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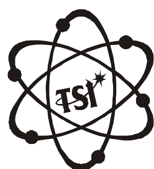

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Selective isolation and screening of laccase producing *Streptomyces*

Ronak Chhaya*, H.A.Modi
Department of Life Sciences, School of Sciences, Gujarat University, Ahmedabad, 380009, Gujarat, (INDIA)
E-mail: ronakcruxr2000@yahoo.co.in

ABSTRACT

A novel streptomycetes, designated strain was isolated from various soil types near Ahmedabad and Anand city, Gujarat, India. Pretreatment of wet heating for 4 h at 70^0C and phenol treatment of soil suspension were the most effective method for isolation. Strain produced a branched substrate mycelium and aerial hyphae that developed into short, compact, spiral spore chains with grey rugose spores at the tips of the aerial hyphae. Bennet's agar was found the most suitable medium for the recovery of rare *Streptomyces*. Their morphological characteristics were determined through electron microscopy. *Streptomyces* were cultivated on solid media containing indicator compounds used were tannic acid, Guaiacol, o-anisidine and p- anisidine for Laccase producing *Streptomyces* also showing positive Bavendamm's reaction. Results of morphological and biochemical characteristics and the 16S rRNA gene partial sequences indicated this strain belonged to the genus *Streptomyces*. The strain formed a monophyletic line in a phylogenetic tree of 16S rRNA gene sequences with other *Streptomyces* reference strains. Growth curve analysis by dry mass method was studied.. Growth condition was optimized using an agro waste such as rice bran and wheat bran by solid state fermentation. Enzyme assay was carried out using ABTS.
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KEYWORDS

Isolation;
Phenol oxidase reaction;
16S r DNA;
Biomass;
Solid state fermentation;
Laccase.

INTRODUCTION

Actinomycetes are widely distributed in natural and man-made environments, and play an important role in the degradation of organic matter. They are also well known as a rich source of antibiotics and bioactive molecules, and are of considerable importance in industry. When conventional isolation techniques were applied, most of the isolates recovered have been identified as genus *Streptomyces*, which are the dominant actinomycetes in soil. Several factors must be considered for the purpose of screening such as choice of screening source, pretreatment, selective medium, culture condition, and recognition of candidate colonies on a primary isolation plate. *Streptomyces* are gram-positive mycelium-forming, soil bacteria that play an important role in mineralization processes in nature and are abundant producers of secondary metabolites. *Streptomyces* species produce spores from aerial filaments called sporophores which rise above the colony

and form spores called **conidia** by simple cross-wall divisions of the filament. Much of the earthy smell of soils arises from chemicals called geosmin given by *Streptomyces* species. Streptomycetes are metabolically diverse and can "eat" almost anything, including sugars, alcohols, amino acids, organic acids, and as agents for bioremediation. The aerial spores of most actinomycetes genera were found to resist desiccation and show a slightly higher resistance to wet or dry heat than the corresponded subsequently; employing pretreatments of soil by drying and heating stimulated the isolation of rare actinomycetes. An alternative approach was to make the isolation procedure more selective by adding chemicals such as phenol to the soil suspension. Microbes that produce laccases have been screened for either on solid media containing coloured indicator compounds that enable the visual detection of laccase production[21] or with liquid cultivations monitored with enzyme activity measurements. As laccases oxidize various types of substrates, several different compounds have been used as indicators for laccase production. Several methods like use of screening reagents such as tannic acid and gallic acid[9] have nowadays mostly been replaced with synthetic phenolic reagents, such as guaiacol. With Guaiacol, O- anisidine and p- anisidine a positive reaction is indicated by the formation of a reddish-brown halo [21]. ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] gives a green colour zone around the colony indicate presence of lignolytic enzyme. While with tannic acid the positive reaction is a dark-brown coloured zone[9]. In this study, laccase-producing *Streptomyces* species were isolated from various soil samples using different indicator compounds in agar plates. In addition, the production of laccase by the positive strains was monitored in liquid cultures. The laccases that were produced in significant amounts were preliminarily characterized. Solid-state (substrate) fermentation (SSF) has been defined as the fermentation process occurring in the absence or near-absence of free water. SSF processes generally employ a natural raw material as carbon and energy source. SSF can also employ an inert material as solid matrix, which requires supplementing a nutrient solution containing necessary nutrients as well as a carbon source. Solid substrate (matrix), however, must contain enough moisture. Depending upon the nature of the substrate, the amount of water absorbed could be one or several times more than its dry weight, which leads relatively high water activity on the solid/gas interface in order to allow higher rate of biochemical process. Low diffusion of nutrients and metabolites takes place in lower water activity conditions whereas compaction of substrate occurs at higher water activity. Hence, maintenance of adequate moisture level in the solid matrix along with suitable water activity is essential elements for SSF processes.

