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Isolation And Biochemical Characterization of Potential Isolates of Actinomycetes for The Production of Tyrosinase from the Campus of Davangere University

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Abstract

Aim: Tyrosinase is a important industrial enzyme which is produced by actinomycetes. Tyrosinase has many applications in medical, industrial and agriculture sector. The present basic study aims to isolate the potential actinomycetes from the campus of Davanagere University.

Methodology and Results: Exploration of untapped environments for the isolation and characterization of actinomycetes is one in all the priority areas of research. Many culture-based studies have revealed that actinomycete recovered from the varied habitats comprises substantial synthesis potentials for the assembly of assorted bioactive compounds. Detection of a typical catalytic zone developed on Tyrosine Gelatin Beef extract (TGB) agar indicating the tyrosinase activity by actinobacteria may be a unique approach. 15 isolates of actinomycetes obtained from soils of Davangere University, India and were proved to be promising during primary and secondary screening for tyrosinase activity, supported the degree of catalytic zone and also intensity of color. Thus, observation of catalytic zone additionally to this the intensity of color could be a novel approach for the detection of tyrosinase activity. TGB medium has been proved as best suited (high intensity of color and maximum catalytic zone) for tyrosinase activity, from among eight media, including two conventional and 7 modified media, assessed during primary and secondary screening. An isolate of actinobacterium MSV1 and MSV3 has been proved as best with the most production of tyrosinase in TGB (15.16 IU) media. The foremost potential isolates were subjected for the biochemical characterization studies.

Conclusion, significance, and impact of study: The basic research to find the potential isolate for the production of tyrosinase was successfully conducted and the potential isolate will be explored further.

Keywords
Actinomycetes, streptomyces, Media, catalytic zone, tyrosinase

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1. INTRODUCTION:

The Actinomycetes are Gram positive bacteria having high G+C (>55%) content in their DNA. Actinomycetes were originally considered to be an intermediate group between bacteria and fungi but now are recognized as prokaryotic organisms. They are saprophytic bacteria found cosmopolitan in soil, water and colonizing plants. Several species of Streptomyces genus produce bioactive molecules like antibiotics, pigments and lots of extracellular enzymes. Their capacity to provide tyrosinase was studied in an exceedingly lesser extent. Additionally, this group of actinomycetes is additionally able, when are cultivated on organic media, to synthesize and excrete dark pigments, melanin or melanoid, which are considered as useful criteria in taxonomic studies. (Subhash *et al*., 2016).

Tyrosinase could be a rate-limiting oxidase enzyme which controls the assembly of melanin. It's directly involved in two melanin synthesis reactions; monophenol hydroxylation and O- diphenol to o-quinone which further goes through more reactions leading to the formation of melanin. Scientific investigations have proved the presence of this copper-containing enzyme in animal and plant tissues catalysing by Oxidation from tyrosine melanin and other pigments. In plants, it's the enzyme answerable for the undesired brown colour when plants are left within the air like potato. Tyrosinase is found within the skin melanocytes specifically in melanosomes. TYR gene is that the gene accountable for encoding Tyrosinase in humans (Abdirahman *et al*., 2017)

Melanin formation from tyrosine is catalysed by the enzyme tyrosinase or oxygen oxidoreductase. Tyrosinase a copper-containing enzyme that has been found cosmopolitan in microorganisms. Tyrosinase catalyses the o-hydroxylation of monophenols into their corresponding o-diphenols (monophenolase or tyrosine hydroxylase (TH) activity), and also the oxidation of o diphenols to o-quinones (diphenolase or dopa oxidase activity), using molecular oxygen, which then polymerise to make brown or black pigments. The synthesis of o-diphenols (catechols) could be a potentially valuable catalytic ability and thus tyrosinase has attracted plenty of attention with relevance biotechnological applications. An enzyme tyrosinase has remarkable properties and lots of applications in several sectors as mentioned. Different aspects of an enzyme tyrosinase are to this point studied to some extent throughout the planet by scientists. (Subhash *et al*., 2016).

2. MATERIAL AND METHOD:

Isolation of actinomycetes:

Soil samples were collected from different region around Davanagere University such as are Chanut form, near channel, compost and the soil which is adhered to the roots of plants were collected from 4 to 5 cm depth into sterile plastic bags, to avoid external contamination. The collected soil samples were brought to the laboratory and treatment of soil sample was done.

