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Isolation and Identification of Antibiotic Producing Microorganisms



اعداد الطالبات :

1. العنود الشعلان
2. ربي سليمان المحيميد
3. رنيم محمد العتيبي
4. زهرة ناصر

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Introduction:

Antibiotics are antimicrobial compounds produced by living microorganisms. These compounds were used therapeutically and sometimes prophylactically in the control of infectious diseases. Over 4000 antibiotics have been isolated before, but only 50 have achieved wide usage. The other antibiotic compounds failed to achieve commercial importance for some reasons such as toxicity to human and animals, ineffectiveness or high production costs (Smith, 1996).

Many antibiotics were produced by microorganisms as secondary metabolites. The isolation of antibiotics from microorganisms is relatively easy as compared to chemical synthesis of antimicrobial agents. The isolation of antibiotics from microorganisms improved the discovery of novel antibiotics that could act as better chemotherapeutic agents (Y. Kulkarni and Y. Aynihjri, 1995).

Different antibiotics have different modes of action, owing to the nature of their structure and degree of affinity to certain target sites within bacterial cells according to the mode of action an antibiotic might be undergo each of the following groups : Inhibitors of cell wall synthesis(Penicillins, Cephalosporins, Bacitracin and Vancomycin) , Inhibitors of cell membrane function (Polymixin B and Colistin) , Inhibitors of protein synthesis(Aminoglycosides, Macrolides, Lincosamides, Streptogramins, Chloramphenicol, Tetracyclines) , Inhibitors of nucleic acid synthesis (Quinolones, Metronidazole, and Rifampin) , Inhibitors of other metabolic processes (Dixon , 2006).

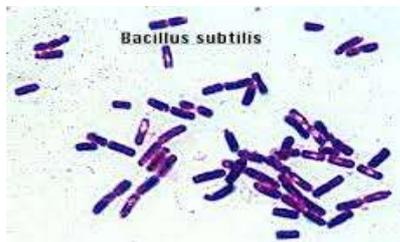
Actinomycetes are the most known subgroup of bacteria for antibiotic production

Antibiotic Producing Microorganisms :

study of bacterial isolates from soil of a selection of seven bacteria isolates from soil with a broad spectrum against bacterial testing was diagnosed using a variety of morphological tests and biochemical as well as have been diagnosed with isolates that *Bacillus subtilis* and studied the effect of different temperatures and the effect of salinity on the growth . (Muthana, 2008)

A trial to find out a new antimicrobial agent producing bacteria from soil samples screened for their antimicrobial activity against the pathogenic bacteria. This study indicates that microorganism *Bacillus polymyxa*_ isolated

from the soil could be an interesting source of antimicrobial bioactive substances . (Mashoria et al., 2014).

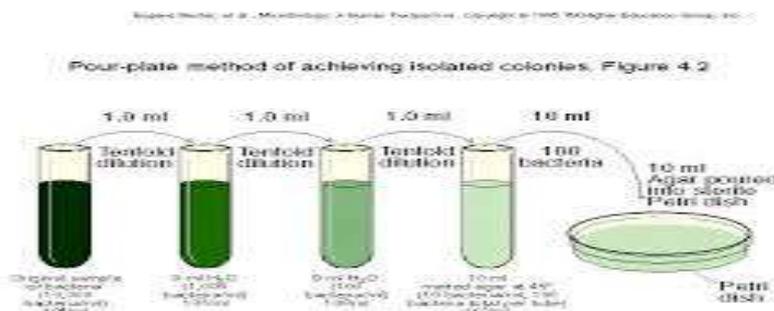


Bacillus subtilis



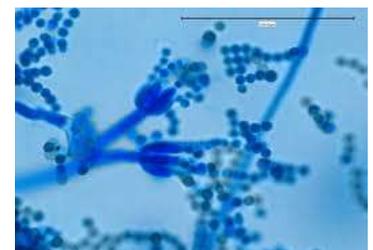
Bacillus polymyxa

It was declared that strains of antibiotic producing fungi are present in the soil, which it is possible to be harnessed by the pharmaceutical industries for the production of antibiotics from local soil samples were collected from ten different locations in Pour plate method involving serial dilution was used for the isolation of fungi .