EXPERIMENTAL

Chemicals

2,2-Azino-bis (3ethylbenzthiozoline-6-sulphonic acid) (ABTS) was purchased from Sigma (St. Louis M.O., U.S.A.). Yeast Malt agar, Casein enzyme hydrolysate, Yeast extract powder, sodium chloride, dextrose, Guiacol and actinomycete isolation agar were procured from Hi-Media (Mumbai, India). O-anisidine and p-anisidine were procured from CDH (Mumbai, India). Tannic acid was procured from Merck (Whitehouse Station, NJ, USA). All other chemicals were of analytical grade procured from Qualigens (Mumbai, India). Guaiacol was added to the media before autoclaving, o- anisidine and p- anisidine after autoclaving as sterile-filtered acetone solutions. Tannic acid was autoclaved separately before addition to the media. ABTS was added after autoclaving as sterile-filtered water solutions.

Sample collection and pretreatment of soil

Streptomyces were isolated from 20 soil samples collected in sterile flask, from different region including reserved forest near "Thol bird" century, garden area near Ahmedabad city, and from agriculture soil in Anand in two seasons, autumn and winter in 2008-09 and 2009-10 at Gujarat, India, respectively. All samples were collected from the depth of 6 to 8 cm and then cultivated on Bennet's agar (g/1000 ml) containing casein hydrolysate, 5; with added glucose, 10 ; and NaCl, 2 ;. Yeast extract, 2 ; is used as nitrogen source and agar,3 %. This medium was supplemented initially with 0.0001 g/1000 ml streptomycin and neomycin antibiotics respectively. Soil samples were air dried for three days under room temperature and sieved to get

rid of large particles, and suspensions were prepared by heating 1g of soil in 10 ml distilled water or saline solution of soil at 70^0 C for 4 h to reduce the proportion of other bacteria. The samples were serially diluted up to 10^{-3} g of soil per ml of saline solution. Aseptically, 0.1 ml of each dilution was spreaded on Bennet's medium containing streptomycin (0.0001g/1000 ml). Streptomycin was added in the medium to prevent the other bacterial contamination, and not added further in screening medium. Further sub culturing was done on Bennet's medium without adding antibiotic. Five different pretreatment methods as described in TABLE 1 were carried out in the first 24 h after sampling. The plates were incubated at 30^0 C for 1 week and isolates that show tough lathery colony were selected and purified by streak plate method and then subjected to Gram staining and observe under an oil immersion microscope. The Gram positive isolates were then maintained as 5 % glycerol stock solution. The pH of the medium was adjusted to 7.2 to 7.5. Bennet's medium was sterilized at 121^0C and 15 lbs pressure for 30 min. After 1 week incubation the colonies resembling *Streptomycetes* were isolated. The identification was based on an appearance of hyphae and spore chains through microscopic examination.

Morphology

Morphological and cultural characters of the selected S*treptomyces* strains were studied by inoculating the selected strain into sterile ISP media. Morphological properties such as colony characteristics, type of areal hyphae, and growth of vegetative hyphae, pigmentation and spore formation were observed [Figure 1]. A Bennet's agar (adjusted to pH 7.2) was used for the sporulation purpose. An actinomycete isolation agar (g/1000 ml) containing Sodium caseinate, 2; L-asparagine, 0.10 ; Sodium propionate, 4 ;. Dipotassium phosphate, 0.50; Magnesium sulphate, 0.10; Ferrous sulphate, 0.001 and agar, 3 % was also employed at primary screening. The taxonomic properties of the strain were determined according to the International *Streptomyces* Project (ISP). ISP Medium No. 2 i.e. Yeast Malt extract agar containing (g/1000 ml) Peptic digest of animal tissue, 5; Yeast extract, 3; Malt extract, 3 ; Dextrose, 10 and agar 3 % was used to study the morphology of the spore-bearing aerial hyphae which was determined by microscopic examination of the culture surface. The texture of strain R1 to R20 was studied and different morphological characters including development of mycelia, pigmentation and sporulation stage studied through dark field and phase contras microscopy.

