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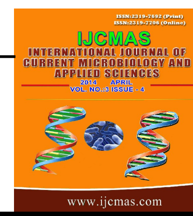

Original Research Article

Isolation, identification, bioprocessing and characterization of secondary metabolites for its antimicrobial and genotoxicity from the soil screened microorganism

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Keywords

Fermentation, actinomycetes, genotoxicity, DNA damage/ protective activity, antimicrobial activity, TLC

A B S T R A C T

Out of 77 samples that were collected in and around Ooty and hydrabad, 2 samples showed the presence of antimicrobial producing microorganism which were named as RRK1, RRK2. Further studies were carried out on 2 isolates (RRK-1 & RRK-2) based on the fact that they possessed prominent and broad spectrum antimicrobial activity. All the test micro organisms after being obtained in the pure culture, were stored on ISP-slants. Fermentation was carried out using the fermentation medium for the period of 144 hours. The fermented medium was extract with butanol, ethyl acetate and methanol and each fractions were tested for their antimicrobial properties against various selected microorganisms. All the extracts showed significant anti microbial activity against *E.coli, B.subtilis, s.aureus, a.niger, candida albicans* where as ethyl acetate extracts showed good activity effect against test fungi and bacteria. In genotoxicity studies, the results showed that various fractions obtained by extracting the fermented broth from both the strains seems to be capable of protecting the DNA rather than standard (Doxorubicin- 10 µg/ml). Through the automatic microorganism detector, the isolated microorganism RRK1 and RRK2 were found to be *Bacillus subtilis*, *Staphylococcus aureus* respectively.

Introduction

In nature, Actinomycetes are the most widely distributed group of organism whose primary inhabit is soil. Routine screening is going on Actinomycetes for new bioactive compounds because they have provided many important bioactive compounds of high commercial value. Approximately two thirds of naturally occurring antibiotics included many of medical importance have been isolated from actinomycetes and these screening has been remarkably successful. From actinomycetes mostly from the genera Streptomyces and Micromonospora, almost 80% of the world's antibiotics are known to come.

In many bacterial pathogens, over prescription and the improper use of antibiotics has led to the generation of antibiotic resistance. Now- a- days, when compared to the rate of discovery of new drugs and antibiotics, the drug resistance strains of pathogens emerge more quickly. For the production of antibiotics, many scientist and pharmaceutical industry have actively involved in isolation and screening of actinomycetes from different untouched habitats. A major global healthcare problem in the 21st century is serious infections causing microorganism have become resistant to commonly used antibiotics. A virulent pathogen that is responsible for a wide range of infection is staphylococcus aureus which has developed resistance to most classes of antibiotics. Because of such type of resistance developed by the microorganism, there is a need to rediscover new drugs against the resistant pathogen (Narendran Kuamr *et al*., 2010).

It is still important to search for novel antibiotics and other bioactive microbial metabolites for potential agriculture pharmaceutical and industrial applications. Useful bioactive compounds are produced by the members of actinomycetes genus streptomyces. For the isolation of uncommon and less studies rare actinomycetes, improved methodologies should be used in order to avoid re-isolation strains that produce know bioactive metabolites. Apart from pretreatment techniques, enrichment techniques that appropriately supplemented agar media with selective antimicrobial agents are adopted to screening the newer organism streptomyces are common in natural soil habitats which are usually a major component of total actinomycetes populations. (Masayuki Hayakawa., 2008). There has been an intensive search in recent years for the microorganism which produce antibiotics during their growth on artificial media (Nancy *et al*., 1962, Davies and Williams, 1970).

Materials and Methods

Screening of soil isolates

For screening and identification of potential microorganism producing antimicrobial substances, intelligent screening is a more rational approach. (Srividya A.R *et al*., 2011). From different places in Ooty and Hydrabad, soil samples were collected by digging 10-15 cm depth of the soils. These soils were air dried for a week, then it was crushed and sieved. (Narendran Kumar et al., 2010, Davis and Williams., 1970). The soil samples were selected on the basis of colour and pH. Description of the soil samples as tabulated in Table No.1.

Method (Narendra kumar *et al*., 2010; Mohamed E. Osman *et.al*., 2011)

1gm of each sample was added to 1gm of $CaCO_3$ in a dried flask. 100ml of sterile water with two to four drops of Tween 80 was added. The flasks were kept on a shaker for half an hour. These flasks were considered as stock culture. From the stock culture, 1ml was taken and diluted with sterile distilled water to get 10^{-1} to 10^{-5} concentrations of original sample. Then the last three dilutions were placed on sterile petridishes for crowded plating. In some plates the dilutions were mixed with antibacterial (Ampicillin 25 µg/ml) and antifungal (Clotrimazole 50 µg/ml) agents in starch casein medium. All plates were kept at 37°C in the incubator and observed for growth every day. After five days, each plate were observed few actinomycetes colonies which were

isolated (pin point colonies) and preserved in actinomycetes agar slants.