Culture enrichment:

The collected soil samples were processed for culture enrichment.1 gm of collected soil samples were mixed with 1 gm of calcium carbonate (CaCo3) in 1:1 ratio in 50 ml of distilled water. This mixture was kept in rotary shaker at 180 rpm for 3 days at 400 c temperature. After 3 days 1 ml of culture is serially diluted up to the 7th dilution. 0.1 ml of 5th and 6th dilution was taken and spread evenly over the surface of Starch casein agar medium. Plates were incubated at 40^0 C for 7 days. After 7 days incubation, whitish colonies, characteristic of actinomycetes were observed with aerial mycelium and substrate mycelium (Bertoka *et al*., 2016).

Screening of actinomycetes for extracellular tyrosinase:

Qualitative screening:

The leading cultures of actinomycetes preserved were screened for the synthesis of extracellular tyrosinase on tyrosine gelatine beef extract medium by standard plate culture method (Shivaveerakumar *et al.,* 2013). Three days old cultures were independently streaked on TGB medium and incubated at 400 c for 4 days. Visual observations on fourth day of incubation exhibiting development of light brown and dark brown colours were recorded. The formation of brownish zone of catalysis was also recorded accounting to the level of synthesis of tyrosinase by the test cultures (Shivaveerakumar *et al.,* 2013).

Characterization of Isolates

All the isolates were morphologically and biochemically characterized.

Gram Staining:

Smear was prepared by spreading the broth culture on a glass slide fallowed by heat drying. The smear was covered with crystal violet for 30-60 seconds, decolorized with alcohol, and washed with water. Finally, the smear was stained with safranin counter stain for 2 min. After washing and drying, the slides were viewed at 100x under light microscope.

Morphological Characters:

Actinomycete isolates were inoculated on Starch casein agar medium and incubated for 5 days at 300c. The colonies were observed under a high-

power magnifying lens and colony morphology was noted with respect to colour, aerial and substrate mycelium, branching, and the nature of colony.

Phenotypic characterization

The classification of actinomycetes was originally based largely upon the morphological observation. So, morphology is still an important characteristic for the description of taxa, and it is not adequate in itself to differentiate between many genera. In fact, it was the only characteristic, which was used in many early descriptions, particularly of Streptomyces species in the first few editions of Bergey's Manual. These observations are best made by the variety of standard cultivation media. Several of the media suggested for the International Streptomyces project (Shirling and Gottlieb *et al.,* 1966) and by (Pridham *et al*., 1957) have proven to be useful in our hands for the characterization of strains accessioned into the ARS Actinomycetes culture collection. It includes some basic tests, Aerial mass colour, reverse side pigment, Melanoid pigments, Spore chain morphology and Spore morphology.

Aerial mycelium

For the grouping and identification of actinomycetes the Chromogen city of the aerial mycelium is an important character. The colour of the mature sporulating aerial mycelium is white, gray, red, green, blue and violet following Prasuer (1964). When the aerial mass colour falls between two colours series, both the colours are recorded. In the cases where aerial mass colour of a strain showed intermediate tints, then in that place both the colour series should be noted (Gottileb *et al.,* 1966).

Substrate mycelium

The actinomycetes produce different coloured pigment in the reverse side of the colony this helps to the identification of the actinomycetes. The colour of the mature sporulated substrate mycelium is yellow, white, red, Gray, green.

Biochemical Characterization:

After the preliminary studies, the isolates which were found to be positive were selected for biochemical studies. Biochemical tests generally used are nitrate reduction test, catalase test, Carbohydrate fermentation test.

Catalase test:

Place a drop of of 3% H_2O_2 on clean grass free slide. Take a loop full of culture and mix with H2O2. Observe the rapid evolution of oxygen as evidenced by bubbling shows the positive result and no bubble formation shows the negative result.

Nitrate reduction test:

Nitrate disc was taken, add one drop of water on the nitrate disc and take on loop full of culture mix it with water. Allow it for few seconds and observe the changes of the colour in nitrate disc.

Carbohydrate fermentation test:

The carbohydrate fermentation test is used to determine whether bacteria can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. It tests for the presence of acid and/or gas produced from carbohydrate fermentation. Basal medium containing a single carbohydrate source such as Glucose, Lactose, Sucrose, or any other carbohydrate is used for this purpose. A pH indicator (such as Andrade's solution, Bromcresol purple (BCP), Bromothymol blue (BTB) or Phenol red) is also present in the medium, which will detect the the lowering of the pH of the medium due to acid production. Small, inverted tubes called Durham tube is also immersed in the medium to test to produce the gas (hydrogen or carbon dioxide).

Quantitative screening:

All the test cultures of actinomycetes were subjected to quantitative screening to produce tyrosinase under submerged bioprocesse in TGB broth.1 ml of 3 days old cultures were inoculated independently into 100ml TGB broth an incubated for 120 hrs. The quantity of tyrosinase produced was determined by modified standard enzyme assay protocol (Shivaveerakumar *et al*., 2014).