Phenol oxidase reaction test

To test for Phenol oxidase activity the strain was grown on Bavendamm's medium, containing 0.01 % tannic acid and 1.5 % malt extract agar sterilized at 15 lbs pressure for 30 min. *Streptomyces* were inoculated as 8 mm plug in diameter cut from the growing edge of actively growing culture on Bennet's medium. The plates were incubated at 30^0 C in darkness and examine daily up to 7 days. Performed the same for 0.01 % guaiacol, o- anisidine and p- anisidine. The colour of the agar medium was used as the indication of Phenol Oxidase activity which was further to be studied and useful for Laccase production.

Estimation of biomass by dry weight method

Due to the filamentous growth analysis of growth characteristics by growth curve is difficult. In the present study we refer a dry weight method in order to investigate the growth characteristics of filamentous bacteria in Bennet's broth containing solid substrates like wheat bran and rice bran. Biomass was determined by weighing the dry mycelia after growth. The attached mycelia were squeezed to remove the medium, washed with the distilled water, and dried completely at 60°C. Biomass was calculated by subtracting the initial weight of the solid substrate (5 grams) measured for the abiotic (unioculated control) from the final weight[17]. Dry weight method for measurement of biomass in shaking as well as in static condition is studied. Each flask was inoculated with four mycelia agar plugs of 8 mm in diameter, cut from the edge of an actively growing colony and kept on rotary shaker incubated under at 30°C. After 24 h of incubation flask 1 is filtered. Prior to weighing the cell sediment, cells were dried at room temperature overnight. Repeat the process for 7 consecutive days. Samples were collected at 24 h each up to 7 days.

Molecular characterization of strain NBRC 12753 and phylogenic classification

NBRC 12753 was grown on Bennets agar plates

for 5 days at 28ºC. The mycelia at the edge of the colonies were taken by sterile loop and resuspended in 200 µl of Tris- EDTA buffer (10 mM Tris-HCl and 1 mM EDTA) in an Eppendorf tube. The mixture was frozen at -70^{0}C and thawed by boiling at 94^{0}C for 10 min. After 10 min of centrifugation at 8,000 rpm, the DNA in the supernatant was collected and used as the DNA template for PCR. A 16S ribosomal DNA sequence of isolate NBRC 12753 was amplified using universal primers (27 forward, 926 forward, 685 reverse and 1492 reverse). Sequencing of the PCR-amplified product was conducted using primers: 27F (5'AGA GTT TGA TCC TGG CTC AG 3'), 926F (5' AAA CTC AAA GGA ATT GAC GG 3'), 685R (5' TCT ACG CAT TTC ACC GCT AC 3') and 1492R (5'TAC GGY TAC CTT GTT ACG ACT T 3') on an automated AVI- 310 Genetic analyzer (Applied Biosystems, USA). The obtained 16S rRNA nucleotide sequence (1392 bp) was deposited at the National Center for Biotechnology Information (NCBI) Gen Bank database under accession number JQ086575. Closely related hologes were identified by comparing the partial 16S rRNA sequence with sequences deposited in the Gen Bank database by BLAST analysis (www.ncbi.nlm.nih.gov). The 16S rRNA gene partial sequence of strain NBRC 12753 (1392 bp) was aligned with other nucleotide sequences, and the phylogenic tree was observed.