Preliminary morphology observation
(Sirinin Chatujinda *et.al*., 2007)

From the various soil samples, only 2 micro organisms were isolated, which showed pin point white colonies and zone of inhibition in the crowded-plate. These isolates were inoculated in slant prepared with actinomycetes media for further purification. To obtain pure cultures, the isolates were once again streaked on starch-casein media by multiple streaking methods. The purified culture were preserved on actinomycetes ISP-Agar slants and kept under refrigeration.

Microscopical characterization (Srividya *et al*., 2009(ii))

Microscopical studies of isolated strains were carried out by agar block method and inclined cover slip method. In the first method, the isolated strains were prepared in thin agar block and examined under light microscope.

Test for microbial sensitivity using different strains of bacteria and fungi

Based on the results of preliminary morphological observations 2 isolates were selected and tested for microbial sensitivity using different strains of bacteria and fungi by crowded plate method. Each isolates were grown on nutrient agar medium while testing against bacteria at 37°C for 24hours and on SDA agar medium in case of yeast and fungi for 48hours at 28°C.

The following test organisms were used:

Bacteria

Gram positive: *Bacillus subtilis, Staphylococcus aureus*, Gram negative: *E.coli, Pseudomonas aeruginosa*

Fungi

Aspergillus Niger, Aspergillus flavus

Yeast

Candida albicans, Candida krusei.
Based on their antimicrobial properties, isolates were chosen for further biochemical characterization.

Taxonomic studies for the isolated strains (Srividya et al., 2009 (ii))

Taxonomic studies such as melanoid formation, nitrate reduction, milk coagulation and peptonization, starch hydrolysis, gelatin liquification, acid production. Morphological and cultural characteristic were studied by using International streptomycetes Project medias.

Morphological and cultural characteristics (Srividya *et al*., 2008 (i))

Among the two isolates which were taken for primary screening (antibiotic production), good antibacterial and antifungal antibiotics producing capacity were selected for further studies. In the present work for macro and micro morphology and cultural characterization of the selected isolates, the following ISP (international streptomyces project) cultural media were employed.

1) ISP-1- Tyrosine - yeast extract broth
2) ISP-2- Yeast extract - malt extract agar
3) ISP-3- Oat meal agar
4) ISP-4- Inorganic salts starch agar

5) ISP-5- Glycerol- asparagine agar
6) ISP-6- Peptone- yeast extract agar
7) ISP-7- Tyrosine agar

Bennet agar

The isolates were categorized into chromogenic and non-chromogenic groups depending upon melanoid pigments produced in peptone iron agar (ISP-6). The previously mentioned ISP- media 2,3,4,5,6 and 7 were employed to study the morphology and culture properties. 8ml of each sterile medium were poured into culture tubes and slants were prepared. These slats were inoculated with the isolates and incubated at 28°C for 2-3 weeks. After 14 and 21 days, cultural characteristics such as aerial mycelium colour, substrate mycelium, reverse colour, diffusible pigments, growth and micro morphology of spore bearing hyphe were studied. Cultures forming a greenish brown to brown to black diffusible pigments or distinct colour pigments were considered as positive. Absences of diffusible pigments were considered as negative. The observation of these culture characters were recorded in the Table No. 7&8.

Carbon utilization test

Assimilation is the utilization of carbon source by microorganism in the presence of oxygen. Positive assimilation of growth was identified by the change in pH of the medium. To melted utilization of agar containing bromocresol purple dye, a ml of soil isolates suspension was added and poured inside the sterile petri plates. The agar was allowed to solidify at room temperature. In that disc containing 4 % solutions of various sugars like glucose, sucrose, lactose, maltose, d-mannitol, fructose and dextrose were placed onto the surface. The plates were incubated and observed daily up to 10-15 days. The presence of growth around the disc along with the change in color of the medium from purple to yellow shows the carbohydrates assimilation of soil isolates (Srividya *et al* 2009.i)

Composition of seeded and Fermentation medium (**Srividya** ***et al*****., 2009** (i))

Based on the carbon utilization test, the seeded and fermentation media was designed to carryout the production of antimicrobial substances in the laboratory. The seeded medium composed of glucose 10 g, soluble starch-10 g, yeast extrat powder- 5 g, beef extract-3 g, CaCo3- 2g for one litre of medium. The fermentation medium consists of soluble starch-20 g, sucrose 15 g, glucose- 5 g, soya bean meal-20 g, yeast extract powder- 5 g, $CaCO_3$-3.2 g, $MgSO_4.7H_2O$-2.5 g, K_2HPO_4- 5 g, 2-0.2 g, NaCl-0.01 g, $FeSO_4.4H_2O$-0.002g, silicon oil as an antifoaming agent at the concentration of 0.3 ml

Fermentation process (Srividya ***et al*****., (ii) 2009)**

10 ml of the seed medium was inoculated with a loopful of strains isolated from the soil in a 250 ml conical flask. Then the flask was incubated at 28 ^{0} C for 48 hours. The contents of the flask was then transferred to 1 litre flask containing 100 ml of the seeded medium and the flask was incubated at 28 ^{0} C which was then used as incoulum to initiate the fermentation in a 5 litre containing 3 litres of fermentation medium. With sufficient aeration and agitation at 200 rpm at 28 ^{0} C, the fermentation was carried out until the pH reaches the neutral. By centrifuging the

fermented broth at 5000 rpm for 10 minutes, culture growth was estimated by noting the percentage of packed cell volume, change in pH and antibiotic production.