The media used for the isolation were Malt Extract Agar (MEA), Potato Dextrose Agar (PDA) and Plate Count Agar (PCA). The genera were *Penicillium sp.* , *Aspergillus fumigates* , and *Aspergillus niger* . All the fungal isolates were found to inhibit the growth of at least one of the pathogens which are : *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

(Trakia Journal of Sciences, 2011).



Penicillium sp.

In regardto actinomycetes one hundred seventy five strains

,with potential antibiotic producing, were isolated from 38 different soil samples from different locations .

It showed broad-spectrum antibacterial and antifungal properties which can be further exploited for industrial and biological applications .

(Kumari et al. ,2013).

Materials and Methods:

A- Isolation of Antimicrobial Agent Producing Microorganisms:

- 1- **Fungi: soil samples** were collected from ten different locations in Nigeria of 250 g each was collected into sterile plastic containers and transported to microbiology laboratory for isolate and defined.

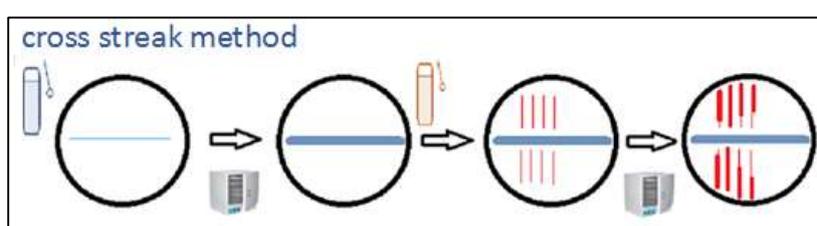
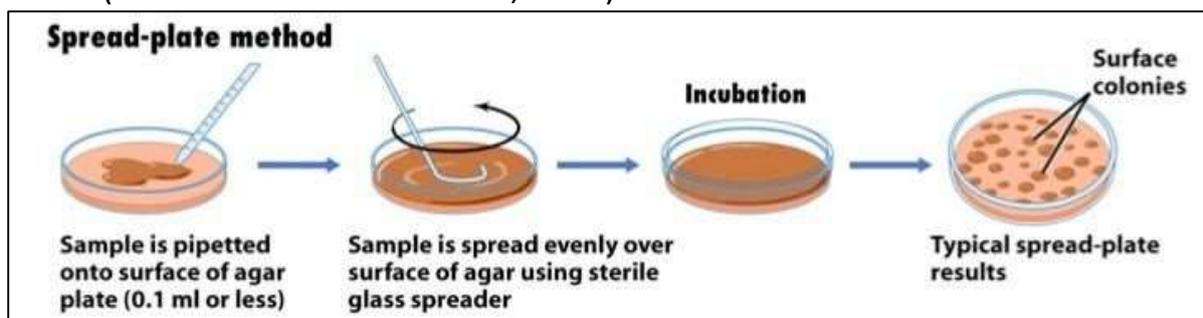
Isolation of Fungi put 10 gm of the soil samples were diluted in 90 ml of sterile distilled water. Ten-fold serial dilution was carried out, 0.1 ml of 10⁻³ and 10⁻⁴ dilution were planted out in duplicate unto Sabouraud Dextrose Agar and Malt Extract-Yeast Extract Agar using a spread plate technique supplemented with 50 mg/ml of streptomycin to inhibit the growth of bacteria, also the pH of the medium was adjusted to 5.8 to encourage the growth of the fungi. The plates were incubated at room temperature (28 C) for 96 hours (Ogbonna, 2013).

The temperature of the soil at the ten different sites was determined by the use of thermometer. The thermometer was inserted into the soil up to depth of 5 cm and allowed to stay for 10 minutes, after which the temperature reading was obtained. The average of three consecutive readings was recorded for each site (Makut, 2011).

The soil pH values were determined by digital pH meter using standard methods of Watson and Brown. Using this method, 3g of soil sample was weighed into a beaker containing 3 ml of distilled water, which was stirred for five seconds and allowed to stand for 10 minutes. The electrode of the pH meter was then inserted into the slurry and swirled carefully. The reading was taken thereof and the average of the consecutive readings was recorded for each site (Makut, 2011).

Actinomycetes: Speead Plate Method: Single colonies of actinomycetes were isolated by serial dilution and spread-plate method using Starch Casein Agar (SCA) Medium . Soil samples (0.1 gram) were suspended in 9.9 ml normal saline (0.87% NaCl, w/v) and serially diluted. Then, 0.1 mL

of inoculums from desired dilution was spread onto sterile SCA agar plates. After incubation at $35 \pm 1^\circ\text{C}$ for 3-4 days, Screening of single colonies for antimicrobial activity was performed by cross streak method (Waksman and Lechevalier, 1962).



