomyces spp. isolated from the Nahoon beach, a coastal shore of Indian Ocean in the Eastern Cape Province of South Africa.

The ethyl acetate extracts of the isolates exhibited activities against at least 6 and up to 26 of the 32 test bacteria screened. IR spectra analysis was done to characterize the crude extracts and the possibility of terpenoid, long chain fatty acids and secondary amine derivatives compounds in the extracts presence was revealed.

Basically, antitumor compounds are produced naturally mainly by microorganisms. In fact, actinomycete is the major producer of various natural products with different properties including antiturnor activity (Olano et al, 2009). A report by Yuan & Crawford (1995) explained an experiment of coating pea seeds with spores/mycelia of Streptomyces Iydicus WYEC108 to inhibit Phytium ultimum, a kind of fungus in an oospore-enriched soil. They discovered that less than 40% of the coated seeds were infected but all uncoated seeds were infected by the fungus 48 hours after planting More recently, an Iranian Streptomyces plicatus strain 101 was reported having chitinolytic activity and antifungal inhibitory effects on mycelial growth of Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium graminearum,

F. solani, Rosellinia necatrix and Pythium aphanidermatum mainly by extracellular chitinase production (Baharlouei et al., 2010).

1.4.2 Actinomycetes enzymes

Actinomycetes enzymes are the most significant products after antibiotics Microbial enzymes are widely used in food processing, detergent manufacturing, the textile and pharmaceutical industries, medical therapy, bioorganic chemistry and molecular biology (Peczynska-Czoch & Modarski, 1988). Actinomycetes were best known as antibiotics sources for many years. These recent years, they also have been detected to be a potential source of a wide range of important enzymes.

Rifaat et al. (2005) investigated twenty producing cellulase free-xylanase Streptomycetes strains isolated from Egyptian soils. They reported two most active strains and have been identified as Streptomyces albus and Streptomyeces chromofuscus. The increment of the enzyme activity was found when both isolates were grown on yeast extract.

Optimum production of xylanase was recorded after five days of fermentation. Xylanase produced with Streptomyces chromofuscus showed higher bleaching activity than that from Streptomyces albus. The enzyme enhanced the liberation of reducing sugars, which improved pulp bleachability. Kim et al. (2003) reported the isolation and purification of chitinase from culture filtrate of Streptomyces sp. M-20. The enzyme was active optimally at pH of 5.0 and at 30'C, and stable from pH 4 to 8, and up to 40^{o}C. The purified chitinase showed antifungal activity against Botrytis cinerea, and lysozyme activity against the cell wall of Botrytis cinerea. Besides, the chitinase activity was completely inhibited by Hg+, Hg2+ and p-chloromercuribenzoic acid. Narayana & Vijayalakshmi (2009) reported that under submerged fermentation, the production of chitinase by a terrestrial Streptomyces sp. ANU 6277 was promoted by utilization of starch and yeast extract as carbon and nitrogen sources.

In a study of mannanase screening in actinomycetes, Montiel et al. (1999) reported high levels of the enzyme produced in Streptomyces scabies CECT3340 and S. ipomoea CECT341 in liquid culture. The mannanase potential in bleachability improvement of pine kraft pulp was tested. Obviously, it released the colour and chromophoric material from the pine kraft pulp besides increased the pulp brightness. The optimization of mananase

production in Streptomyces sp. PG-08.3 from Rajasthan dessert, India was investigated by Bhoria et al. (2009). Increment of guar gum concentration in the growth media demonstrated to enhance the production of mannanase.

Adriana et al. (2005) published a first report of two Antartic actinomycetes which produced keratinolytic enzymes to enable their growth on keratin-containing wastes. Streptomyces flavis 2BG (mesophilic) and Microbispora aerata IMBAS-11A (thermophilic) demonstrated as very promising strains for effective processing of native keratinous wastes. Pettett & Kurtboke (2004) explained that keratin-degrading and antibiotic producing actinomycetes such as Streptomyces, Saccharomonospora, Nocardioides, Nocardiopsis and Nonomuraea have potentials in turning poultry farm feather waste by composting into odourless and complete biological degradation in pathogen-free biofertilizer.

1.43 Actinomycetes as agents of biodegradation/bioremediation

Zhou& Zimmermen (1993) reported the role of actinomycetes as agent of decolourization in industrial effluents containing water-soluble synthetic reactive dyes. The report exhibited the capability of actinomycetes in decolorizing between 17% up to 73% of the effluent, by decolourization or adsorption of the dyes to the cells. Azo dyes, azo-copper complex, anthraquinone, formazan-copper complex and phthalocyanine dye were among the included dye in this report. Streptomyces spp. have also reported to decolourize paper mill effluent obtained after semichemical alkaline pulping of wheat straw (Hernandez et al, 1994). The highest decolourization level reported were 60-65%.