Down stream processing of fermented broth (Srividya *et al*., 2009 (i))

After completion of fermentation process, the fermentation medium was centrifuged to separate the medium and mycelium. one part of the fermented broth was extracted with equal volume of n- butanol and another part with ethyl acetate. The mycelium was extracted with methanol. The extracts obtained from the fermented broth and mycelium were concentrated at 40 ^{0} C to obtain the crude extracts. The crude extracts was subjected to thin layer chromatographic analysis to characterize the extracts.

Thin Layer Chromatograhic Analysis

Preparation of the sample solution

A pinch of the sample substance was dissolved in 1ml of water and mix thoroughly to get a clear solution. These test samples were spotted on different TLC plates using cleaned, rinsed capillary tubes. The spots made about 2 cm above the bottom of the plates and the spots were allowed to dry. The plates were kept inside the TLC chamber containing the appropriate mobile phase, which were saturated with the vapors of the mobile phase. The mobile phase was allowed to rise to a height of 10cm from the bottom and then removed, allowed to dry and kept inside the iodine chamber for the detection of spots. The Rf values of the different samples were then measured. The values are given in Table No. 12

Antimicrobial studies (Srividya et al., 2009 (i))

Antimicrobial studies of antibiotics of actinomycetes against various strains of bacteria and fungi were carried out by two-fold serial dilution technique. The concentration of the working stock culture was 10^{6} – 10^{8} cfu/ml. The antibiotics were screened against the following organism.

1. *Escherichia coli*
2. *Staphylococcus aureus*
3. *Bacillus subtilis*
4. *Aspergillus niger*
5. *Aspergillus flavus*
6. *Candia albicans*

Each test organism was streaked on nutrient agar and SDA for bacteria, fungi and then incubated for 24hours and 48 hours respectively.

From the 24 hours, culture 2-3 loop full of organism were transferred aseptically into 100ml of nutrient broth and SDB for bacteria and fungi respectively. Then the 100ml broth was incubated for 24 hours. This is called stock culture. The stock culture is standardized using the followed procedure.

Standardization of stock culture

Few drops of tween 80 was added to 100ml of Millipore water and sterilized by autoclaving. From this 9ml was transferred to 10 serial test tubes and labeled from 10^{-1} – 10^{-10}. 1ml of stock culture was added to first tube 10^{-1} and proceeded with ten-fold serial dilution technique. Then 0.2ml of each dilution was plated in triplicate using NA and SDA plates. Then the plates were incubated for 24hours at 37°C and 48 hours at 28°C for bacteria and fungi respectively. From the number of colonies

obtained from each dilution, the cfu/ml of the stock culture was calculated. Depending on the number of microorganism present, further dilutions were made. This is called seeded broth. The test antibiotics were diluted in Dimethyl sulphoxide (DE 150) to get a final concentration of 1mg/ml.

Cup plate method

Each organism was grown on nutrient broth & SDB medium 24hours. And 0.1 ml of organism was spread on the plates. And make the cups with help of borer in that Instead of soil isolates the various extract were poured into the cups. zone of inhibition was measured on Nutrient agar media against bacteria after 24 hours of incubation at 37°C and after 48 hours of incubation on SDA agar medium for yeast and fungi at 28°C. The results were tabulated in Table No.9&10.

Genotoxicity studies DNA damage/protective activity (Srividya *et. al*., 2013)

DNA damage/ protective activity of extract (RRK-&RRK-2) was performing using super coiled plasmid DNA pBR [322]. The mixture consist of 10µl of extract of different concentration (10-40µg/ml). Plasmid DNA (0.5µl) was incubated for 10min at room temperature. Followed by addition of 10µl Fenton's reagent (30mM H_2O_2, 50µm ascorbic acid & 80µM $FeCl_3$. The final volume of mixture was made up to 20µl & incubated for 30min at room temperature 37°C. The DNA was analyzed on 1% agarose gel using ethidium bromide staining & photographs in gel documents. Figure No.2

Results and Discussion

The screening of soil isolates

70 soil samples from in around Ooty and Hyderabad were collected. Based on the crowded plate method primary screening was done. 2 micro organisms were found to possess antimicrobial activity. Further studies were carried out on 2 isolates (RRK-1 & RRK-2) based on the fact that they possessed prominent and broad spectrum antimicrobial activity. All the test micro organisms after being obtained in the pure culture, were stored on ISP-slants.