Enzyme assay:

The level of tyrosinase produced was visually correlated with the intensity of brown colour developed in the medium. The exact amount of tyrosinase produced in the medium was determined by uv-vis spectrophotometric analysis.10ml of spent broth was carefully withdrawn and centrifuged at 13000 rpm for 15 mi. The supernatant thus obtained was used as crude extract of tyrosinase. Standard reaction mixture was prepared using 1 ml of crude enzyme ,1ml of tyrosine and 0.5 ml of sodium phosphate buffer (pH 6.8) (Horowitz *et al.,1963).* The components of standard reaction mixture were brought to the room temperature independently before preparing the reaction mixture. The mixture was allowed to react for 15 min and absorbance of reaction mixture was monitored at 275nm (Majumdar *et al.,* 2018)

The calculation of enzyme activity was done by using the following formula

Enzyme activity=micrograms of substrate catalyzed ×total assay volume/Volume of enzyme× incubation time

3. RESULTS:

Isolation and identification of actinomycetes:

Actinomyces are group of prokaryotic organisms belonging to subdivision of Gram-positive bacteria phylum: actinobacteria (Stackebrandt *et al.,* 1997) they are filamentas bacteria which produce two kinds of branching mycelium. Aerial mycelium and substrate mycelium. In the present study it was aimed to isolate potential actinomycetes for the synthesis of tyrosinase from various habitats of Davanagere University.

Fig 1 indicates various habitats of unique ecological niche from where the soil samples were collected for the isolation of actinomycetes. About five different soil samples were collected from different habitats of davangere university. Around 15 isolates were obtained from the different soil samples collected. Comparatively compost soil sample was proven to have the greater number of actinomycetes.

Although, actinomycetes were isolated on selective specific media it is difficult to distinguish the colonies of actinomycetes from bacteria and fungi. It is for a simple reason that the colonies actinomycetes share the typical colony features of both bacteria and fungi. However, colonies of actinomycetes are yet stands to be distinguished from bacteria and fungi. Actinomycetes grown on starch casein agar were identified based on their typical colony characters (fig 1) and gram staining (fig 2) properties. Dry highly pigmented small to medium size colonies were identified and confirmed as isolates of actinomycetes based on their gram-positive and unique highly branching filamentous nature. These are the typical characters of the isolates to be confirmed as actinomycetes as par the significant features proposed by Pridham and gottileb (1948) Flaig and kutzner(1960) Shirling and gottilb (1966).

Figure 1: Showing different habitats from where the soil samples were collected

A

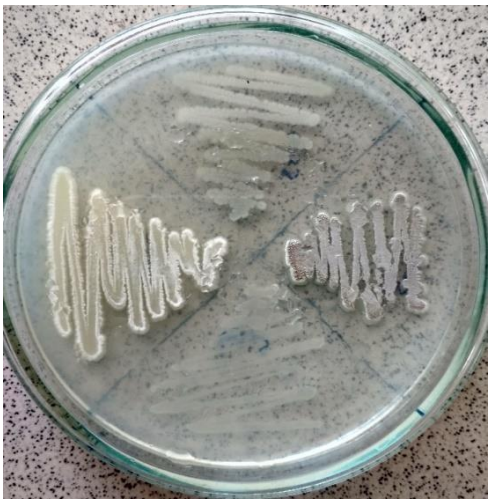
B

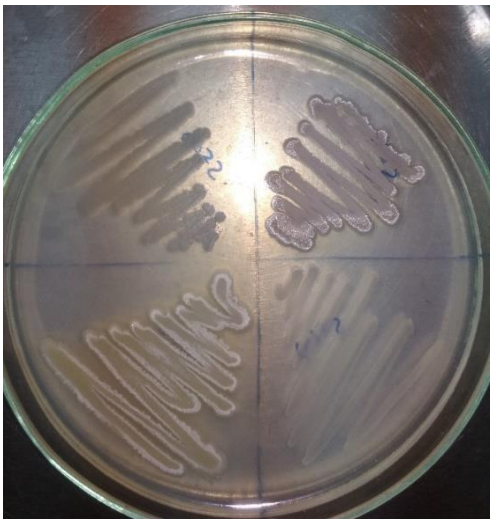
C

D

Figure 2: Showing the morphological observation of isolated actinomycetes.

