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Production and Optimization of Extra-Cellular Enzymes of Actinobacteria Isolated from Textiles Dye Polluted Soils of Tirupur, South India

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Abstract:
The diversity and the distribution of actinobacteria and their antimicrobial activities were studied and reported in the textile dye polluted soil of Tirupur (Dollar City of Tamilnadu), Xouth India. Physico-chemical properties of the soil samples were analyzed. As much as twenty actinobacterial isolates were isolated from ten different soil samples. They were characterized and grouped into 8 genera viz. Streptomyces (5), Pseudonocardia (4), Actinopolyspora (3), Actinoplanes (2), Nocardia (2) Nocardiopsis (2), Streptoalloteichus (2) and Actinomyces (1). All the twenty isolates were screened for their extra-cellular enzyme activity including amylase, cellulase, chitinase, L-asparaginase, keratinase and proteasae. Among the 20 isolates, maximum of 7 isolates had L-asparaginase activity, followed by 6 isolates with chitinase activity, 3 isolates with amylase activity, 2 isolates with protease activity, 2 isolates with cellulase activity, 2 isolates with keratinase activity, while seven isolates did not produce any enzyme. Actinobacteria with strong enzyme activity were characterized based on the morphological, biochemical, cultural and physiological properties. Furthermore, the growth and enzyme productivity of the isolates were optimized with respect to following parameters such as pH, temperature, salinity, media, carbon and nitrogen sources.

Keywords: *Dye polluted soil, actinobacteria, extra-cellular enzymes, Streptomyces spp.*

1. Introduction

Actinobacteria, the filamentous bacteria are primarily saprophytic microorganisms of the soils, where they contribute significantly to the turnover of complex biopolymers such as lignocelluloses, hemicelluloses, pectin, keratin and chitin. Actinobacteria are the most widely distributed group of microorganisms in natural and manmade environment. They are the most economically and biotechnologically valuable prokaryotes (Gandhimathi *et al.,* 2009). They are miniature chemical factories as they have capacity to convert to a variety of raw materials used as substrates in to a series of value added products. The main products of the actinobacteria are antibiotics, polysaccharides, proteins, oils, fatty acids, enzymes, pigments, enzyme inhibitors etc. (Balagurunathan, 2002; Peela *et al*., 2005; Imada *et al*., 2007; Thirumurugan and Vijayakumar, 2015). Metabolites of actinobacterial origin, in particular, antibiotics have been valuable in the field of pharmaceuticals. A rapid increase of discoveries of new antibiotics occurred from late 1910 to 1960s, followed by a gradual fall until 1968 and then again raised in 1970s. But most of the major antibiotics had been discovered by 1960s. However, the rate of discovery of novel substances from microorganisms, especially from actinobacteria of terrestrial origin, has decreased recently, even though most of the actinobacteria were believed to terrestrial origin.

In recent years, microbial metabolites like enzymes and drugs are substituting the chemical catalysts in leather, food, paper, pharmaceuticals and textile industries (Stamford *et al.,* 2001). Majority of the enzymes are derived from plants, animals and microorganisms. Among them, microorganisms are the superior due to their rapid doubling time and enzyme production when compared with plants or animals to meet the existing market demand for industrial enzymes (Kumar and Takagi, 1999). Actinobacteria are capable of producing enzymes with good stability at higher temperature and alkaline conditions. Even though, the production of antibiotics as major bioactive compounds from marine actinobacteria (Berdy, 2005; Bull and Stach, 2007; Meena *et al.,*

2013) the ability to synthesize variety of industrial enzymes can be an attractive phenomenon to accomplish our future demand. A little is known about the diversity and role of actinobacteria from polluted soils (Vijayakumar and Malathi, 2014), which is an extremely/ entirely different location contaminated with several physical and chemical factors. Hence, the present study was made an effort to isolate, screen and optimize the extra cellular enzyme production by actinobacteria from the soil samples collected from textile dye effluent polluted areas of Tirupur.

2. Materials and Methods

2.1. Collection of Soil Samples

The top layer soil samples were collected aseptically in sterile polythene bags from ten different textile dye polluted areas of Tirupur, and they were brought to the laboratory and stored at 4°C for further assay.

2.2. Physico-Chemical Analysis of Soil

The pH of soil and the electrical conductivity of soil were determined as described by Jackson (1973), nitrogen was estimated by alkaline permanganate method (Subbiah and Asija, 1956), available phosphorus was estimated by Bray method (Bray and Kutz, 1945) and the potassium content was determined using flame photometer (Stanfold and English, 1949).

2.3. Pretreatment of Soil Samples

The soil samples were pretreated at 55°C for 6 min. in an oven to reduce the other bacterial and fungal growth (Balagurunathan and Subramanian, 2001).

2.4. Isolation of Actinobacteria

Starch casein agar (SCA) (Kuster and Williams, 1964) medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 min. The medium was supplemented with cyclohexamide 50 µg/ml to prevent the growth of other bacterial and fungal contaminants. The medium was poured into sterile Petri dishes and were allowed to solidify. The collected soil samples were diluted up to 10^{-6} and 0.1 ml of the diluted samples were spread over the SCA plates. The plates were then incubated at 28±2°C for 7-10 days. After incubation, the actinobacterial colonies were observed, purified using pure culture method and maintained in SCA medium for further assay.