Microscopical Observations

Studies were carried out on the microscopical characteristics of the aerial mycelia. The results are tabulated in Table No.2

Microbial sensitivity using different bacteria and fungi were performed by cup-plate method. The results are tabulated in Table No.3 It was found that all the micro organism possessed antimicrobial activity. 2 microorganism were selected from these based on the spectrum and intense of activity. The microorganism RRK-1& RRK-2 was selected for further study because it was found to possess antimicrobial activity.

Taxonomical characterization

Various biochemical tests were performed. The results are tabulated in Tables No.4 The results co-related satisfactorily with the specifications of Bergey's manual of determinative bacteriology.

Morphological and cultural characteristics

An elaborate study on morphology and cultural characteristics of the 2 microorganism was carried out on ISP 2-ISP 7 and various media designed to check the carbon utilization. The cultural characteristics were shown on the Table No.7 All microorganisms showed nitrate reduction and starch hydrolysis. Only RRK-1 & RRK-2 showed melanin formation

Bioprocessing

The 2 microorganism were used for fermentation of antibiotics. During the process parameter like PCV & pH were monitored. Suitable additions of 1N NaOH were made to neutralize the increasing acidity. Increase in the concentration of antimicrobial substance was studied by withdrawing samples of fermentation broth at regular intervals and checking activity against the test microorganism *E.coli* and *B.subtilis* (these organisms were chosen on the basic of marked susceptibility seen in the preliminary studies). Fermentation was allowed to process till the fermentation broth became neutral and PCV attained the stationary phase. The results were tabulated on Table No.8

TLC studies

TLC studies were carried out to check the purity of the extract to separate the various fraction, and also to check each chromatographic spot for antimicrobial activity. Results of studies were tabulated in Table No.12

TLC studies showed a single spot for each fraction which indicate that consists of a single component using the solvent system given in Table No.12. The Rf values were placed on Table No.12. After studying the purity of the samples, UV and IR analysis were carried and the results were tabulated on Table No.12. The three fractions i.e. A-N-Butanol, B-ethyl acetate, C-methanol thus obtained were subjected to antimicrobial studies against *E.coil, B.subtilis, S.aureus, A.niger, A.flavus, Candida albicans*. The results are tabulated in Table No.12 All the extracts showed significant anti microbial activity against *E.coli, B.subtilis, s.aureus, a.niger, candida albicans* where as ethyl acetate extracts showed good activity effect against test fungi and bacteria.

Genotoxic activity

DNA damage protective activity of RRK-1 & RRK-2 was carried out. By using pBR322 DNA. Then DNA was analyzed on agarose gel using ethidium bromide staining & photographs in gel document was placed Figure 2. The results showed that various fractions obtained by extracting the fermented broth from both the strains seems to be capable of protecting the DNA rather than standard (Doxorubicin- 10 µg/ml).

In the research for new antibiotics, the leading position of Japan, US, England remains unchanged. Antibiotics continue to play crucial role in the development of tissue culture techniques and basic screenings, primarily in biochemistry, molecular biology, microbiology and genetics including genetic engineering and to a lesser degree, pharmacology and organic chemistry. The continuing success of a biotechnologist in the search of microbial metabolites for antibiotics compounds useful in combating human,

Table.1 Description of soil samples

Name of the soil isolate	Place of soil sample collection	Description of the soil	Soil pH
RRK1	Hyderabad, Andrapradesh	Reddish brown in color	6.8
RRK2	Elkhil, Ootacamund	Brown in color	7.2

Table.2 Microscopic characteristic of aerial mycelium

Name of the soil isolates	Description of the structure
RRK1	Rod shaped, chain like arrangement
RRK2	Rod shaped, chain like arrangement

Table.3 Microbial sensitivity test using different bacteria and fungi

Soil isolates	*E. coli*	*Pseudomonas auroginosa*	*Bacillus subtilis*	*Staphylo coccus aureus*	*A. niger*	*A. flavus*	*Candida albicans*	*Candid krusi*
RRK1	21mm	28mm	18mm	25mm	14mm	3mm	Nil	15mm
RRK2	25mm	30mm	19mm	25mm	18mm	5mm	Nil	15mm

Table.4 Biochemical tests

Soil isolates	Melanin formation	Nitrate reduction	Hydrogen sulphide production	Starch hydrolysis	Gelatin liquification test	Acid production
RRK1	+	+	+	+	+	+
RRK2	+	+	+	+	-	+

Table.7 Cultural characteristics of RRK-1

Medium	Cultural characterization			
ISP-2	G: Good	AM: Pale white	R: Pale white	SP: Pale white
ISP-3	G: Good	AM: Pale white	R: Reddish white	SP: Brick red
ISP-4	G: Good	AM: Pinkish red	R: Pale yellow	SP: yellow
ISP-5	G: Good	AM: Pale yellow	R: Pale white	SP: Pale yellow
ISP-6	G: Good	AM: light pink	R: Pinkish red	SP: light pink
ISP-7	G: Good	AM: pale white	R: Pale white	SP: Yellow