(A) **(B)**





Morphological identification:

Actinomycetes are characterized by the formation of normally branching threads or rods. The hyphae are generally non-septate; under certain special conditions, septa may be observed in some forms. The sporulating mycelium may be branching or non-branching, straight or spiral shaped. The spores are spherical, cylindrical, and oval shaped isolates also observed for several characteristics such as presence or absence of aerial mycelium. Presence of sclerotic, spore chain morphology. In the present investigation fifteen test isolates of actinomycetes were identified based on morphological features. Almost all the isolates exhibit small, circular, and smooth dry colonies but few isolates also exhibit medium, circular and rough dry colonies which were shown in Figure 2.

Biochemical characterization:

Gram staining:

Isolated bacteria were subjected to the Gram's staining (Figure 3) and observed under microscope (Oil immersion); it was found that all the isolates are gram positive in nature.

Visual observation:

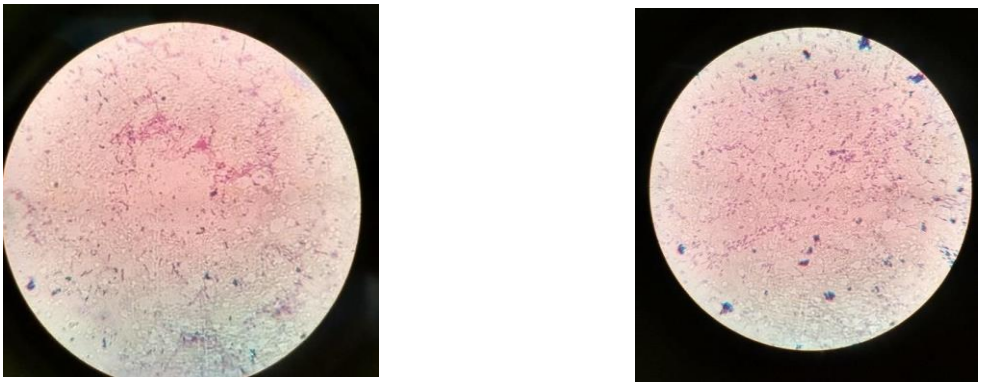
Out of 15 isolates 6 were identified as arial mycelium showing the colour of the mature sporulating aerial mycelium as white, gray, red, green, blue, and violet. Remaining 9 isolates are showing substrate mycelium. The visual observation of each colony is depicted in Table 1.

Table 1: Colony morphology of actinomycetes.

Colony characters	MSV1	MSV2	MSV3	MSV4	MSV5	MSV6
Shape	Round	Round	Round	Round	Round	Round
Margin	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	Flat	Flat	Raised	Convex	Flat	Raised
Size	Small	Large	Large	Modarate	Modarate	Modarate
Texture	Rough	Rough	Rough	Rough	Rough	Rough
Appearance	Dull	Dull	Dull	Dull	Dull	Dull
Pigmentation	Creamy	White	White	Light pink	White	White
Optical property	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque

Figure 3: All the strains showing Gram's nature and spore chain morphology was observed.

(A) **(B)**



Catalase test:
The rapid evolution of oxygen is not evidenced by bubbling hence the result is recorded as negative. All the isolates are showing negative for catalase test as depicted in Table 2.

Table 2: Biochemical characterisation of actinomycetes

Gram staining	+	+	+	+	+	+
catalase	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-
Maltose utilisation	+	+	+ With gas	+	+With gas	-
Glucoseutilization	-	-	-	-	+	-

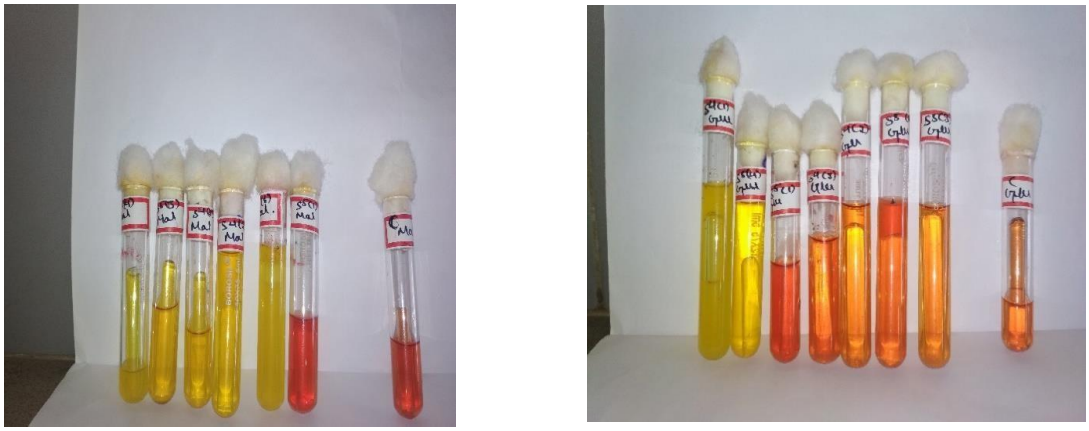
+: Positive - : Negative

Nitrate reduction test:
All the isolates are subjected to nitrate reduction test, all the isolates are showing negative result. nitrate reductase enzyme that hydrolyzes nitrate (NO_3^-) to nitrite (NO_2–) is not produced hence all the isolates showed negative result for nitrate reduction test.