2.5. Characterization of Actinobacteria

2.5.1. (Colony Morphology)

Colony morphology of actinobacteria were recorded with respective to colour of aerial spore mass and reverse side of the SCA medium, size and nature of colonies and diffusible pigmentation on culture medium (Shrilling and Gottlieb, 1966). Morphologically distinguishable colonies were considered as separate isolates and they were selected for further studies

2.5.2. Light Microscopy

The microscopic morphology of the actinobacterial isolates were studied by using cover slip culture technique. Actinobacterial culture plates were prepared by streak plate method and sterile cover slips (2-4) were inserted at an angle of 45°C. The plates were incubated at 28±2°C for 4-8 days. The cover slips were removed and observed under the high power magnification. The morphological features of spores, sporangia and aerial and substrate mycelium were observed and recorded (Pridham *et al.,* 1958; Vijayakumar *et al.,* 2007).

2.6. Screening of Extra-Cellular Enzyme Producing Actinobacteria

2.6.1. Amylase Activity

Starch agar medium was prepared, sterilized at 15 lbs pressure and poured into sterile Petri dishes. The actinobacterial cultures were streaked onto medium and incubated at 28±2°C for 7 days. Plates were then flooded with Lugol's iodine. Hydrolysis zones were observed and the results were recorded (Ellaiah *et al*., 2002).

2.6.2. Cellulase Activity

Mineral salt agar medium was prepared, sterilized and poured into sterile Petri dishes. The actinobacterial cultures were then inoculated onto medium and incubated at 28±2°C for 7 days. Plates were then observed for hydrolysis zones and the results were recorded (Tai *et al*., 1989).

2.6.3. Chitinase Activity

Chitin mineral agar medium was prepared, sterilized and poured into sterile Petri plates. The cultures were then inoculated in the medium and incubated for 7 days at 28±2°C. After incubation, hydrolysis zones were observed and the results were observed (Abdel-Fatah, 1995).

2.6.4. L-asparaginase Activity

The actinobacterial cultures were streaked on modified nutrient agar medium and the plates were incubated for 7 days at 28±2°C. After incubation, the colours of the colonies were observed. Pink colouration of the media indicated positive results and yellow colouration of the colonies indicated negative results (Dhevagi and Poorani, 2006).

2.6.5. Keratinase Activity

The actinobacterial cultures were streaked on modified Kosmatchev agar medium and the plates were incubated for 7 days at 28±2°C. After incubation, hydrolysis zones were observed and the results were noted (Abdel-Hafez, 1995).

2.6.6. Protease Activity

Skim milk agar medium was prepared, sterilized and poured into sterile Petri plates. The actinobacterial cultures were streaked and incubated for 7 days at 28±2°C. After incubation, formation of hydrolysis zones was observed (Ellaiah *et al*., 2002). The isolates with maximum (zone of clearance) enzyme activities were selected for further characterization studies.

2.7. Cultural Characterization of Actinobacteria on Different Media

Selected actinobacterial isolates were inoculated on eleven different culture media namely SCA, starch nitrate agar, nutrient agar, yeast extract malt extract agar (ISP 2), oat meal agar (ISP 3), inorganic salt starch agar (ISP 4), glycerol asparagine agar (ISP 5), potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA), beef extract agar and brain heart infusion agar. The plates were incubated at 28±2°C for 7 days. After incubation, the colony morphology with respect to colour of aerial spore mass, size and nature of colony, reverse side colour and pigmentation on different media were recorded (Vijayakumar *et al.,* 2012).

2.8. Biochemical Characterization

Biochemical tests namely production of indole, methyl-red, Voges-Proskauer test, utilization of citrate, production of hydrogen sulfide, urease, catalase, oxidase and melanin =pigment, hydrolyses of starch, casein, lipid and gelatin, haemolysis assay, reduction of nitrate and triple sugar iron agar test were conducted for the characterization of selected actinobacterial isolates.

2.9. Chemotaxonomy of Actinobacteria

2.9.1. Analysis of Whole Cell Sugar

One ml of 1N H_2SO_4 was added to 50 mg freeze-dried actinobacterial cultures in a vial and heated at 100°C for 2 h. The mixture was centrifuged at 3000 rpm for 10 min. The pH of the supernatant was adjusted to 5 with barium hydroxide, followed by centrifugation at 6000 rpm for 10 min. the supernatant was filtered. 5 µ of the fluid and 3 µl of 1% standard sugar solution (glucose, galactose, arabinose and ribose), and the filtrates were spotted separately onto a TLC silica gel plate. The plate was developed in the solvent (acetonitrile: water 92.5: 7.5 v/v) for 20 min. and dried for 2 h. It was sprayed with aniline phthalate and heated at 100°C for 4 min. on a hotplate and observed the colour changes (Lechevalier and Lechevalier, 1970).

2.9.2. Analysis of Cell Wall Amino Acids

One milliliter of 6 N HCl was added to 50 mg freeze dried cultures in a vial with a screw cap and heated at 100°C for overnight. The mixture was completely dried at 45°C in vacuum. Two milliliter of distilled water was added to the vial and then dried off. This step was repeated several times to remove HCl. The final dried material was dissolved in 0.2 ml of distilled water. Five micro liter of the sample and 1 µl of standard diaminopmelic acid solution were separately spotted onto silica gel plates (Becker *et al*., 1965). The plate was developed in the solvent (methanol: distilled water: 6N HCL: pyridine = 80: 26: 4: 10) for 3 h, air dried in an chemical hood for 2 h, and sprayed with 0.1% ninhydrin, followed by heating at 120°C for 10 min. on a hot plate.

2.10. Physiological Characterization

2.10.1. Effect of pH (Flowers and Williams, 1977)

Modified Bennett broth was prepared and sterilized. The pH of the broth was adjusted to 6, 7, 8 and 9 using 0.1N HCl and NaOH. The actinobacterial cultures were inoculated into the test tubes with broth and incubated at 28±2°C for 7 days. The growth was recorded after incubation.