AM: Aerial Mycelium, G: Growth, R: Reverse colour, SP: Soluble pigments

Table.8 Cultural characteristics of RRK-2

Medium	Cultural characterization			
ISP-2	G: Good	AM: Pale white	R: Pale white	SP: Pale white
ISP-3	G: Good	AM: Pale white	R: Reddish white	SP: Brick red
ISP-4	G: Good	AM: Pinkish red	R: Pale yellow	SP: Yellow
ISP-5	G: Good	AM: pale yellow	R: Pale white	SP: Pale yellow
ISP-6	G: Good	AM: light pink	R: pinkish red	SP: Light pink
ISP-7	G: Good	AM: Pale white	R: Pale white	SP: Yellow

AM: Aerial Mycelium, G: Growth, R: Reverse colour, SP: Soluble pigment

Table.9 Fermentation parameters RRK1

S.No	Time	Broth packed cell volume (gm)	Percentage of packed cell volume (%)	pH	Amount of iN NaOH added to neutralize the pH
1	24hrs	0.280	18	7.0	……
2	48hr	0.281	23	7.0	……
3	72hrs	0.285	27	6.0	2ml
4	96 hrs	0.318	33	7.1	0.5ml
5	120hrs	0.324	42	6.5	1.5ml
6	144hrs	0.324	43	7.0	……

Table.10 Fermentation parameters RRK2

S.No	Time	Broth packed cell weight (gm)	Percentage of packed cell volume (%)	pH	Amount of 0.1 N NaOH added to neutralize
1	24hrs	0.233	21	6.5	0.5ml
2	48hr	0.249	27	7.0	……
3	72hrs	0.269	35	6.0	2ml
4	96hrs	0.325	41	7.0	0.5ml
5	120hrs	0.357	43	6.5	0.5ml
6	144hrs	0.357	43	7.0	……

Table.9 Microbial sensitivity of fermentation isolated using different bacteria

Name of the sample	Zone of inhibition in mm		
	E.coli	*B.subtilis*	*S.aureus*
RRK-1-A	15	21	21
RRK-1-B	21	20	20
RRK-1-C	18	19	21
RRK-2-A	15	19	25
RRK-2-B	31	25	25
RRK-2-C	21	23	24

Table.10 Microbial sensitivity of fermentation isolated using different fungi

Name of the sample	Zone of inhibition in mm		
	E.coli	***B.subtilis***	***S.aureus***
RRK-1-A	18	11	15
RRK-1-B	21	16	19
RRK-1-C	20	25	24
RRK-2-A	19	15	19
RRK-2-B	25	28	25
RRK-2-C	35	32	26

Table.11 Description and yield of products of downstream processing

Name of compound	Nature and colour of compound	Quantity of the isolated fractions
N-Butanol:		
RRK-1	Reddish brown semisolid	2.603gm
RRK-2	Yellowish green powder	753mg
Ethyl acetate:		
RRK-1	Reddish brown semisolid	328mg
RRK-2	Greenish yellow powder	671mg
Methanol:		
RRK-1	Brown semisolid	5.382gm
RRK-2	Green semisolid	5.067gm

Table.12 Result for TLC studies for RRK1

Sample	Solvent system	Rf value
RRK-1-A	Methanol : Glacial acetic acid 7 : 3	0.09
RRK-1-B		0.38
RRK-1-C		0.38
RRK-1-A	Butanol : acetic acid : water 9 : 0.5 : 0.5	0.42
RRK-1-B		0.37
RRK-1-C		0.34
RRK-1-A	Ethyl acetate : Butanol : phosphoric acid 8 : 2 : 0.1	0.36
RRK-1-B		0.46
RRK-1-C		0.33

Table.13 Result for TLC studies for RRK2

Sample	Solvent system	Rf Value
RRK-2-A	Methanol : Glacial acetic acid 7 : 3	0.28
RRK-2-B		0.37
RRK-2-C		0.59
RRK-2-A	Butanol : acetic acid : water 9 : 0.5 : 0.5	0.17
RRK-2-B		0.31
RRK-2-C		0.37
RRK-2-A	Ethyl acetate : Butanol : phosphoric acid 8 : 2 : 0.1	0.46
RRK-2-B		0.35
RRK-2-C		0.42