2- **Bacteria:** Soil samples were collected from different localities of Bhopal region in India. Each 1 g of the sample was suspended in 9 ml sterile distilled water and shaken vigorously for 2-3 minutes. The soil suspension was serially diluted in sterile normal saline (0.85%) and the dilution from 10^{-3} and 10^{-10} were then plated on overlaid Nutrient agar 0.8% with seeded test organisms and incubated at 37°C for 12 to 24 hours, to screen for antagonistic bacteria. Colonies giving a clear zone of inhibition were isolated and re-streaked over a fresh media plate.

B- Pathogens Antibiotic Sensitivity Tests:

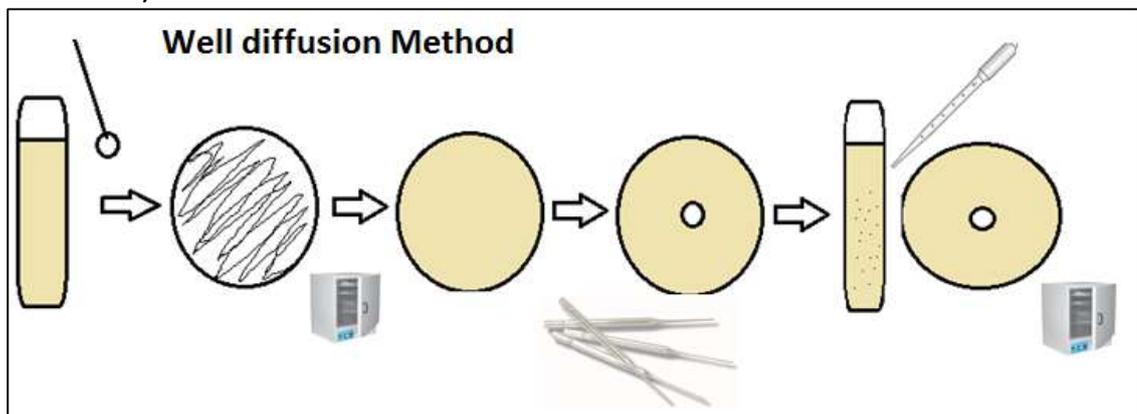
1- **Fungi: Disc Diffusion Method :** The fungal isolates were tested against pathogens and their antibiotic sensitivity was determined. The bacterial lawn of each organism was prepared on the nutrient agar plates. One drop of fungal culture was added to sterile filter paper disc (size:5mm) and allow to dry after each addition. The discs were then placed on air dried surface of the medium. The plates were incubated at 37°C for 24 hours. After incubation the diameter of inhibition zones around the discs was measured (Helen,2012).

The bacterial strains used were *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Makut,2011)

Pure isolates of the fungi were identified microscopically on the basis of their cultural,morphological and physiological characteristic and

microscopically using lactophenol staining technique. Identification of the isolates was accomplished by using the dichotomous key and picture key of known fungi class (Ogbonna,2013).

- 2- **Actinomycetes: Well diffusion Method:**The antibiotic producing potential of the selected actinomycete strain was determined on an agar plate. The diameter of the zone of complete inhibition was measured to the nearest millimeter. Test organisms used for bioassay were Gram-positive bacteria (*Streptomyces lividans* TK23 MTCC4, *Staphylococcus aureus* MTCC96) and Gram-negative bacteria (*Escherichia coli* MTCC739) and a fungus (*Candida albicans* MTCC227) All test microorganisms were procured from Microbial Type Culture Collection (MTCC) and GeneBank, Institute of Microbial Technology (IMTECH), Chandigarh, India. The isolates were characterized for taxonomic identification based on the parameters described in Bergey's Manual of Determinative Bacteriology (Holt, 1994). To optimize the conditions for antibiotic production, the selected strain was grown in eight different media, Glycerol Asparagine Broth (GAB), Starch Casein Broth (GSB), Yeast Extract Malt Extract Broth (YMB), Czapek Dox Broth (CDB), Nutrient Broth (NB), Oat Meal Broth (OMB), Starch Broth (SB) and Soyabean Meal Broth (SMB) as well as at different pH ranges (5.0-10.0) and different temperature (25, 30, 35, 42 and 50°C).