2.10.2. Effect of temperature (Shimizu et al., 2000)

Modified Bennett broth was prepared and sterilized. The actinobacterial cultures were inoculated into the broth. The test tubes were incubated at 18, 28, 38, and 48°C for 7 to 14 days. After incubation the growth was recorded.

2.10.3. Effect of NaCl (Tresner et al., 1968)

The basal medium was prepared in 4 batches and supplemented with 0, 1, 2 and 3% of NaCl. The medium was autoclaved and poured into the Petri plates. Agar plates were streaked with actinobacteria and incubated for 7-14 days. After incubation, the tolerance limit of actinobacteria against NaCl was determined.

2.11. Optimization of enzyme activity

2.11.1. Effect of pH

The production media namely starch broth, mineral salt broth, modified Kosmatchev broth, modified nutrient broth, chitin mineral broth and milk casein broth were prepared for production of amylase, cellulase, keratinase, L-asparaginase, chitinase and protease enzyme analyses respectively. The initial pH of the media was adjusted 6, 7, 8, and 9. Agar blocks from previously grown culture were inoculated into the broth and incubated for 7 days. After incubation, the culture filtrates were centrifuged, and optical density (OD) value of the supernatant was measured at 450 nm in UV Spectrophotometer (Elico: model SL-159). One unit of enzyme was expressed as the amount of enzyme required for increment of 1.0 absorbance unit per min (Adhi *et al.,* 1989).

2.11.2. Effect of temperature

The different enzyme assay media were prepared and the actinobacterial cultures were inoculated, and incubated at different temperature such as 18, 28, 38, and 48°C for 7 days. The enzyme activities of the actinobacteria and enzyme quantities were measured as previously mentioned.

2.11.3. Effect of NaCl

The different enzyme assay media were prepared with different NaCl concentration (0, 1, 2 and 3%) and the actinobacterial cultures were inoculated and incubated for 7 days. The enzyme activities of the actinobacteria and enzyme quantities were measured as previously mentioned.

3. Results and Discussion

Actinobacteria comprise an extensive and diverse group of Gram positive, aerobic bacteria that play an important role in biogeochemical cycles. Many are well known for their economic importance as producers of biologically active substances such antibiotics, vitamins and enzymes. In the present study, the isolation, identification and screening of enzyme activity of actinobacteria was carried out from the soils of textile dye polluted area of Tirupur. The heat treatment used often as a method for reduction of Gram negative bacteria that commonly occur in the soils and overrun the isolation plates (Jensen *et al.,* 1991; Pisano *et al.,* 1989). Usually, the conventional isolation techniques employed were recovered most of streptomycetes, which are dominant actinobacteria in the soil (Nolan and Cross, 1988)**.** In the present study, a total of 20 different morphologically distinct actinobacteria were isolated from textile dye polluted soil of Tirupur after the pre-treatment of soil by drying at 50°C for 2 h. Among the 20 isolates, 25% of the isolates were belonged to *Streptomyces* (n=5)*, Pseudonocardia* (20%; n=4), *Actinopolyspora* (15%; n=3), *Actinoplanes* (10%; n=2), *Nocardia* (10%; n=2), *Streptoalloteichus* (10%; n=2), *Nocardiopsis* (5%; n=1) and *Actinomyces* (5%; n=1) (Table 1; Fig. 1). The earlier (Vijayakumar *et al.,* 2007; 2012a; b) and present studies reported that among the actinobacteria, *Streptomyces* was reported as predominate genera in the terrestrial as well as marine soil samples when compared to other genera.

S. No.	Name of the isolate	Isolate code assigned	Colour of aerial mycelium	Colour of substrate mycelium	Colony size (mm)
1.	*Pseudonocardia* sp.	ESP1	White	Creamish yellow	1.5
2.	*Pseudonocardia* sp.	ESP2	White	Creamish yellow	1.5
3.	*Actinoplanes* sp.	ESP3	White	Yellow	2.0
4.	*Streptomycetes* sp.	ESP4	White	Yellowish white	1.0
5.	*Streptomycetes* sp.	ESP5	White	Yellow	1.0
6.	*Nocardia* sp.	ESP6	White	Yellow	3.0
7.	*Actinopolyspora* sp.	ESP7	White	Yellow	2.5
8.	*Actinopolyspora* sp.	ESP8	White	Yellow	2.5
9.	*Actinoplanes* sp.	ESP9	Grey	Yellow	1.5
10.	*Streptomyces* sp.	ESP10	White	Creamish yellow	1.0
11.	*Actinopolyspora* sp.	ESP11	White	Yellow	2.5
12.	*Pseudonocardia* sp.	ESP12	White	Creamish yellow	1.5
13.	*Actinomyces* sp.	ESP13	Pink	Red	1.5
14.	*Streptomyces* sp.	ESP14	White	Light brown	1.0
15.	*Streptomyces* sp.	ESP15	White	Yellow	1.0
16.	*Pseudonocardia* sp.	ESP16	White	Yellow	1.5
17.	*Nocardia* sp.	ESP17	White	Yellow	3.0
18.	*Streptoallotecichus* sp.	ESP18	White	Yellow	5.5
19.	*Nocardiopsis* sp.	ESP19	Light brown	Yellow	3.0
20.	*Streptoalloteichus* sp.	ESP20	Grey	Light brown	5.5