Figure.1 Plates showing the zone of inhibition produced by soil isolates

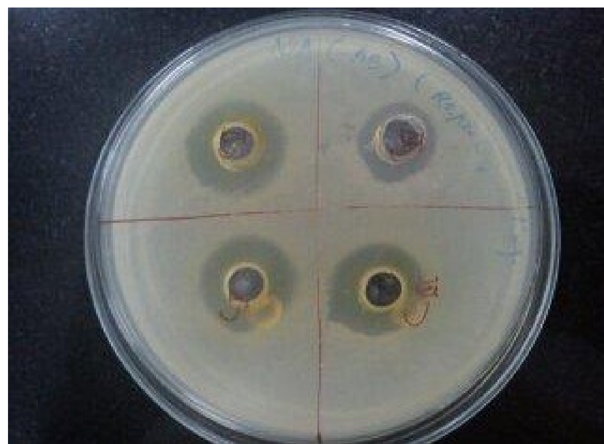
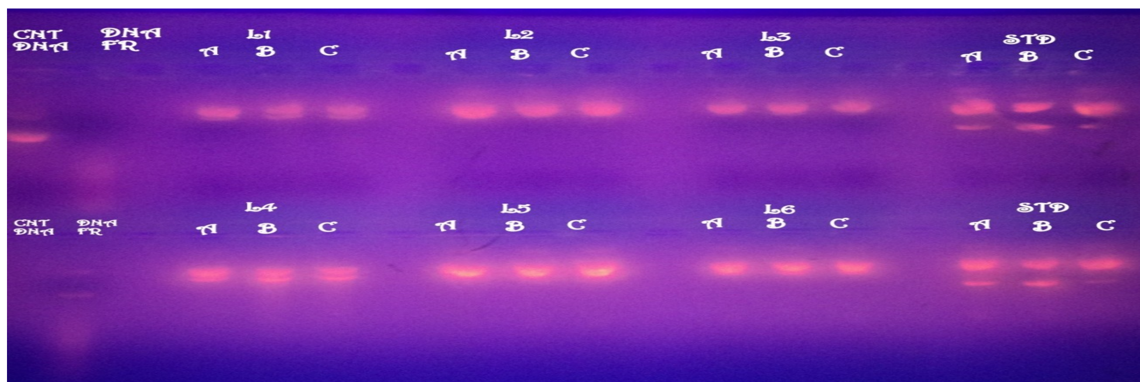
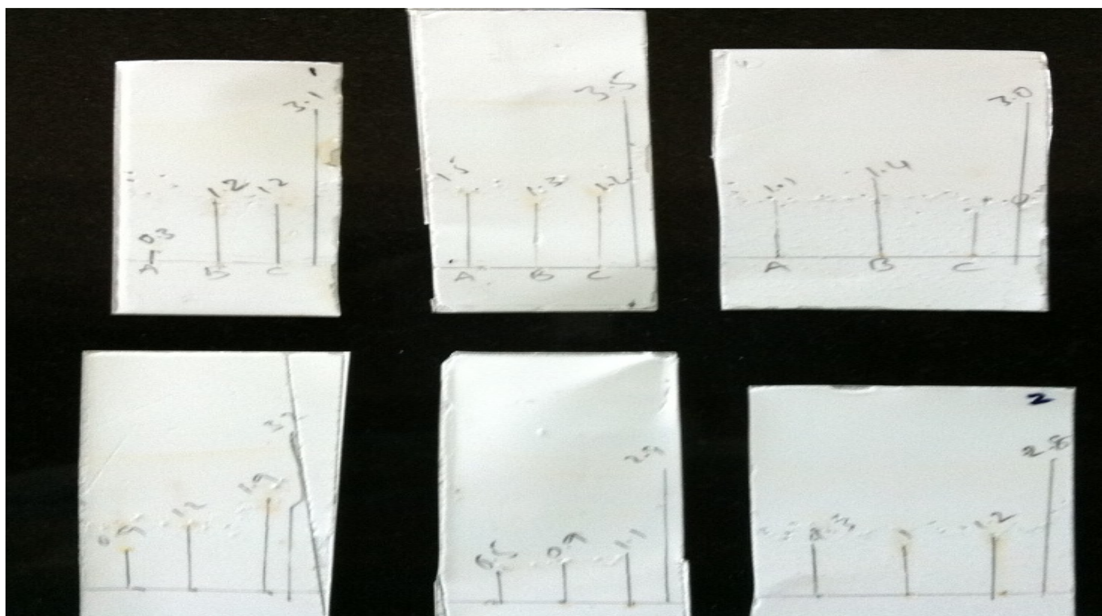


Figure.2 DNA Damage-protective activity



First lane- control DNA; Second lane- DNA treated with Fenton's reagent alone.
Li- RRK1-A- A lane- 250 μg/ml, B lane - 500 μg/ml. C lane - 1000μg/ml
L2- RRK1-B- A lane- 250 μg/ml B- lane- 500 μg/ml C- lane- 1000 μg/ml
L3- RRK1-C- A Lane- 250 μg/ml B-lane- 500 μg/ml C- lane- 1000 μg/ml
Second line; L1- RRK2-A- A lane 250 μg/ml, B lane - 500 μg/ml. C lane - 1000μg/ml
L2- RRK2-B- A lane- 250 μg/ml B- lane- 500 μg/ml C- lane- 1000 μg/ml
L3- RRK2-C- A Lane- 250 μg/ml B-lane- 500 μg/ml C- lane- 1000 μg/ml

Figure.3 Results for Thin layer Chromatography



animal and plant diseases for stimulating the belief that microorganism constitute an inexhaustible reservoir of compounds, with all agricultural and medical applications other than pathogenic micro organisms. This has already been proved extensively..