- 3- **Bacterial:** The isolated bacterial strains were inoculated in nutrient broth media for 48 hours. The cultures were centrifuged at 6000 rpm for 10 min Duplicate plates were used for each target organism. 100 µl of bacterial culture supernatant were added to the wells in the plates. The detection of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activities.. This supernatant was used to study antibacterial activity of isolated bacteria among Pathogenic bacterial species. The test bacteria include *Salmonella typhi* (MPCST-109), *Serratia ficaris*(MPCST-076), *Streptococcus faecalis* (MPCST-

072), *Pseudomonas vesicularis* (MPCST-088), *Staphylococcus cohnii* (MPCST-121) *Escherichia coli* and *Pseudomonas aeruginosa* .

Effects of enzymes on antimicrobial activity :each bacterial supernatant were treated with 2 mg/ml⁻¹ trypsin (Hi-Media) at 37°C for 1h.

Results:

1- Fungi:

The results of the Physico-chemical properties of soil samples in Table1 show The pH values of the soil samples show that (J) was the most acidic with pH of 5.7 and (A) was the most alkaline with PH of 8.3. (D) and (F) were slightly alkaline (7.0 and 7.4 respectively), while other locations had pH values that ranged from 5.9 to 6.5. The temperature of the soil environments at the time of this investigation (rainy season) revealed that the soil environment had temperature range between 25C and 26C

| Locations | PH | Temperature |
|-----------|-----------|-------------|
| A | 8.3 ± 1.7 | 26 ± 0.5 |
| B | 5.9 ± 0.7 | 25 ± 0.5 |
| C | 6.3 ± 0.3 | 26 ± 0.5 |
| D | 7.0 ± 0.4 | 26 ± 0.5 |
| E | 6.7 ± 0.1 | 25 ± 0.5 |
| F | 7.4 ± 0.8 | 25 ± 0.5 |
| G | 6.5 ± 0.3 | 26 ± 0.5 |
| H | 6.0 ± 0.6 | 25 ± 0.5 |
| I | 6.5 ± 0.1 | 26 ± 0.5 |
| J | 5.7 ± 0.9 | 26 ± 0.5 |

Table 1.Physico-Chemical Properties of soil sample of the different locations(Makut,2011)

the results of the characterization and identification of fungi isolates Which have the ability to produce antimicrobial were *Absidia corymbifera* , *Alternaria alternate*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium herbarum*, *Curvularia lunata*, *Penicillium sp.*, *Rhizopus stolonifera*, *Trichoderma viride*(Ogbonna,2013).

Fig-1 : *Aspergillus sp*(Helen,2012).

In table2 shows that *Aspergillus niger* and *Penicillium sp* had the highest percentage frequency of occurrence of 60% each, while *Aspergillus fumigatus*,

Curvularia lunata and *Trichoderma viride* respectively had 40%. Other fungal isolates, namely *Absidia corymbifera*, *Alternaria alternata*, *Aspergillus flavus*, *Cladosporium herbarum* and *Rhizopus stolonifer*, all had 30% as their percentage occurrence frequency

| Fungal Isolates | Sites | | | | | | | | | | Occurrence Frequency (%) |
|------------------------------|-------|---|---|---|---|---|---|---|---|---|--------------------------|
| | A | B | C | D | E | F | G | H | I | J | |
| <i>Absidia corymbifera</i> | - | + | - | - | - | - | + | - | - | + | 30 |
| <i>Alternaria alternata</i> | + | - | + | - | - | - | - | - | + | - | 30 |
| <i>Aspergillus flavus</i> | - | - | + | - | + | + | - | - | - | - | 30 |
| <i>Aspergillus fumigatus</i> | - | + | - | - | + | - | - | + | - | + | 40 |
| <i>Aspergillus niger</i> | + | - | + | + | - | + | + | - | + | + | 60 |
| <i>Cladosporium herbarum</i> | - | + | - | - | + | + | - | - | - | - | 30 |
| <i>Curvularia lunata</i> | - | + | - | + | - | - | + | - | + | - | 40 |
| <i>Penicillium sp.</i> | + | - | + | + | + | + | - | + | - | - | 60 |
| <i>Rhizopus stolonifer</i> | - | - | - | + | + | - | - | + | - | - | 30 |
| <i>Trichoderma viride</i> | + | - | - | + | - | - | + | + | - | - | 40 |

Table 2. Percentage Frequency of Occurrence of Fungal Isolates(Makut,2011).