Table 1: Cultural characteristics of actinobacterial isolates

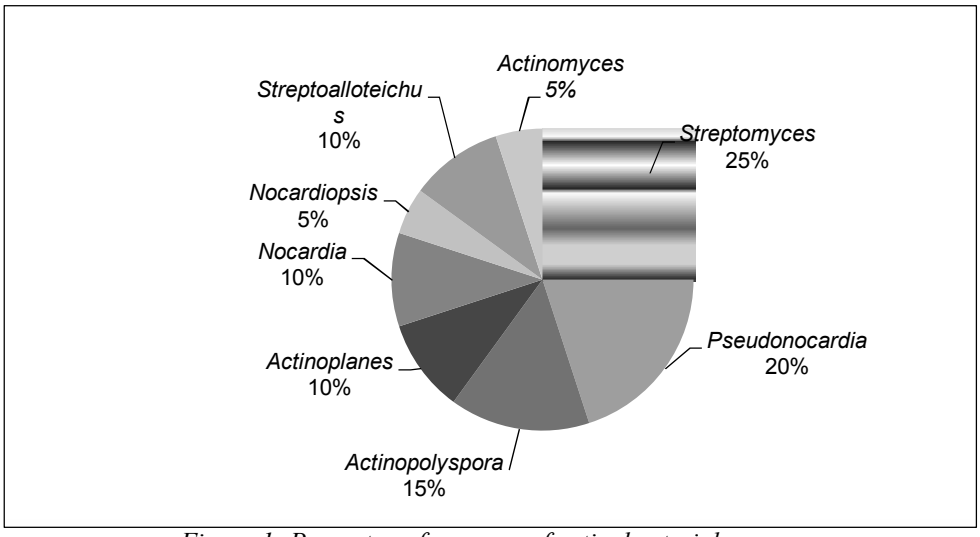

Figure 1: Percentage frequency of actinobacterial genera

Soil actinobacteria showed their optimum growth in neutral and slightly alkaline conditions and isolation procedures have been traditionally based on this neutrophilic character. Previous reports showed the existence of a large diversity of acidophilic actinobacteria that was different with respect to morphological and physiological properties from neutrophilic species. Acidophilic isolates grow in the pH range of 3.5 – 6.5, with optimum growth between 4.5 and 5.5, while neutrophiles grow in the pH range of 5.0 and 9.0 (Flowers and Williams, 1977; Mikami *et al.,* 1982). In the present investigation, it was found that the pH of the soil was recorded as 8.6, electrical conductivity 3.35, soil texture was found to be sandy soil type, lime status was found to be calcareous, N and K contents of the soil was recorded as 18.5kg/ha^{-1} and 369 kg/ha^{-1} (data not shown) respectively. Similar type of work has been reported by several workers (Ghanem *et al*., 2000; Cholarajan and Vijayakumar, 2013; Vaijayanthi and Vijayakumar, 2014). The ecological parameters such as pH, temperature, total N, P and K and organic matter have significantly influenced the occurrence of actinobacteial population.

Actinobacteria contributes significantly to the turnover of complex biopolymers such as cellulose, lignocelluloses, hemicellulose, pectin, keratin and chitin. Actinobacteria are important source of enzymes which are involved in the degradation of cellulose, pulps, agro-wastes, textile cellulosic wastes, industrial and municipal wastes (Williams *et al*., 1989). In the present study, among the 20 isolates of actinobacteria, maximum of 7 isolates had L-asparaginase activity, followed by 6 isolates showed chitinase activity, 3 isolates with amylase activity, 2 isolates with protease activity, 2 isolates with cellulase activity and 2 isolates with keratinase activity, whereas 7 isolates of actinobacteria did not showed any enzyme activity (Fig. 2). Of these, *Streptoalloteichus* sp. (ESP18) and *Nocardiopsis* sp. (ESP19) exhibited maximum activity for all the enzymes tested. The screening of the production of extra-cellular enzymes by actinobacteria has been reported by many workers. Amylase activity of actinobacteria (Ellaiah *et al*., 2002), cellulase activity (LI X, 1997), chitinolytic activity (E1-Fiky *et al*., 2003), keratinolytic activity (Chitte *et al*., 1999), L-asparaginase activity (Dhevagi and Poorani, 2006) and protease activity (Nikolova *et al*., 2005). Based on the present and previous reports, it has been reported that most of the actinobacterial isolates exhibiting good enzymatic activities and acted as a major source of industrially important enzymes.

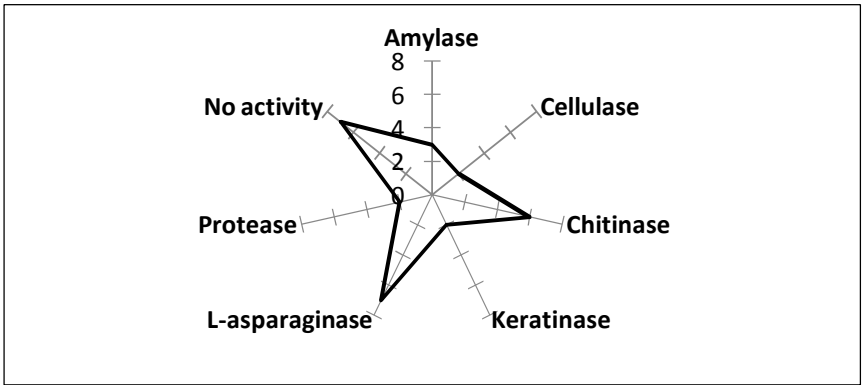

Figure 2: Screening of enzyme activity of actinobacteria

Identification of microorganisms, especially actino bacteria, could be achieved by morphological, cultural, biochemical, physiological and molecular characteristics. Formation of colonies on culture media, production of vegetative and aerial mycelia and arrangements of sporophores were the most important features of identification of *Streptomyces* (Waksman, 1961; Kuster, 1963). In the present study, the majority of the isolates produced white coloured aerial mycelium, yellow coloured substrate mycelium and none of the