Solid substrate utilization for optimization of growth p-meters

Five grams of substrate were added to a 250 ml Erlenmeyer flask and was moistened with a salt solution containing (g/1000 ml) yeast extract, 1; $(NH_4)_2SO_4$; 0.2, $MgSO_4$; 0.2, $CaCO_3$; 0.04 and $CuSO_4$; 0.002. Total 11 ml of the moistening solution was added to the substrate and the initial moisture level in the substrate was adjusted at the ratio of 1:3. After sterilization by autoclaving at 121 °C for 30 min, the medium was cooled to room temperature and inoculated with 4 mycelia agar plugs of 8 mm and incubated under static condition at 30 °C for 96 h. The use of cheap and easily available agro industrial residues for the production of value added products is one of the suggested advantages of solid-state fermentation. Different agro-industrial residues were screened to identify the suitable substrate for laccase production in solid-state fermentation. The substrates used were wheat bran and rice bran. The fermented material was extracted with 20 ml of Sodium acetate buffer (pH 5) to get a final extraction volume of 30 ml. The contents were mixed thoroughly by keeping the flasks on a rotary shaker at 200 rpm for 1 h. After 1 h the contents of the flask were filtered using musclin cloth. The enzyme extract obtained after filtration was then centrifuged at 10,000 rpm for 20 min at 4^0 C. The supernatant was collected and used for enzyme assay.

Enzyme assay

Laccase activity (E.C.1.10.3.2) was measured by monitoring the oxidation of 500µM 2, 2- Azino-bis (3ethylbenzthiozoline-6-sulphonic acid) (ABTS). Boost in absorbance for 2 min was measured spectrophotometrically (Make:- Wensor, Model:- WSP-UV 800A) at 420 nm (ε =36000cm-1 M-1). The reaction mixture contained 100µl of 50mM ABTS and 800µl of 20mM Sodium Acetate butter (pH-5.0) and 100µl of appropriately diluted enzyme extract. One unit of enzyme was defined as amount of enzyme that oxidized 1µM of substrate per minute ($\varepsilon = 3.6 \times 10^4 M^{-1}cm^{-1}$) (Niku Paavola et al.1988)[3].

RESULTS & DISCUSSION

Effect of pretreatment

When the forest soils were cultured without pretreatment the number of colonies recovered was in the order of other *Streptomyces*, Actinomycetes and other bacteria. When the soil was air dried, other bacteria number were decreased, and *Streptomyces* colonies were increased. All kind of colonies including *Streptomyces* were decreased when the soil was dried at 100^0 C for 1 h heating the soil suspension at 70^0 C for 4 h inhibited the fungal and bacterial colonies, thus the recovery of *Streptomyces* was increased up to 50 % of the total microorganisms. 1.5 % Phenol treatments of soil suspension lowered the number of fungi and other bacteria, and support the growth of *Streptomyces* and less for *Actinomycetes*.

Effect of morphology

The isolated bacteria was found to be gram positive. Initiation of sporulation starts after 24 h and pigmentation was started after 72 h. Complete sporuation

occured on 6th day of inoculation. From the general morphological characteristics, two strain R1 and R2 ware selected respectively to study further. Phase contrast & dark field microscopy (Make:Olympus,Type: B x 51) upto six consecutive days carreid out and observation related to mycelia formation, pigmentation and sporulation was noted (Figure 1). Growth properties and biochemical characteristics of *Streptomyces* species are given in TABLE 3. The substrate mycelium colour varied from grey to dark grey depending on the medium composition and the age of the culture. Pure cultures were obtained from selected colonies for repeated sub culturing. Strain R1 and R2 streaked on different media and day wise growth observed visually on ISP medium No.2(Yeast Malt Extract Agar), Actinomycete agar and Bennet's agar. (TABLE 2) The isolates were maintained as suspensions of spores in 5 % glycerol (v/v) at deep freezer. Growth characteristics and biochemical criteria were studied (TABLE 3). Out of 20 strain 6 strain were isolated from forest soil, 8 strains were found from garden soil and 6 strains were found from agricultural soil.(TABLE 4).

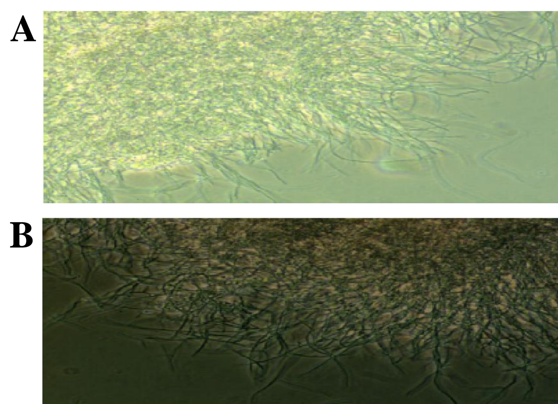

Figure 1 : Phase contrast microscopy (A) and dark field microscopy (B).