Actinomycetes are also responsible in pesticides degradation with various different chemical structures, including organochlorines, s-triazines, triazinones, carbamates, organophosphates, organophosphonates, acetanilides, and sulfonylureas (De Schrijver & De Mot, 1999; Nawaz et al, 2011). Indigenous soil actinomycetes had been reported to degrade the herbicide Diuron in soil (Esposito et al., 1998). Diuron, a kind of phenylurea is widely used as weed biocontrol on non-crop areas and certainly on crops like cotton, pineapple, citrus and sugar-cane at low concentration. In vitro, by applying Diuron, the selected actinomycetes exhibited up to 37% level of herbicide degradation in seven days.

Rubber-degrading actinomycetes are widespread in nature due to natural rubber degradation as sole carbon source is actinomyctes privilege (Jendrossek et al., 1997). This was concluded after isolation of variety of rubber-degrading actinomycetes (up to 105cfu/g) from soil

samples of Hevea brasiliensis plantation and from waste water ponds of a rubber-producing company in Malaysia. Among the actinomycetes, Streptomyces were found to be dominant followed by Micromonospora, Actinoplanes, Nocardia, Dactylosporangium and Actinomadura. Novel rubber-degrading Gordonia species from fouling water inside a deteriorated automobile tyre: G. polyisoprenivorans (Linos et al., 1999) and G. westfalica (Linos et al., 2002) have been published.

MATERIALS AND METHODS

Sample Collection

The agricultural soil samples were collected from different sites in Thaluru and medikonduru villages of Guntur district. These samples were transferred to the laboratory by sterile dried polyethylene containers and kept at room temperature until used.

Collection of samples from different places;

Sl.no	Source	Place	Character
1	Chilli soil	Thaluru	Brown and muddy
2	Cotton soil	Thaluru	Brown
3	Chilli soil	Medikonduru	Brown and muddy
4	Cotton soil	Medikonduru	Brown

Isolation media

1 Half strength nutrient agar medium.

2 Starch casein agar medium.

Composition of half strength nutrient agar medium

Peptone - 2.5g

Beef extract - 1.5g

Nacl - 2.5g

Agar - 20g

Sterile water - 1L

PROCEDURE

Preparation of agar medium

Required amount of given chemicals were taken and dissolved in 100ml of sterile water. Mix thoroughly with stirrer until the mixture was dissolved. Heat on water bath for 15m. The mixture was suspended in 5 boiling tube. Each boiling tube contains 20ml of mixture (agar medium). The boiling tubes were fully covered with cotton plug. The 5 boiling tubes were

kept in to the beaker and covered with aluminium foil. The beaker was kept in autoclave for 45m for the purpose of sterilization.

Determination of pH of soil samples

The pH of the clear supernatant soil suspensions were determined using pH papers.

pH of the soil samples

Sl.no	Samples	Ph
1	Chilli soil(A)	7.0(neutral)
2	Cotton soil(B)	6.5(acidic)
3	Chilli soil(C)	6.5(acidic)
4	Cotton soil(D)	6.5(acidic)

Isolation of actinomycetes

- 1g of soil was taken and it was suspended in 20ml of sterile water in 100ml conical flask
- It was kept for 1 hour in incubator formed suspension.
- Half strength agar medium and starch casein agar medium were prepared and sterilized by autoclave 121°c for 20 min.
- Each soil suspension was serially diluted by using 1ml sterile pipette from 10^{-1} to 10^{-9} in sterile water tubes.
- The last three dilutions were inoculated (1ml from each dilution) in to sterile isolation media 45°C, mixed thoroughly and poured into sterile petri dishes under aseptic conditions.
- The inoculated plates were incubated at room temperature for 7-10 days.
- Well isolated colonies were observed and they were sub cultured to the agar medium slants of the above media.

Determination of Starch activity

The amylase activity was determined for all isolates (20) using starch agar medium of the following composition by cross streak method.

Composition of Starch agar medium

Yeast extract	-	1g
Starch	-	0.3g
Peptone	-	1g
Potassium hydrogen phosphate	-	0.5
Agar	-	15g

Distilled water - 1 litre

Procedure

The weighed quantity of all the ingredients were dissolved in distilled water and pH was adjusted according to the P^H of the soils collected using pH Paper. Then the required quantity of the agar was added and the beaker was kept in hot water bath and allowed the agar to melt. Then the medium was distributed in to the 250ml conical flask each contained 50ml. A total of 4 flasks were arranged and they were sterilized by autoclave at 121°C for 20 mins.

The sterile molten starch agar medium was poured into sterile petri dishes (20) under aseptic conditions and allowed the medium to solidify. The solidified agar medium plates were inoculated separately with all 20 isolates. The inoculated plates were then incubated at room temperature for 7 days. After incubation period the plates were flooded with weak iodine solution. The clear hydrolysing zones were observed and measured the hydrolysing zone and growth zones. The ratio was calculated.

In the next phase of our work, we selected the best isolate which exhibited excellent amylase activity. The effect of temperature on growth and preliminary morphological studies of the isolate were conducted. The results given in the table.