isolates produced diffusible pigments. The potential isolate *Streptoalloteichus* sp. (ESP18) produced white coloured aerial mycelium and yellow coloured substrate mycelium. Similarly, another potential isolate *Nocardiospsis* sp. (ESP19) produced light brown coloured aerial mycelium and yellow coloured substrate mycelium on SCA medium. Microscopically, *Nocardiopsis* sp. (ESP19) produced oval shaped spores whereas *Streptoalloteichus* sp. (ESP18) produced cylindrical spores (Table 2). Similar type of work has been reported by many workers (Pridham and Tresner, 1974; Kim *et al*., 1999; Vijayakumar *et al.,* 2007; Thirumurugan and Vijayakumar, 2015). The two selected potent enzyme producers of the present study were grown on various culture media like *viz*. SCA, nutrient agar, starch nitrate agar, ISP 2, ISP 3, ISP 4, ISP 5, SDA, PDA, beef extract agar and brain heart infusion agar. They showed differences in colony colour such as yellow, white and brown series. Both the isolates varied in the colour of the aerial and substrate mycelium, size of the colony and none of the isolates produced diffusible pigments. Correspondingly, the cultural characteristics of the actino bacteria was studied on various culture media and reported by many workers (Pagani and Parenti, 1978; Vijayakumar *et al.,* 2012; Thirumurugan and Vijayakumar, 2013). Thus, it has been reported that the composition of the culture medium has a great influence on the morphological appearance of the organisms

Properties	***Streptoallotecichus* sp. ESP18**	***Nocardiopsis* sp. ESP19**
Morphological properties		
Sporophore morphology	Cylindrical	Oval
Colour of aerial mycelium	White	Light brown
Colour of substrate mycelium	Yellow	Yellow
Spore mass	White	Brown
Spore shape	Oval	Cylindrical
Gram staining	+	+
Acid fast staining	Non acid fast	Non acid fast
Biochemical properties		
Indole	-	-
Methyl red	-	-
Voges proskauer	-	-
Citrate	+	-
H_2S production	+	+
Nitrate	+	-
Urease	+	+
Catalase	+	+
Oxidase	-	-
Casein	+	+
Starch	+	+
Gelatin	+	+
Haemolysis	β – haemolysis	β – haemolysis
Triple sugar iron	Ak/Ak	Ak/Ak
Melanin	-	-
Lipid	-	-
Di-aminopimelic acid	+	+
Cell wall sugars	+	+
***Physiological properties:* Temperature**		
18°C	No growth	No growth
28°C	No growth	No growth
38°C	Excellent growth	Excellent growth
48°C	Excellent growth	Excellent growth
pH		
6	Poor growth	Poor growth
7	Good growth	Good growth
8	Moderate growth	Moderate growth
9	Poor growth	Poor growth
Sodium chloride (%)		
0	Excellent growth	Excellent growth
1	Moderate growth	Moderate growth
2	Poor growth	Poor growth
3	No growth	No growth

Table 2: Phenotypic characteristic features of selected actinobacteria
(+ = positive; - = negative; Ak/Ak = alkaline bud acid slant)

Biochemical characters of the actinobacteria are play an essential role for species/ strain level identification/ differentiation (Kim and Goodfellow, 2002; Nikolova *et al.,* 2005; Oskay *et al*., 2004). In the present study, it was found that citrate utilization and nitrate reduction tests were positive only for *Streptoallotichus* sp. (ESP18) but not *Nocardiopsis* sp. (ESP19). Both the isolates produced H_2S,

catalase and urease and both the isolates hydrolyzed casein, starch and gelatin. Further, both the isolates were given positive results for haemolysis and TSI tests. Whereas, both the isolates ESP18 and ESP19 did not produce indole, oxidase and melanin pigment. Similarly, both the isolates were given negative results for methyl red and Voges-Proskauer tests and hydrolysis of lipid. The qualitative analysis of cell wall amino acids and sugars were considered as most useful techniques for the identification of actinobacteria to generic level. The most useful diagnostic marker is the diaminopimelic acid, which occupies the anchor position in the tetra peptidoglycan. In the present study, the cell wall of *Nocardiopsis* sp. (ESP19) was found no sugars but the cell wall of *Streptoallotechus* sp. (ESP18) was to found to have cell wall sugars like galactose, mannose, galactose, mannose and rhamnose. The biochemical characteristics like cell wall amino acids and whole cell sugars of actinobacteria have also been studied and reported by several workers (Balagurunathan and Subramanian, 2001; Kokare *et al*., 2004; Vijayakumar *et al.,* 2012c).

Several physiological properties are very significant for the identification of actinobacteria at genus level but not species level. But up to certain extent they can be used as markers by which an individual strain can be recognized. The present study revealed that both the selected isolates were grown well at the pH ranged between 6.0 and 9.0 and the temperature ranged between 38 and 48°C. The inhibitory effect of compounds was studied and found that both the isolates were able to tolerate NaCl ranged between 1% and 2%. Both the isolates could not tolerate at 3% level of NaCl. The utilization of carbon and nitrogen sources also used to confirms the identity of actinobacteria (Augustine *et al.,* 2005; Dhevagi and Poorani, 2006). Influence of various physiological parameters on the growth of actinobacteria have already been studied and reported by Kim *et al.* (1999); Shimizu *et al.* (2000); Remya and Vijayakumar (2008). The present study revealed that carbon sources namely starch, maltose and mannitol were utilized by both the isolates ESP18 and ESP19, whereas dextrose and sucrose were utilized by the isolate ESP18 alone. The nitrogen sources (amino acids) namely L-asparagine, L-cysteine and L-tyrosine were utilized by both the isolates, whereas L-arginine and L-histidine were utilized by none of the isolates (Table 3). Based on the morphological, biochemical and physiological properties the isolate ESP18 was found similar to *Streptoalloteichus hindustanus* and the isolate ESP19 was found similar to *Nocardiopsis mutabilis.* The identity of both the isolates was also confirmed by Bergey's Manual of Systemic Bacteriology (Williams *et al.,* 1989) and Bergey's Manual of Determinative Bacteriology (Pridham and Tresner, 1974).