Actinomycetes are prolific producers of antibiotics and other bioactive compounds. They have provided over two-thirds of the naturally occurring antibiotics discovered and continue to be a major source of novel and useful compounds (Becker *et al* 1964) Re isolation of strains has, however, increased the difficulty of isolating cultures that produce novel and useful secondary metabolites (Yedir *et al*., 2001). This has necessitated the development of more directed and innovative selective isolation techniques in natural product screening programmers (Xu *et al*., 1996).

Antibiotics are one of the pillars of modern medicine (Ball *et al.,* 2004), but the rate of loss of efficacy of old antibiotics is outstripping their replacement with new ones for many species of pathogenic bacteria (Hancock, 2007). The emergence of antibiotic resistant bacteria is a problem of growing significance in dermatological and surgical wound infections (Colsky *et al.,* 1998; Giacometti *et al*., 2000). In general, the most important resistance problems in the management of wounds have been observed with *S. aureus* and coagulase-negative staphylococci among the Gram-positive species and with *E.coli*, *Klebsiella pneumoniae* and *P. aeruginosa* among the Gram-negative species (Filius and Gyssens, 2002).

Considerable research is being done in order to find new chemotherapeutic agents isolated from soil (Rondon *et al.,* 2000; Crowe and Olsson, 2001; Courtis *et al.,* 2003). Soil microbial communities are among the most complex, diverse and important assemblages of organisms in the biosphere; and they participate in various biological activities. Accordingly, they are

an important source for the search of novel antimicrobial agents and molecules with biotechnological importance (Hackl *et al*., 2004). One of the areas in soil where one can find abundance in microbial populations is the rhizosphere. It is a thin layer of soil adhering to a root system which is rich in microbial diversity. The magnitude of this area depends on the plant and the size of the roots that the plant posses (Rondon *et al*., 1999; Rondon *et al*., 2000 and Dakora & Phillips, 2002). Many groups of microorganisms like Gram-positive, Gram-negative bacteria and fungi have the ability of synthesizing antimicrobial agents and the top cultivable antimicrobial agent producers present in soils are the actinomycetes (Pandey *et al*., 2002).
Accordingly, in the present investigation, we tried to find a new antimicrobial agent producing microbe from soil microbiota of local habitat. In addition, we aimed at selection and identification of the most potent antimicrobial producing one for its genotoxicity effect on the DNA. From the 70 soil samples, that underwent primary screening, 2 soil samples resemble the presence of actinomycetes in the crowded plates. The colony of the particular organism was isolated and grown in pure culture and microbial sensitivity test was performed using cup plat method.

Microscopical, biochemical & culture characterizations were studied to confirmed that the chosen microorganism RRK-1 & RRK-2 belongs to the antibiotics producing group. Fermentation was designed to ensure rapid and healthy growth. Downstream processing yielded 6 fractions all of which had antimicrobial activity. So it is worthy to perform exhaustive studies to establish the exact identity of the soil screened microorganism and through the automatic microorganism detector, the isolated microorganism RRK1 and RRK2 were found to be *Bacillus subtilis*, *Staphylococcus aureus* respectively.

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References

Aszalos, A., Davis, S., and Frost D. 1968. The characterization of the new Viridomycin. Journal of chromatography. 37:487-498.

Ball, A. P., Bartlett, J.G., Craig, W.A., Drusano, G.L., Felmingham, D., Garau, J.A., Klugman, K. P., Low, D.E., Mandell, L.A., Rubinstein, E., and Tillotson, G.S. 2004. Future trends in antimicrobial chemotherapy: expert opinion on the 43rd ICAAC. J. Chemother. 16: 419-436.

Becker, B., Lechevalier, M.P., Gordon, R.E., Lechevalier, H.A. 1964 . Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. Appl Microbiol Biol. 12: 421-423.

Colsky, A.S., Kirsner, R.S., Kerdel, F.A. 1998. Analysis of antibiotic susceptibilities of skin wound flora in hospitalized dermatology patients: The crisis of antibiotic resistance has come to the surface. Arch. Dermatol. 134 (8): 1006-1009.

Crowe, J., Olsson, S. 2001. Induction of lactase activity in *R. solani* by antagonistic *Pseudomonas flouescens* strains and a range of chemical treatments. Appl. Environ. Microbiol. 67: 2088-2094.

Davies, F.L., and Williams, S.T. 1970. Studies on Ecolgy of actinomycetes in soil.I. the occurrence and distribution of actinomycetes in a pine forest soil. Soil biology and Biochemistry. 2: 227-238.