The results of the Zone of Inhibition in Table3 shows that all the fungal isolates have antimicrobial activity against at least three of the test pathogens, which indicates that these fungi produce some form antimicrobial substance(s) was responsible for inhibiting the test organisms. *Absidia corymbifera*, *Aspergillus niger* and *Penicillium sp.* Were found to inhibit all the four test organisms, while the remaining isolates inhibited at least three of the pathogens tested. *Absidia corymbifera* was found to inhibit *C. albicans*, *E. coli*, *P. aeruginosa* and *S. aureus* with inhibition zones of 10mm, 15mm, 10mm and 11mm, respectively. *Alternaria alternate* inhibited all test organisms except *S. aureus* with inhibition zones of 10mm against *E. coli* and 6mm against *C. albicans* and *P. aeruginosa*. The three species of *Aspergillus* isolated showed variable extents of inhibition. *A. flavus* was found to inhibit *C. albicans*, *E. coli* and *S. aureus* by producing inhibition zones of 12mm, 8mm and 12mm, respectively, but had no effect on *P. aeruginosa*. *A. fumigatus* inhibited *C. albicans*, *P. aeruginosa* and *S. aureus* by producing inhibition zones of 11mm, 13mm and 14mm, respectively. Similarly, inhibition zones of 10mm, 15mm, 7mm and 16mm were respectively produced against *C. albicans*, *E. coli*, *P. aeruginosa* and *S. aureus* by *A. niger*. *Cladosporium herbarum* was also found to inhibit *C. albicans* (8mm), *E. coli* (14mm) and *S. aureus* (11mm). *C. albicans*, *E. coli*, *P. aeruginosa* and *S. aureus* were inhibited by *Curvularia lunata* by producing inhibition zones of 9mm, 18mm and 6mm, respectively. Inhibitory activity of

Penicillium sp. was observed against *C. albicans* (12mm), *E. coli* (13mm), *P. aeruginosa* (6mm) and *S. aureus* (8mm). *Rhizopus stolonifer* inhibited *C. albicans* (9mm), *P. aeruginosa* (7mm) and *S. aureus* (6mm) while *Trichoderma viride* showed inhibition zones of 7mm, 18mm and 9mm respectively against *C. albicans*, *E. coli*, *P. aeruginosa*(Makut,2011).

| Fungal Isolates | Zone of Inhibition (mm) of Test Organisms | | | |
|------------------------------|---|----------------|----------------------|----------------------|
| | <i>C. albicans</i> | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>Staph. aureus</i> |
| <i>Absidia corymbifera</i> | 10 ± 0.6 | 15 ± 3.9 | 10 ± 2.7 | 11 ± 2.6 |
| <i>Alternaria alternata</i> | 6 ± 3.4 | 10 ± 1.1 | 6 ± 1.3 | 0 ± 0.0 |
| <i>Aspergillus flavus</i> | 12 ± 2.6 | 8 ± 3.1 | 0 ± 0.0 | 12 ± 3.6 |
| <i>Aspergillus fumigatus</i> | 11 ± 1.6 | 0 ± 0.0 | 13 ± 5.7 | 14 ± 5.6 |
| <i>Aspergillus niger</i> | 10 ± 0.6 | 15 ± 3.9 | 7 ± 0.3 | 16 ± 7.6 |
| <i>Cladosporium herbarum</i> | 8 ± 1.4 | 14 ± 2.9 | 0 ± 0.0 | 11 ± 2.6 |
| <i>Curvularia lunata</i> | 9 ± 0.4 | 18 ± 6.9 | 0 ± 0.0 | 6 ± 2.4 |
| <i>Penicillium sp.</i> | 12 ± 2.6 | 13 ± 1.9 | 6 ± 1.3 | 8 ± 0.4 |
| <i>Rhizopus stolonifer</i> | 9 ± 0.4 | 0 ± 0.0 | 7 ± 0.3 | 6 ± 2.4 |
| <i>Trichoderma viride</i> | 7 ± 2.4 | 18 ± 6.9 | 9 ± 1.7 | 0 ± 0.0 |

Table3: Zone of Inhibition(mm) of Test Organisms (Makut,2011).