TABLE 1 : Recovery of microorganisms (CFU/g dry soil) on Bennet's agar from soil.

Pretreatment	Bacteria	Rare actinomycetes	Streptomyces	Fungi
Control	6.2 x 10^5	1.4 x 10^3	1.1 x 10^3	3.2 x 10^3
Control + Antibiotic[a]	1.8 x10^3	2.5 x 10^2	1.8 x10^3	1.2 x 10^2
Control + Antibiotic[b]	1.2 x10^4	1.8 x 10^6	2.5 x10^6	--
Dry heat 100^0C, 1 h	4.4 x 10^3	3.6 x 10^2	1.4 x 10^4	1.1 x 10^2
1.5 % Phenol treatment	1.9 x 10^3	1.7 x 10^3	5.1 x 10^5	--
70^0 C for 4 h	1.7 x 10^2	2.6 x 10^3	5.7 x 10^4	--

Antibiotic[a] :- Streptomycin (0.0001 g/1000 ml), Antibiotic[b] :- Neomycin (0.0001g/1000 ml)

TABLE 2 : Growth of *Streptomyces* on different medium.

Medium	Age (days) Strain R1	Color of mycelium Strain R1	Age (days) Strain R2	Color of mycelium Strain R2
ISP- No. 2	2	Yellow; dark cream	2	Yellow
	4	White; grey	4	Creamish
	6	Creamish -grey	6	Light grey
Actinomycetes agar	2	Pale yellow	2	Pale yellow
	4	White	4	Creamish
	6	grey	6	Grey
Bennet's agar	2	Pale yellow	2	Pale yellow
	4	White; grey	4	Creamish light grey
	6	Dark grey	6	Dark grey

TABLE 3 : Growth and biochemical characteristics of strain R1.

Parameter	Description of Strain R1
Colony morphology	Gram positive, filamentous with brown aerial mycelium and hard substrate mycelium, with tough leathery texture
Growth temperature	26-32^0 C
Growth pH	7.2-7.7
Biochemical characteristics	Oxidase Positive

TABLE 4 : Characterization of the *Streptomyces* isolates.

Isolates no.	Type of The sample	Morphology /Colour	Pigmentation
Strain R1	Forest soil	Smooth, grey	Dark brown
Strain R2	Forest soil	Smooth, light grey	Brown
Strain R3	Garden soil	Serrated white	Yellow
Strain R4	Agricultural soil	Smooth pink	Brown
Strain R5	Forest soil	Serrated blue	Bluish black
Strain R6	Garden soil	Smooth grey	Bright yellow
Strain R7	Garden soil	Smooth bright green	No pigment
Strain R8	Garden soil	Smooth, grey	Yellow
Strain R9	Garden soil	Smooth white	Yellow
Strain R10	Garden soil	Smooth, grey	Cloudy grey
Strain R11	Forest soil	Smooth, light grey	Dark grey
Strain R12	Agricultural soil	Serrated grey	Grey
Strain R13	Forest soil	Smooth, grey	Bright yellow
Strain R14	Garden soil	Smooth, grey	No pigment
Strain R15	Agricultural soil	Smooth, grey	Light brown
Strain R16	Agricultural soil	Smooth, light grey	Light brown
Strain R17	Agricultural soil	Serrated grey	No pigment
Strain R18	Garden soil	Smooth, grey	No pigment
Strain R19	Forest soil	Smooth, grey	No pigment
Strain R20	Agricultural soil	Serrated, white	Bright yellow

FULL PAPER

Effect of phenol oxidase reaction test

Dye based plate assay is the common method used for the screening purpose. The degradation of dye and the formation of clear zone around the colony on the agar plates could be correlated to the ability of the strain to produce lignin degrading enzyme. Strain R1 produced a strong brown colour reaction while Strain R2 produces less effective colour reaction. Here most powerful reaction is observed in plate containing 0.1 % O-anisidine and 0.1 % p-anisidine. The compounds such as P-anisidine and O- anisidine are further used for optimization study. The plates are also incorporated with ABTS for confirming further presence of Laccase. Out of 20 strains only 2 strains are found better for further study due to its color reaction. Strain R1 produce most powerful zone of color at initial 24 h compared to others and so finally was selected for fermentation and laccase production in secondary (TABLE 5) screening study.