S. No.	Name of the carbon/ nitrogen source	*Streptoallotecichus* sp. ESP18	*Nocardiopsis* sp. ESP19
Carbon source utilization			
1.	Starch	+++	+++
2.	Dextrose	++	-
3.	Fructose	+	-
4.	Maltose	++	+
5.	Mannitol	+++	+++
Nitrogen (amino acid) source utilization			
6.	L-arginine	+++	+
7.	L-asparagine	+++	+++
8.	L-cystine	+++	+++
9.	L-histidine	+++	-
10.	L-tyrosine	+++	+++

Table 3: Utilization of carbon and nitrogen sources

3.1. Optimization of enzyme activity

The enzyme activity has been observed in both solid and liquid media. Enzyme activity is often influenced by the component of medium and cultural conditions, such as aeration, agitation, pH, temperature, salinity, time course, nitrogen and carbon sources and suitable media, which often vary from organism to organism. In the present study, the required conditions have been optimized for the enzyme production using the terrestrial actinobacteria. Temperature is an important factor influencing not only in the diversity, distribution, physiology, morphology, sporulation, biochemistry of the microorganisms but also greatly in the production/synthesis of their metabolites. In the present study, both the isolates showed maximum enzyme activities at the temperature ranged between 38 and 48°C whereas minimum enzyme activities were found at 28°C and poor enzyme activities found at 18°C. The maximum production of amylase 1.6 U/min, chitinase 1.4 U/min and L-asparaginase 1.7 U/min was achieved at 48°C by the isolate ESP18, whereas the maximum production of cellulase 1.3 U/min, keratinase 0.9 U/min and protease 0.9 U/min at 48°C was achieved by the isolate ESP19 (Fig. 3) The isolates produced enzymes moderately at other incubation temperatures. The similar type of work has been carried by Rawashdeh *et al.* (2005). It is also clear from the present study that temperature plays an important role on the enzyme production.