2- Actinomycetes:

Antibiotic production in Different Growth Media at Varying pHs and Temperatures Antibiotic activity is defined and measured in terms of its ability to inhibit microbial growth of bacteria, fungi and protozoa (Lancini et al., 1995). Screening of the isolates for antimicrobial properties revealed that about 33% of Actinomycetes isolates in the present study were active against at least one of the four test microorganisms, One isolate, MP 525, was able to inhibit the growth of all four test organisms (*S. lividans*, *S. aureus*, *E. coli*) and *C. albicans*. The level of antibiotic production in different growth media was listed in Table 4. Results revealed that Starch Casein Broth (SCB) was found to be the most suitable media for antibiotic production. In contrast, Glycerol Asparagine Broth (GAB) and Czapek-Dox Broth (CDB) media were able to produce much less antibiotics.

| Media | <i>Streptomyces lividans</i> MTCC4 | <i>Staphylococcus aureus</i> MTCC96 | <i>Escherichia coli</i> MTCC739 | <i>Candida albicans</i> MTCC227 |
|-------|---------------------------------------|--|------------------------------------|------------------------------------|
| GAB | 1 | 2 | 3 | 1 |
| SCB | 10 | 20 | 20 | 24 |
| YEMEB | 4 | 7 | 6 | 4 |
| CzDB | 0 | 0 | 2 | 2 |
| NB | 9 | 13 | 12 | 8 |
| OMB | 8 | 12 | 12 | 8 |
| SB | 5 | 8 | 7 | 5 |
| SMB | 9 | 10 | 12 | 8 |

Table4. production of antibiotics by actinomycete strain MP525 in different media, shown by the zone diameter (mm) of inhibition against each of the test organism. (Kumari et al. 2013)

In addition, antibiotic yield varies at different pH and temperatures as shown in Table 2 and 3, respectively. The maximum antibiotic production was observed in the cultures grown at pH 7.0 Table 5 as well as at 35°C temperature Table 6.

| pH | <i>Streptomyces lividans</i> MTCC4 | <i>Staphylococcus aureus</i> MTCC96 | <i>Escherichia coli</i> MTCC739 | <i>Candida albicans</i> MTCC227 |
|----|---------------------------------------|--|------------------------------------|------------------------------------|
| 5 | 10 | 8 | 8 | 3 |
| 6 | 18 | 18 | 17 | 7 |
| 7 | 20 | 20 | 22 | 8 |
| 8 | 19 | 19 | 17 | 7 |
| 9 | 15 | 14 | 14 | 6 |
| 10 | 12 | 12 | 10 | 4 |

Table5. production of antibiotics by actinomycete strain MP525 in different pH, shown by the zone diameter (mm) of inhibition against each of the test organism (Kumari et al. 2013).

| Temp | <i>Streptomyces lividans</i> MTCC4 | <i>Staphylococcus aureus</i> MTCC96 | <i>Escherichia coli</i> MTCC739 | <i>Candida albicans</i> MTCC227 |
|------|---------------------------------------|--|------------------------------------|------------------------------------|
| 25°C | 2 | 1 | 2 | 3 |
| 30°C | 6 | 12 | 14 | 16 |
| 35°C | 10 | 20 | 20 | 24 |
| 42°C | 8 | 15 | 15 | 20 |
| 50°C | 0 | 0 | 0 | 0 |

Table6. production of antibiotics by actinomycete strain MP525 in different temperatures, shown by the zone diameter (mm) of inhibition against each of the test organism (Kumari et al. 2013).

3- Bacterial:

Out of 28 isolates bacteria tested, 12 (42.87%) isolates were found to exhibit antibacterial activity against pathogenic strains of bacteria. Isolate PBR-11 showed the largest antimicrobial spectrum, exhibiting inhibitory activity against 6 pathogens, *Pseudomonas aerogenosa*, *Salmonella typhi* (MPCST- 109), *Escherichia coli*, *Pseudomonas vesicularis* (MPCST-088), *Serratia ficaria* (MPCST-076), and *Streptococcus faecalis* (MPCST- 072). The highest zone of inhibition was shown by PBRI-4 against *Pseudomonas vesicularis* as shown in Table 7.