Effect of growth curve by dry weight method

Every organism has a range of ideal conditions at which members of its species will grow optimally. Temperature and pH concentration within the media is often carefully controlled in industrial applications as deviations from the optimal conditions often have profound effects on the growth rate and activity of the organism. The temperature of the environment directly affects the activity and growth of cells. This optimum temperature for growth may not be the same as the temperature at which metabolites are produced most efficiently. Similarly to temperature, the hydronium ion concentration (pH) of the media influences the growth rate of bacteria by affecting the activity of cellular enzymes. To achieve the highest growth rate, current literature recommends growing *Streptomyces* strain R1 (*Streptomyces chartreusis*, NCBI accession number: JQ086575) in both static and shaking condition in media with pH maintained is 7.0 and temperature is 30 ^{0} C. The consumption of substrates such as sugar is necessary for growth and is therefore also affected by these p-meters. A graph plotted taking time on X-axis and weight of dried mycelium on Y-axis. After getting results and as per Figure (2,3), strain R1 gives higher mass in shaking condition when compaire with its static condition.

TABLE 5 : Result of plate assay method for ligninolytic enzymes production using dyes.

Isolates no.	Guaiacol	Tannic acid	O- anisidine	P- anisidine	ABTS
Strain R1	+++	+++	+++	+++	+++
Strain R2	++	++	+	+	+
Strain R3	++	+	+	+	+
Strain R4	+	-	+	++	-
Strain R5	+	-	+	+	-
Strain R6	+	-	+	+	-
Strain R7	+	-	+	-	-
Strain R8	+	-	+	+	-
Strain R9	+	-	+	+	-
Strain R10	+	+	+	+	-
Strain R11	+	+	+	+	-
Strain R12	-	-	+	-	+
Strain R13	-	-	-	-	-
Strain R14	-	+	+	-	-
Strain R15	-	-	-	-	-
Strain R16	-	-	+	-	-
Strain R17	-	-	-	+	-
Strain R18	+	-	+	-	+
Strain R19	-	-	-	-	-
Strain R20	-	-	-	-	-

+++ = within 48 h;++= within 72 h, + =within 96 h; -= No result found after 96 h

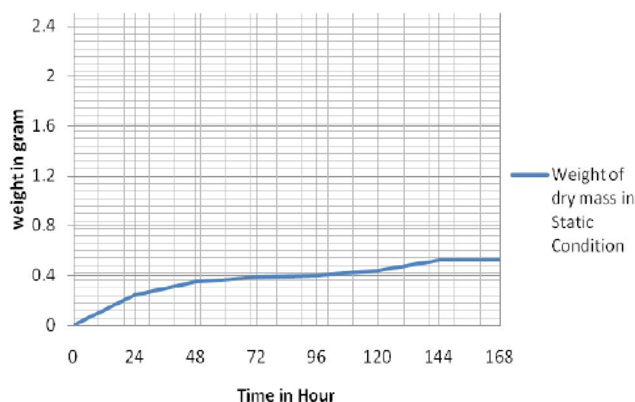

Figure 2 : Weight V/s time- static condition.

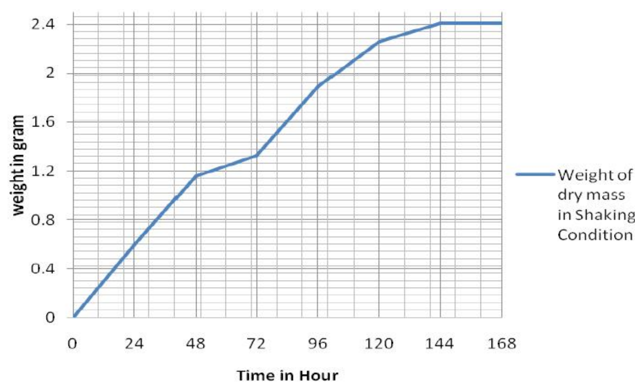

Figure 3 : Weight V/s time- shaking condition.