Carbohydrate fermentation test:
Maltose utilization:
Out of 6 isolates 3 shows positive result, 2 shows positive result with gas production and 1 show negative result. The colour of the medium turns from red to yellow due to the production of acid which is recorded as positive (Figure 4).
Glucose utilization:
Out of 6 isolates 2 shows positive result and remaining are negative. The colour of the medium turns from red to yellow due to the production of acid which is recorded as positive (Figure 4).

Figure4: A represents maltose utilization test and B represents glucose utilization test

(A) **(B)**



Screening:

Screening large number of isolates from natural sample aiming at potential bioactive molecule is a troublesome task and critical. The isolates under screening unless are subjected a phase wise screening, it is is difficult to distinguish efficient isolates. This can be achieved by subjecting the isolates for or both qualitative and quantitative screening. Out of 15 isolates 6 were known to be positive for tyrosinase enzyme production. They showed blackish brown pigmentation on tyrosine agar medium while deep red colour were observed in the tyrosine broth. The identified and selected 6 isolates of actinomycetes were subjected for qualitative screening to assist their ability of synthesizing tyrosinase on TGB media.

Qualitative screening:

The isolates are streaked on TGB medium and incubated at 40^0C for 4 days. On fourth day of incubation the development of light brown and dark brown colours were observed. The zone of catalysis is measured and depicted in table 3.

Table 3: Qualitative screening of actinomycetes for the synthesis of tyrosinase on TGB media

Sl no	Name of isolates	Catalyses zone in mm
1	MSV1	10
2	MSV2	23
3	MSV3	25
4	MSV4	11
5	MSV5	15
6	MSV6	17

Table 3 explains all the 6 isolates of actinomycetes and their ability to to synthesise tyrosinase at 120 hour of incubation period. Formation of of zone of catalyzes on the TGB agar from 0- 10(poor)10-20 (modarate) 20-30(maximum) tyrosinase production on TGB medium.

Figure 5 indicates the dark brown colour development and zone of catalyses. The test isolates of actinomycetes were coded with different alphabets and numbers based on the researchers involved in in the process of isolation and characterization of actinomycetes. This illustrates the isolates for tyrosinase activity by the zone of catalyzes with pigmentation. The qualitatively potential isolate was employed to produce tyrosinase in TGB media.

Quantitative screening:

The selected six test isolates of actinomycetes based on the maximum activity exhibited at 120 hours of incubation were selected and subjected for quantitative screening. The level of tyrosinase produced was visually correlated with the intensity of brown colour developed in the medium shown in figure 6.

Enzyme assay:

The exact amount of tyrosinase produced in the medium was determined by UV-Vis spectrophotometric analysis.10ml of spent broth was carefully withdrawn and centrifuged at 13000 rpm for 15 mi. The supernatant thus obtained was used as crude extract of tyrosinase. Standard reaction mixture was prepared using 1 ml of crude enzyme ,1ml of tyrosine and 0.5 ml of sodium phosphate buffer (pH 6.8).

The components of standard reaction mixture were brought to the room temperature independently before preparing the reaction mixture. The mixture was allowed to react for 15 min and absorbance of reaction mixture was monitored at 275 nm (Majumdar *et al*., 2018).