| Name of reference strain | NAME OF COLONIES (Zone of inhibition (mm)±S.D) | | | | | | | | | | | |
|------------------------------------|--|------------|------------|------------|------------|-------------|------------|------------|-----------|------------|------------|------------|
| | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 |
| <i>Staphylo coccus ficaria</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17.05±0.12 | 21.55±0.20 | 0 |
| <i>serratia ficaria</i> | 0 | 0 | 11±0.32 | 0 | 12.27±0.22 | 0 | 0 | 0 | 0 | 17.17±0.29 | 24.52±0.05 | 0 |
| <i>Staphylo coccus colint (12)</i> | 0 | 0 | 11.82±0.23 | 0 | 13.15±0.15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Pseudomonas vesicularis</i> | 0 | 26.1±0.52 | 0 | 43.52±0.25 | 0 | 0 | 0 | 0 | 0 | 13.52±0.17 | 34.05±0.09 | 0 |
| <i>E.coli</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17.37±0.26 | 0 | 30.17±0.23 | 22.45±0.12 | 0 |
| <i>Pseudomonas aerogenosa</i> | 36.52±0.35 | 26.77±0.38 | 10.17±0.16 | 31.2±0.21 | 12.07±0.11 | 16.57±0.17 | 0 | 0 | 30.5±0.36 | 0 | 20.52±0.17 | 30.2±0.21 |
| <i>Salmonella typhi ae</i> | 0 | 0 | 0 | 0 | 12.05±0.07 | 18.175±0.08 | 15.52±0.17 | 0 | 0 | 16.5±0.08 | 24.25±0.26 | 28.57±0.17 |

Table 7: antibacterial activity of isolated bacterial strains different pathogenic strains of bacteria.(Mashoria et al. 2014)

Although the identification of these isolated bacterial colonies were not yet done up to the species level, but their morphological and biochemical characteristics indicate that they belong to the genus *Bacillus* and coccus. Different antimicrobial compounds are produced by members of the genus *Bacillus*, most of these identified as peptides, lipopeptides and phenolic derivatives (Nakano and Zuber.,1990).

Different antimicrobial substances produced by *Bacillus* spp. isolated from arthropods were recently described, including aromatic acids, acetylamino acids (amino acid analogs), and peptides (Gebhardt et al., 2002).



(a)

(b)

Fig.2 antagonistic affects of bacterial suspension on different pathogenic bacteria :(a) *salmonella typhi* (b) *streptococcus fecalis* (Mashoria et al. 2014).

Most of these substances were partially or completely inactivated by proteases and TCA, suggesting that a protein moiety is involved in the activity. This may indicate that the inhibition was due to the presence of bacitracin-like substances .The antimicrobial substances showed high thermal resistance and low molecular weight, which are characteristics of small hydrophobic peptides that constitute class II bacteriocins (Riley and Wertz, 2002).



(a)

(b)



(c)

(d)

Fig.3 antagonistic effect of bacterial suspension after treatment with trypsin

Against : (a) *streptococcus fecalis* (b) *salmonella typhi* (c) *serratia ficaris* (d) *Pseudomonas vesicularis* (Mashoria et al. 2014).

Discussion:

All the fungal isolates inhibited *Candida albicans* while all, except *Aspergillus fumigatus* inhibited *Escherichia coli*. Of all the isolates, only three, *Aspergillus flavus* did not inhibit *Pseudomonas aeruginosa*. This investigation reveals that all fungal species isolated from the soil environment do produce some form of antimicrobials. Although, this investigation is a primary study, further investigations needs to be embarked upon to determine the type of antimicrobial substance produced or the type of effect they cause on the pathogens, whether static or tidal.

The best genus of actinomycete that produce antibiotic is Streptomyces, Starch Casein Broth (SCB) was found to be the most suitable media for antibiotic production because the environmental factors that affect the timing and extent of production of antibiotics and method of cultivation, such as carbon and nitrogen sources, oxygen tension, temperature, pH and other secondary metabolites, The best growth of Streptomyces on SCA medium with pH 7 and temperature 35°C (Kumari, 2013).

The inhibition was due to the presence of bacteriocin-like substances. The antimicrobial substances showed high thermal resistance and low molecular weight, which are characteristics of small hydrophobic peptides that constitute class II bacteriocins (Riley and Wertz, 2002).

Although the identification of these isolated bacterial colonies were not yet done up to the species level, but their morphological and biochemical characteristics indicate that they belong to the genus Bacillus and coccus. Members of the genus Bacillus, most of these identified as peptides, lipopeptides and phenolic derivatives (Nakano and Zuber., 1990).

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