16s rRNA partial sequence analysis of strain NBRC 12753 and its phylogenetic analysis

Comparison of the 16S rRNA partial sequences of strain R1 NBRC 12753 (Genbank accession number JQ086575) with the GenBank database showed this isolate belongs to the *Streptomyces genus Streptomyces* with > 99% certainty. The strain is similar to several *Streptomyces* strains, such as *Streptomyces matensis* strain NBRC 12889, *Streptomyces griseorubens* strain NBRC 12780 and *Streptomyces variabilis* strain. The phylogenetic tree derived from neighbor-joining analysis showed a correlation between the 16S rRNA sequence of NBRC 12753 and other *Streptomyces* strains like *Streptomyces prasinosporus* strain NBRC 13419, *Streptomyces chrsomofuscus* strain NBRC 12851, and *Streptomyces cinereospinus* strain NBRC 15397 in the NCBI database. It can be divided into five main chains by the *Streptomyces* branches, which are bacteria in the same family. NBRC 12753 belongs to chain 5 as *Streptomyces chartreusis,* with a close relationship. Blast tree (Figure 4) was formed as per NCBI blast tool system.

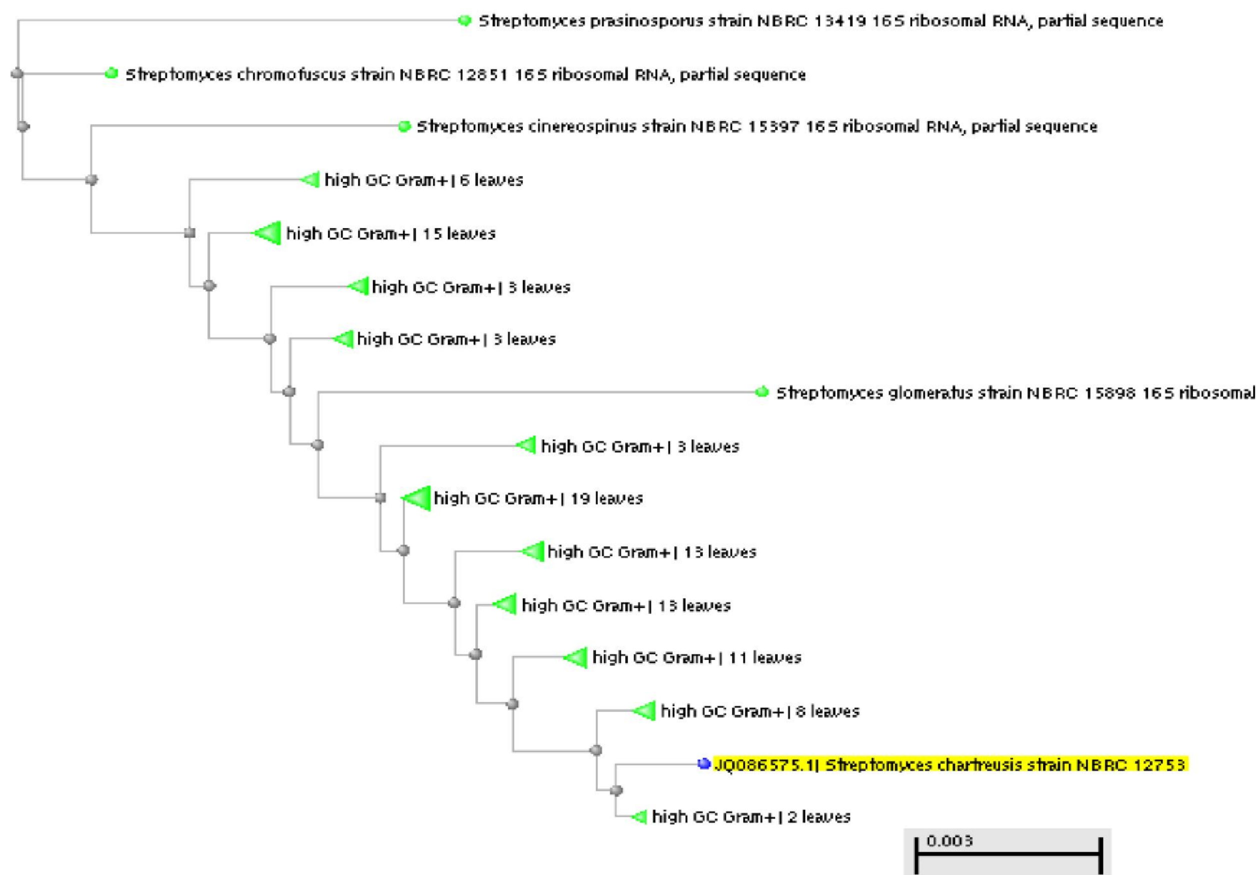

Figure 4 : Blast tree view.