Figure 5: Qualitative screening of actinomycetes for the synthesis of tyrosinase

(A) **(B)**

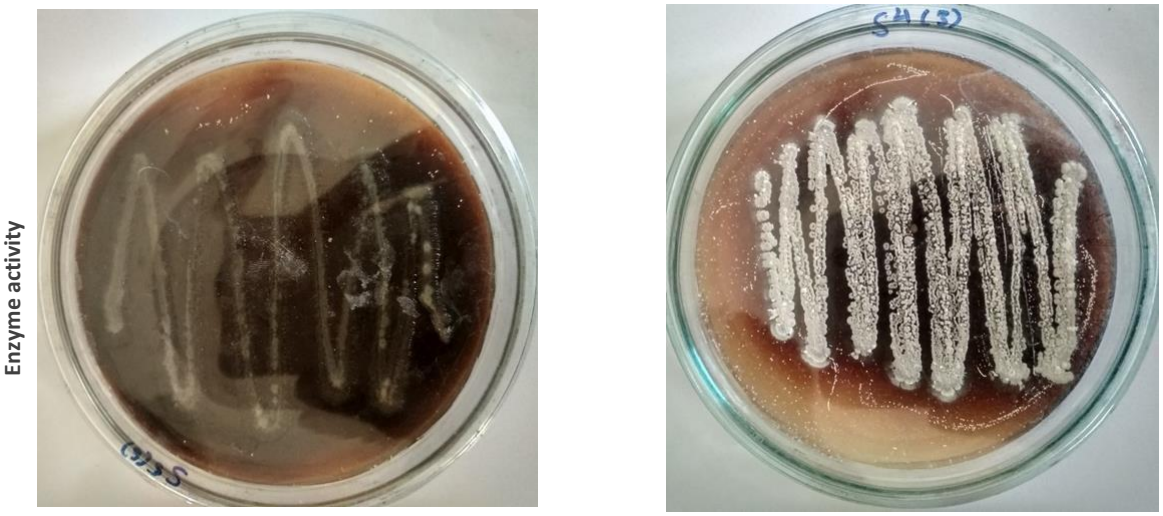


Figure 6: A showing the prepared TGB medium without inoculation and B showing the media after 4 days of inoculation

(A) **(B)**

Figure 7: Showing the Production of tyrosinase enzyme

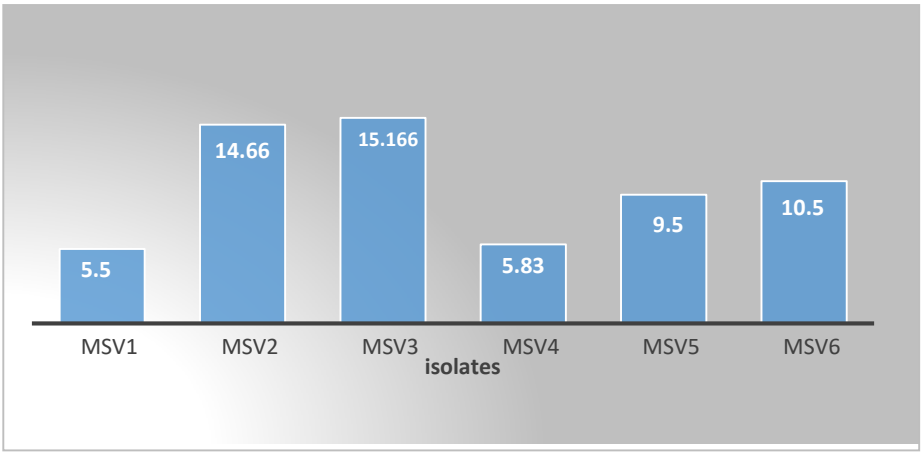


Table 4: Enzyme activity

Sample	Absorbance at 275nm
MSV1	0.125
MSV2	0.290
MSV3	0.301
MSV4	0.136
MSV5	0.176
MSV6	0.222

Table 4 Illustrate the activity of enzyme extracted from all the 6 isolates obtained by taking absorbance in Uv-visible spectrophotometer at 280 nm. The maximum enzyme was seen in MSV3 as 0.301 and minimum activity was seen in MSV1 as 0. 125.The concentration of the enzyme is detected by plotting the absorbance in standard tyrosine curve.

Concentration of tyrosine in the solution:

Enzyme activity =$\frac{\text{Micrograms of substrate catalysed} \times \text{total assay volume}}{\text{Volume of enzyme} \times \text{incubation time}}$

MSV1 = $\frac{33\times2.5}{1\times15}$ = 15.166 µmol/min

MSV2 =$\frac{88\times2.5}{1\times15}$ = 14.66 µmol/min

MSV3 = $\frac{91\times2.5}{1\times15}$ =15.166 µmol/min

MSV4 = $\frac{35\times2.5}{1\times15}$ =5.83 µmol/min

MSV5 = $\frac{57\times2.5}{1\times15}$ = 14.66 µmol/min

MSV6 = $\frac{63\times2.5}{1\times15}$ =10.5 µmol/min

Figure 7 illustrate the activity of enzyme at 72 hours of incubation. The maximum activity of tyrosinase as much as 15.166 µmol/min by MSV3 and minimum as much as 5.83 µmol/min at 72 hours of incubation by MSV4 was recorded. Therefore, it is assumed to be 15.16 is the highest activity recorded.