Filius, P.M.G., Gyssens, I.C. 2002. Impact of increasing antimicrobial resistance on wound management. *Am. J. Clin. Dermatol.* 3 (1): 1-7.

Giacometti, A., Cirioni, O., Schimizzi, A.M., Del Prete, M.S., Barchiesi, F.,D'Errico, M. M., Petrelli, E., Scalise, G. 2000. Epidemiology and microbiology of surgical wound infections. J. Clin. Microbiol. 38 (2): 918-922.

Hackl, E., Boltenstern, S., Bodrossy, L., Sessitsch, A. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Appl. Environ. Microbiol. 7: 5057-5065.

Hancock, R.E.W. 2007. The end of an era? Nat. Rev. Drug. Discov. 6: 28.

Karanagh, F. 1985. Dilution methods of antibiotic assay. Analytical Microbiology, Indiana Polis: Academic press. 207-210.

Masayuki Hayakawa. 2008. Studies on the isolation and distribution of rare actinomycetes in soil. Actinomycetologica. 22: 12-19.

Mohammed E. Osman., Fath Allah H. Ahmed and Walla S.M. Abd El Ali. 2011. Antibiotic production from local streptomycets isolated from Egyptial soil at Wadey E1-Natron.II Noverl antibiotic identification produced by streptomycets plicatus. Australian journal of basic and applied sciences. 5(11): 1906-1910.

Nancy, O., Sturgen and Casida, L.E. 1962. Antibiotic production by anaerobic bacteria. Jornal series of Pennsylvania Agricultural experiment station. 10;55-59.

Narendran kumar, Ravi Kant Singh, Mishra, S.K., Singh, A.K., Pachouri, U.C. 2010. Isolation and screening of soil actinomycetes as a source of antibiotics active against bacteria. International journal of microbiology research. 2(2): 12-16.

Pandey, B., Ghimire, P., Prasad, V., Agrawal, V. 2002 . Studies of the antimicrobial activity of the actinomycetes isolated from the Khumby region of Nepal. 1(4): 76-80..

Rondon, M., August, P., Bettermann, A.D., Brady, S.F., Grossman, T.H., Liles, M.R., Loiacono, K.A., Lynch, B.A., MacNeil, C., Minor, C., Tiong, M., Osborne. J., Clardy, J., Handelsman, J., Goodman, R. 2000 . Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl. Environ. Microbiol. 66: 2541-2547.

Rondon, M., Raffel, S., Goodman, R., Handelsman, J. 1999. Toward functional genomics in bacteria: analysis of gene expression in *Escherichia coli* from a bacterial artificial chromosome library of *Bacillus cereus*. Microbiol. 96: 6451-6455.

Sirinin Chatujinda, Somboon Tanasupawat, Surattanaamoypol, Chaiyo Chai Chantipyuth. 2007. Identification and antimicrobial activities of streptomyces strains isolated from soil. Journal of health research. 21(3): 195-200.

Srividya, A.R., Saritha G.S., and Suresh, B. 2008. Study of the soil isolates for Antimicrobial activity. Indian Journal of Pharmaceutical Sciences. 2(4): 812-815-(iii).

Srividya, A.R., Saritha, G.S., and

Suresh, B.. 2009. Isolation, Characterization, Bioprocessing, and Product evaluation of new Microorganism, Act- 17. The Pharmacist. 4(1): 1-4.(ii)

Srividya, A.R., Dhanabal, S.P., Sathish Kumar, M.N., Vishnuvarthan, V.J. 2013. Genoprotective activity of hydro alcoholic extract of *Curcuma aromatica* and *Curcuma zedoaria* rhizomes and their comparison with Curcumin**.** The Journal of Ethno biology and Traditional Medicine. Photon. 118: 306-321.

Srividya, A.R., Saritha, G.S., and Suresh B. 2009. Isolation, characterization, bioprocessig and product evaluation of new strain of *streptomyces species* from Kerala top soil. Journal of Global Pharma Technology. 1(1): 71-75.-(i)

Srividya, A.R., Shalom, A., Chandrasekhar, R., Vijayan, P and Vishnuvarthtan, V.J. 2012. Cytotoxic, Antioxidant And Antimicrobial Activity of *Polygonum Chinensis* Linn. International Journal of Pharmaceutical Sciences and Nanotechnology. 4(4): 1569-1574.

Srividya, A.R., Lavanya, C.J, Vijayan, P, Vishnuvarthan, V.J. 2011. Screening of pigment producing microorganism from soil. Inventi Rapid Journal of Cosmaceuticals. 2: 11-15.

Xu, L., Li, Q., and Jiang, C. 1996. Diversity of soil actinomycetes in Yunnan China. Appl Environ Microbiol. 62: 244-248.

Yedir, O.Y., Barakate, M., Finance, C. 2001. Actinomycetes of Moroccan habitats: Isolation and screening for antifungal activities. Europ J Soil Biol. 37 (2): 69-74.

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