Figure 5 : Rice bran after 96 h of SSF.

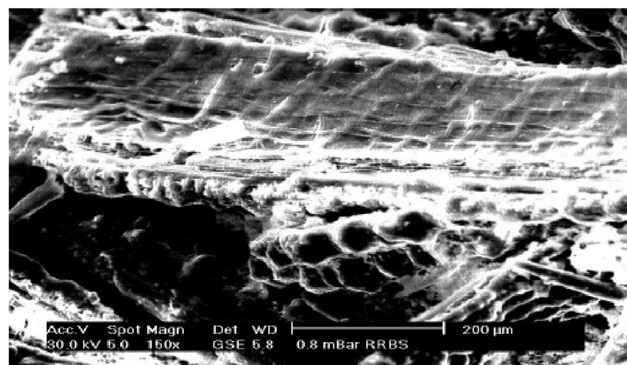

Figure 6 : Scanning electron microscopy of rice bran.

Results of solid substrate utilization for optimization of growth p-meters

Streptomyces produced branch mycelium and spores in characteristics longitudinal pairs in solid substrate like rice bran (Figure 5) and Wheat bran. Optimization of growth p-meters studied for developing the methodology of performance for fermentation of Laccase. Two different agro substrates are used for growth p-meters optimization as a part of primary screening. Rice bran is found more effective and shows excellent growth on third day after inoculation. Wheat bran show slow growth and found less effective for the growth of *Streptomyces chartreusis*. Laccase production was assayed using ABTS Scanning electron microscopy was carried out to study the level of penetration of substrate with inoculum. Scanning electron microscopy was carried out at sophisticated instruments centre (SICART, Anand) using Scanning electron microscope (Make:- Philips, Netherlands, Model:- ESEM EDAX XL- 30). Different magnified angles are used to evaluate the level of penetration of both the substrates (Figure 6) towards an inoculum.

CONCLUSION

Twenty *Streptomycetes* culture were isolated in autumn and winter from varieties of soil samples, which proved to be an ideal source for isolating lignin degrading *Streptomyces*. Screening for laccase-producing microbes on plates containing coloured indicators resulted in isolation of 20 *Streptomyces* strains. Solid substrate proved to be highly interesting sources for laccase producers. This is presumably because of the presence of relatively high concentration of laccase substrates in these environments. In view of the results obtained, it can be concluded that the isolate was able to oxidize phenolic substrates such as Guaiacol and ABTS. Comparison of the reactions with different indicators showed that the o- anisidine, p- anisidine and Guaiacol gave very similar results, thus either one can be chosen for an indicator in future screening procedures. Reactions with Guaiacol also correlated well with reactions on the polymeric dyes. However, tannic acid deviated substantially from the polymeric dyes and Guaiacol. Tannic acid is one of the traditional screening reagents for laccases. Our results suggest, however, that colour reactions with synthetic dyes and Guaiacol are more easily detectable. Findings from morphological, cultural and microscopical studies reveal that the isolated and investigated strain is designated as *Streptomyces* spp. In future we are interested to scale up the production of laccase by using various agro residues in solid state fermentation and also test the ability of this enzyme to degrade the various azo dyes an important enzyme for various industrial applications. Effect of inducers in submerged fermentation and production of laccase should be studied for further optimization process. Solid state fermentation is found as more satiable for the mycelia bacteria. The production of 60 U/g of Laccase from *Streptomyces* chartreusis, using rice bran as the substrate, is a promising result and it suggests that solid state culture is also functional with actinomycetes. The filamentous nature of the organism favors the growth of organism onto solid substrate. Rice bran shows two fold increases in production in compared with wheat bran due to its high lignolytic composition.

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