DISCUSSION:

Several studies reported the isolation of large numbers of actinomycetes from different sources which have got the great economic importance. It has been reported widely that tyrosinase production from actinomycetes have got more importance towards various aspectsas reported by researchers depending on the organisms and source of isolation. Gare Sandeep Subhash isolated total 70 actinomycete from different soil environments. Among 70 isolates, 18 isolates from soil of college region, 17 isolates from fertile soil of Shirala,9 isolates from Sarud, 12 isolates from garden region of college and 14 isolates from Kokrud region. Out of 70, 19 actinomycete isolates were showed blackish brown pigmentation on tyrosine agar and Peptone Yeast Extract Iron Agar while deep red colors were observed in the tyrosine broth. Out of the total 19 reported tyrosinase producer's maximum were from genus Streptomyces followed by *Strepto verticillum*. Percentage of tyrosinase production after qualitative test was recorded as 27.1%.

Gare and Kulkarni (2015) reported C7 as maximum tyrosinase producer and identified isolate C7 based on morphological, cultural, biochemical and 16S rRNA gene sequencing as *Streptomyces luteogriseus.* Gare and Kulkarni (2015) carried out qualitative tests for pigment melanin by inoculating in tyrosine broth supplemented with traces of chloroform and incubating at 30^0 C for 48 hrs. Red color was reported in tyrosine broth indicated positive melanin production. Based on intensity of red color seen in the tyrosine broth Kd8 was selected for further study (Kulkarni *et al* 2015).

Suki roy isolated total of 20 marine actinobacteria from marine water sample. Among these 20 isolates, 2 isolates LK-4 and LK-20 showed positive proteolytic activity in skimmed milk agar were selected for further studies. In the tyrosine agar plate, both the isolates LK-4 and LK-20 showed brown colored pigmentation. The occurrence of a brown

pigmentation around the colonies gave a positive indication for the tyrosinase production.

The colour of the inoculated tyrosine broth changed from light pink to brown and ultimately to deep red with further incubation. The marine actinobacteria *Streptomyces espinosus* strain LK-4 isolated from marine water sample was found to be the proficient producer of tyrosinase as well as potent remover of phenol from aqueous solution. The enzyme was found to be stable even at high temperature, pH and it's also having high enzymatic activity and greater stability than mushroom tyrosinase enzyme (Suki roy *et al*., 2014).

Majumdar isolated Actinomycetes strain A3DR2S from soil was screened for different industrially important enzymes. The result revealed that the isolate produce five enzymes viz, Amylase, Protease, Lipase, Tyrosinase and Asparginase. The study conducted by previous workers reported the production of enzyme Amylase, Protease, Xylanase and Lipase from different Actinomycetes isolated from soil. Melanin biosynthesis (charcoal blackish pigmentation) is a two-step biocatalytic process mediated by tyrosinase enzyme using tyrosine as the substrate material. Biochemically, tyrosinase enzyme possesses both monophenolase activity (hydroxylation of monophenols to o-diphenols) and diphenolase activity (oxidation of o-diphenols to o-quinones). In the first reaction, the tyrosine is converted to L-Dopa (3, 4- dihydroxyphenyl alanine), which upon subsequent catalysis step results in production of melanin. L-DOPA is pivotal in the treatment of Parkinson's disease (Majumdhar *et al* 2017). Both qualitative and quantitative screening is preferred for the production and detection of enzyme. Primary, secondary and if necessary, tertiary, stages are to be followed meticulously to target an efficient isolate for a desired product.

Primary screening does not give much information required in setting up a new fermentation procedure. Secondary screening aids in detecting helpful microorganisms in fermentation procedures and provides answers to many questions which arise during the final sorting out of industrially helpful microorganisms. Secondary screening experiments are conducted on agar plates or in flasks having liquid media or combination of such approaches, can be qualitative or quantitative in its approach. It yields the kinds of information that are required to compute the true potential of microorganisms for industrial usage.

It has been revealed that the production of tyrosinase by a microorganism in various growth media is regulated by composition of the medium, the growth duration, PH and the presence of enzyme inducers (Katz and betankart,1988). The tyrosinase enzyme from various species have been study for more than a century. The first biochemical characterization of a bacterial enzyme was also performed using a tyrosinase from Streptomyces species (Lerch and Ettinger,1972).

4. CONCLUSION:

Detection of an efficient isolate *Streptomyces sp* from Davanagere University soil for the extracellular synthesis of tyrosinase is a major contribution to the world of industrial important microorganisms. Authentication of synthesis of extracellular tyrosinase by unusual and innovative observation of a clear zone of catalysis on tyrosine agar, along with the usual observation of pigmentation, is a novel and significant contribution of the present investigation.

5. REFERENCES:

Baumann, R and R.Hotter (1973). Genetic Analysis in a Melanin-producing Streptomycete, *Streptomyces glaucescens*. *Journal of General Microbiology* 12:463-474.