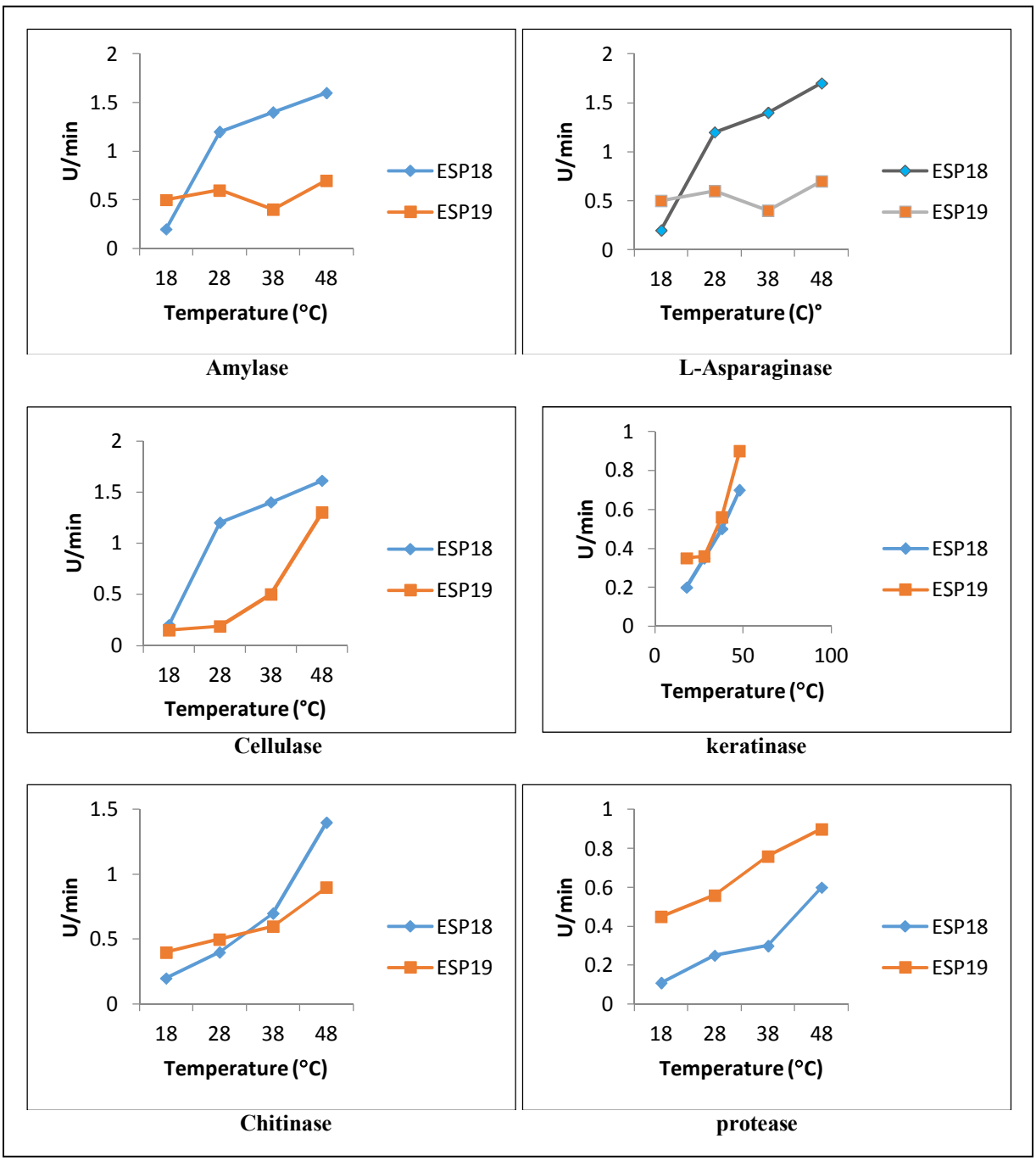

Figure 3: Effect of temperature on the production of enzymes

The changes in the pH of the culture medium will greatly influence production of the enzymes by microorganisms. It is a well known factor for each and every microorganism has an optimum, minimum and maximum pH at which it will grow. The maximum enzyme activity was found at pH 6, 7, 8, and 9 but the production of enzyme was reduced when the pH of the medium was low. The maximum (1.1 U/min) amylase, chitinase (0.9 U/min), keratinase (1.0 U/min), protease (0.9 U/min) and L-asparaginase (1.2 U/min) enzymes production were recorded at pH 9 by the isolate ESP19, whereas maximum production of cellulase (1.6 U/min) was recorded at pH 7 (Fig. 4) In contrast to this, another isolate ESP18 produced all the enzymes at moderate quantity at all the pH tested. From this study it was reported that the actinobacteria prefers neutral or slightly alkaline condition for the production of enzymes. The pH stability of extra-cellular enzymes and role of pH in the production of extra-cellular enzymes was already reported by Meena *et al.* (2013) and Prakash *et al.* (2013).

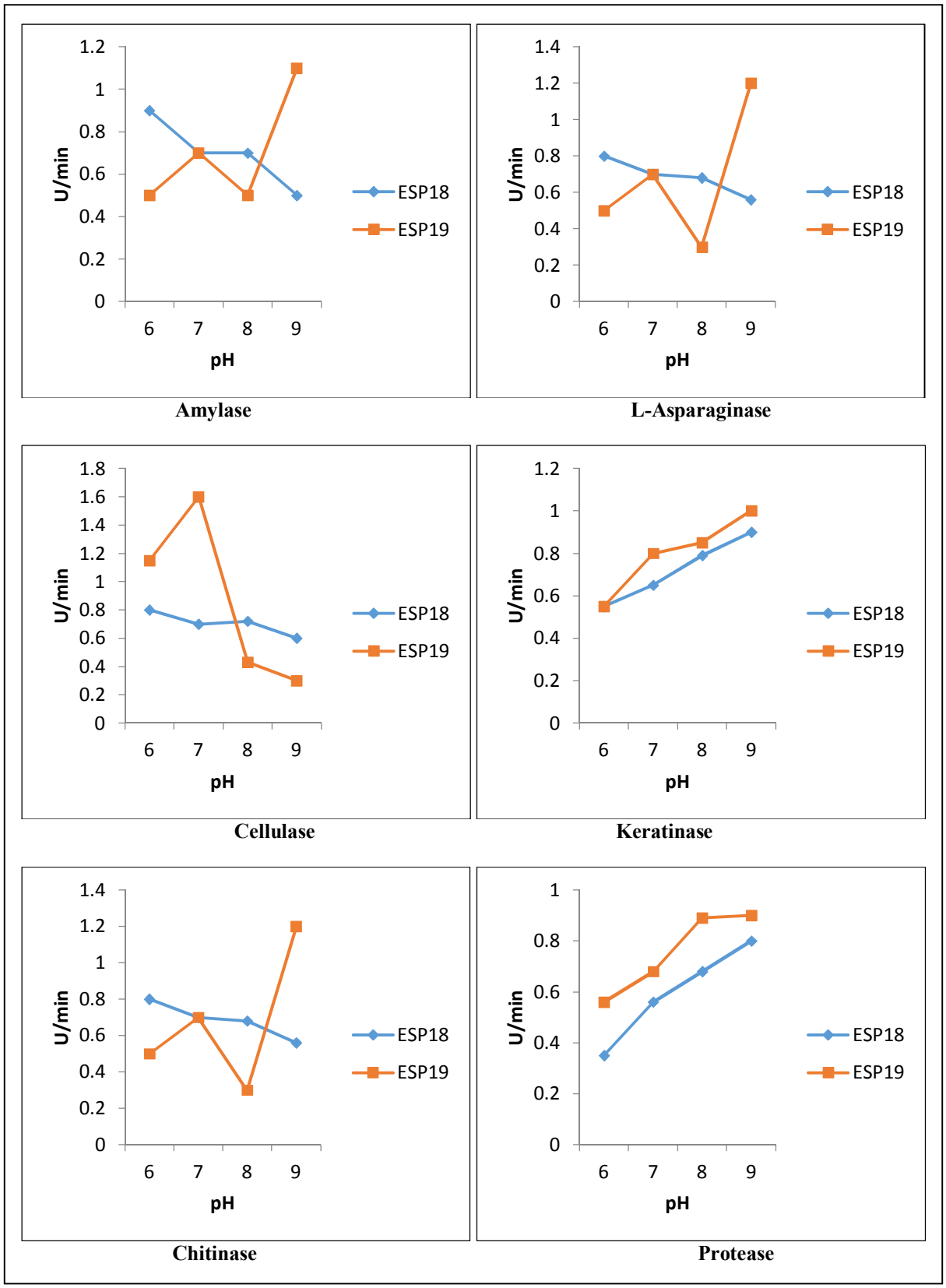

Figure 4: Effect of pH on the production of enzymes Amylase

The ability of microorganisms to tolerate NaCl in media is well known; likewise certain halophilic bacteria are known to develop in pickling brines containing 20-30% NaCl (Tresner *et al.,* 1968). The minimum concentration of salts preferably sodium chloride is essential for the growth of actinobacteria and the production of their metabolites (Mathew *et al.,* 1994). In the present study, it was