RESULTS AND DISCUSSION

A total of 16 isolates were isolated from 4 different soil samples which were collected from the soil of thaluru and medikonduru villages of Guntur district sterile. In the first phase of our work the pH was determined from the collected soil samples as given in the table. All the soil samples were shown pH7 which indicates that they all are neutral in nature. In the next step all the soils were screened by using selective isolation media which resulted in 16 isolates. All the 16 isolates were subjected to starch activity studies as shown in table. All the 16 isolates exhibited starch activity on milk casein in agar medium. Among the 16 isolates A_7 exhibited best starch activity followed by A_{10} and A_{12}

In the next phase of our work the isolate A_{10} was selected for further studies which includes

- Preliminary studies using light microscope
- Effect of temperature on growth
- The morphological studies indicates that the isolate A_7 exhibited spiral spore chain.

Hence it belongs to Streptomyces species.

Different temperature were selected and determined the effect on temperature on growth of the selected isolate A_{10} as indicated in Table. Good growth was observed at room temperature. No growth was observed at 0^0C and 10^0C. At 37^0C slight growth was observed. For the determination of enzymatic concentration in the broth and identification of the isolate detail studies are needed

Table 4: Determination of Starch Activity.

S.no	Isolate no	Hydrolysing Zone (mm)	Growth zone (mm)	Ratio
1	A_1	50	20	2.5
2	A_2	40	15	2.67
3	A_3	54	26	2.08
4	A_4	42	20	2.10
5	B_1	36	17	2.11
6	A_5	39	20	1.95
7	A_6	44	23	1.91
8	A_7	24	12	2.0
9	C_1	33	18	1.83
10	A_8	50	13	3.85
11	D_1	35	16	2.18
12	A9	39	20	1.95
13	D_2	52	25	2.08
14	A_{10}	40	15	2.67
15	A_{11}	32	15	2.13
16	A_{12}	46	21	2.19

Table 5: Effect ot Temperature on Selected Best Isolate A_2.

S.no	Temperature	Growth
1	0^0C	-
2	10^{0C}	-
3	Room temperature	+++
4	37^0C	++

CONCLUSION

- All collected soil samples were exhibited p^H 7. Hence all the soils were neutral in nature.
- 16 isolates were isolated from four different soil samples.
- All 16 isolates exhibited starch activity.
- Among 16 isolates, A_{10} exhibited excellent starch activity followed by A_{10} and A_{12}.The remaining isolates were showed poor to moderate activities.
- Selected best isolate (A_{10}) exhibited good growth at room temperature and showed poor or no growth at 37^0C and 10^0C respectively.

- The preliminary morphological studies of the selected isolate indicates that belongs to Streptomycetes species (because it exhibited spiral spore chains).
- Detailed taxonomic studies are needed for the determination of enzyme concentration in the broth.

REFERENCES

1. Ara 1., N.A. Bukhari, D.R, Wijayanti and M.A. Bakir, 2012, proteolytic activity of alkaliphilic salt tolerant actinomycetes from various regions in Saudi Arabia, African Journal of Biotechnology.
2. Akpinar, O., F, Ucar and T. Yalcin, 2011, Screening and regulation of alkaline extracellular protease and ribonuclease production of Yarrowia lipolytica strains isolated and identified from different cheese in Turkey, Annals of microbiology.
3. Beheshti Maal, K.,G, Emtiazi and I, Nahvi, 2009, Production of alkaline protease by Bacillus cereus and Bacillus polymixa in new industrial culture mediums and its immobilization, African Journal of Microbiology Research.
4. Beheshti Maal, K., G. Emtiazi and I, Nahvi, 2011. Increasing the alkaline protease activity of Bacillus cereus an Bacillus polymyxa simultaneously with the start of sporulation phase as a defence mechanism, African Journal of Biotechnology.
5. Charles, P., V. Devanathan, P.Anbu, T.Ponnus, N.M.Wamy, T.P. Kalaichelvan and et al., 2008. Purification characterization and crystallization of an extracellular alkaline protease from Aspergillus nidulans HA-10, Journal of Basic Microbiology.
6. Chi, Z., C, Ma,P, Wang and H,F,Li, 2007, Optimization of medium and cultivation conditions for alkaline protease production by the marine yeast Aureobasidium pullulans, Journal of Bioresource Technology.
7. Devi, M.K., A,R. Banu, G.R, Gnanaprabhal, B.V.Pradeep and M.Palaniswamy, 2008, purification characterization of alkaline protease enzyme from native isolate Aspergillus niger and compatibility with commercial detergent. Indian journal of science and Technology.
8. Gupta, R., Q.K. Beg and P. LORENZ, 2002. Bacterial alkaline protease molecular approaches and industrial applications. Applied Biotechnology.
9. Kumar, C. and H. Takagi, 1999. Microbial alkaline protease from a bioindustrial view point. Biotechnology Advances.
10. Lowry, O.H., N. Rosebrough, A. Farr and R. Rundall, 1951. Protein measurement with folin phenol reagent. Journal of Biology and Chemistry.