Crameri, L,R..Ettlinger,R..Hutter.,K.Lerch,M.A.Suter and J.Ajetterli (1981) Secretion of Tyrosinase in *Streptomyces glaucescens*. *Journal of General Microbiology*.9:371-379.

Dolashkia,A., Gushterovab,A., Voelterc,W., and Tchorbanova, B (2009). Purification and Characterization of Tyrosinases from *Streptomyces albus*. *Institute of Organic Chemistry with Center of Phytochemistry* 9:724-732.

Francisco, S (1997). Isolation and Charrectarization of A novel meleno gene marine bacterium. *Applied and Environmental Microbiology* 7:3499-3506.

Gao,F., Yuanhua Wu and Wang,W (2014). Identification and antifungal activity of an actinomycete strain against Alternaria spp. *Spanish Journal of Agricultural Research*.12:1158- 1165.

John Lacey., Actinomycetes in Compost (1997). *Ann Agric Environ Med*.11:113-121.

S. Shivaveerakumar, D.N.Madhusudhan, Dayanand agsar, Merja Kontro, (2013). Catalytic zone, a novel screening approach for the extracellular tyrosinase by *Streptomyces vinaceusdrappus* DSV5. Journal of Pure and Applied Microbiology. 7: 2799-2808

S. Shivaveerakumar, D.N. Madhusudhan, H. Raghavendra, Dayanand agsar, (2013). Screening of Streptomyces and process optimization to produce tyrosinase. Journal of Pure and Applied Microbiology. 8(8): 3245-3252.

Krishnamoorthy,S., Natarajan E., Muthukumar B., and Govindaraju, A (2017). Isolation, Separation and Production of Melanin Pigment in Streptomyces sp. from Garden Soil. *International journal of current biotechnology*.5: 6-13.

Mahmoud Al-Ruweidi. Abdirahman Said (2007). Literature Review on Tyrosinase Enzyme. *Ann Agric Environ Med*.11:1-11.

Majumder,D.R., Lambate.,S, Firfire,A., Palekar,R., Shaikh,A., Bhandari,A., and Sayyed,R (2018). Pharmaceutically Significant Enzymes-Tyrosinase and Asperginase from Mesophilic Streptomyces. *World Journal of Pharmaceutical Research* 21:89-101.

Pasindu Chamikara,H.J.M. (2016). Advanced Study on selected taxonomic groups of Bacteria and Archaea. *World Journal of Pharmaceutical Research* 11:1-3.

Pradhan,S., Mishra,B.B.,and Rout,S (2015). Screening of Novel Actinomycetes from Near Lake Shore Sediment of the Chilika Lake, Odisha, India. *International Journal of Current Microbiology and Applied Sciences* 17:66-82.

Pridham,T.G., Anderson,P., Foley,C.,Lindenfelser,H,A., Hesseltine,C,W. and Bendict,R,G (1956). A Section of media maintainence and taxanomic study of Streptomyces.*Antibiotics Ann*.,947-953.

Roy,S., Munjal,M., Das,I., Loganathan,K (2014). Isolation and characterization of tyrosinase produced by marine actinobacteria and its application in the removal of phenol from aqueous environment. *Article in Frontiers of Biology* 4:1-5

Sharma,K., Dangi,P., and Choudhary,M (2014). Actinomycetes: Source, Identification, and Their Applications. *International Journal of Current Microbiology and Applied Sciences* 10:801-901.

Stakerbrandt, E.,Rainey.F.A., and Ward Rainey,NL (1997). Proposal for a new hierarchi classification system,Actinobacteria classification. *International Journal of Systemic Bacteriology* 47:479-491.

Subramani,R., and Albersberg,W (2012). Marine actinomycytes:an ongoing source of novel bioactive metabolites. *Microbiology research* 7:117-122.

Subhash, G.S., Karad, D.D., and Kulkarni,S.W (2016). Screening of Tyrosinase Producing Soil Acti nomycetes from Shirala Region of Maharashtra, India. *International Journal of Current Microbiology and Applied Sciences* 9:345-353.

Uma.T.C., Srinivas Radha., D. Kale (2018). Actinomycetes from Vermicompost as Potential source for Bioactive compounds. *International Journal of Management, IT & Engineering* 8:1- 8.

Zaidi,K.U., Ayesha,S. A., Sharique A. A., and Naaz, I (2014). Microbial Tyrosinases: Promising Enzymes for Pharmaceutical, Food Bioprocessing, and Environmental Industry. *Hindawi Publishing Corporation* 10:1-10.