found that 1-2% level of salinity was optimum condition for the production of enzymes. The maximum content of amylase (0.7 U/min) and L-asparaginase (0.7 U/min) was produced by the isolate ESP18 production media without NaCl concentration. But, cellulase and protease (1.9 U/min) were produced maximum by the isolate ESP19 media without NaCl. Whereas, the maximum quantity of chitinase (1.7 U/min) and keratinase (1.7 U/min) were produced by both the isolates ESP18 and ESP19 in the media without NaCl (Fig. 5). It was also found that 3% salinity minimized the enzyme production as well as the growth of the both isolates. Thus it is obvious that the salinity plays a vital role on the enzyme activity in actinobacteria. The maximum enzyme production of the two potent isolates was expressed as U/min. Similarly, reports on the extra-cellular enzyme production by actinobacteria were reported by several workers (Adhi *et al.,* 1989; Yang and Wang, 1999).

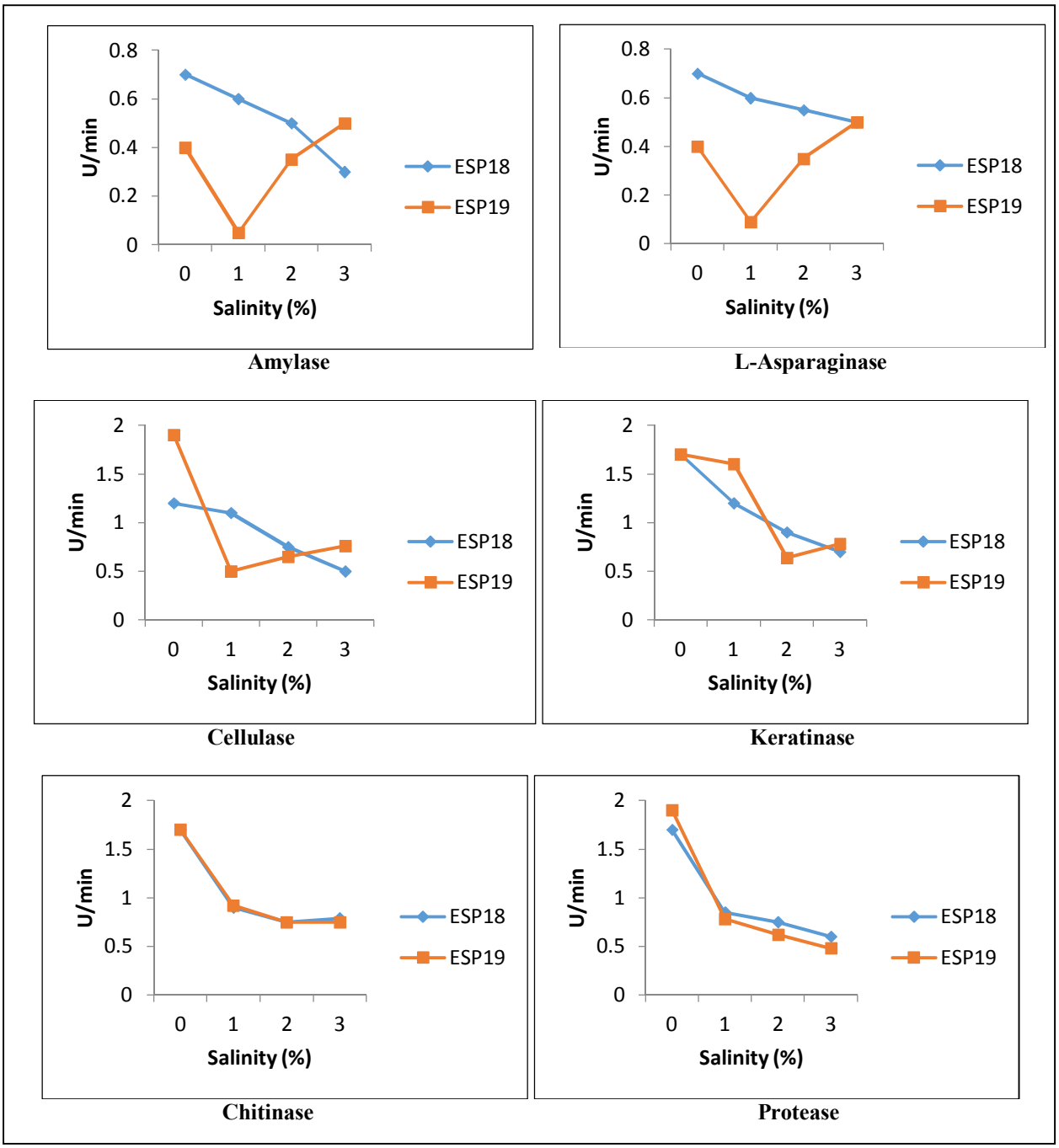

Figure 5: Effect of salinity on the production of enzymes Amylase

Actinobacteria are a reservoir of important enzymes and metabolites due to their versatile genetic repertory. However, many of the rare genera of actinobacteria have been neither explored nor manipulated for their biotechnological and industrial potential. The enzymes of actinobacteria possessed wide applications in particular finishing, modification of cotton, deinking, cleaning, scouring and removal of starch in textile industry. Overall results of the present study concluded that the polluted soils of Tirupur are good source of potential actinobacteria and their novel enzymes. Future course work such as purification and characterization of enzymes from the

actinobacteria and effect of enzymes on different chemicals, dyes, chromogenic compound etc. would make a platform on the applications of actinobacterial enzymes in textile and dye industries.

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