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ISOLATION & PRODUCTION OF ANTIMICROBIAL SUBSTANCES FROM *BACILLUS CEREUS*

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ABSTRACT

In the present study soil samples were collected from different places of Lucknow. These soil samples were designated as sample MRDNS-MB (Maqui berry) and MRDNS-FS (forest soil) for the isolation of microbes which are showing antagonistic activity against multiple drug resistant pathogens. The antagonistic activity was observed through agar well diffusion technique. Among all the isolates MRDNS-FS05 was found to be most effective towards pathogens like *Escherichia coli* and *Pseudomonas aeruginosa* giving zone of inhibition 19.7mm and 14 mm respectively. MRDNS-FS05 cultures was further subject in production media for production, extraction and purification of antibiotics was done using solvents like ethanol, ethyl acetate, chlorofrorm, among which antibiotic extracted with ethyl acetate gave positive result in antibiogram analysis with zone of inhibition of 17mm against *Escherichia coli,* 16.2mm against *Pseudomonas aeruginosa and* 18.5 mm against *Staphylococcus aureus*

Key words: *Escherichia coli*, *Pseudomonas aeruginosa,* Antibiogram analysis, Pathogens

INTRODUCTION

Microorganism that can be divided in to categories according to several criteria .One means of classifying bacteria uses staining to divide most bacteria into two group (Gram positive, Gram negative) according to the properties of their cell wall **[1]**.

Gram positive and Gram negative bacteria are differentiated according to the chemical and corporal properties of their cell wall. Gram negative are more problematic because they have outer cell wall which make them difficult to attack with antibiotics .Encompass species such as *Escherichia coli, Helicobacter, Mirabella, Pseudomonas , Salmonella* and *Shigella* ***[2]***.

Gram positive bacteria encompass species such as *Bacillus, Listeria, Staphylococcus, Streptococcus* and *Enterococcus*.

The term antibiotics have come to the uses to denote a broad range of antimicrobial compound including antifungal and other compounds. The term antibiotic was first used in 1992 by Selman Waksman. Most of antibacterial is semi synthetics modification of various natural compounds. These include for example the β-lactam antibodies which include the penicillin. In general a compound or substance that either kills or inhibits the growth of a microorganism, such as bacteria, fungi and protozoa. Antibiotics have three major sources of origin; (1) naturally isolated; (2) purely chemically synthesized or (3) semi synthetically derived. In addition they can be classified according to their effect on bacteria those that kill bacteria are bactericidal those that inhibit the growth of bacteria are which bacteriostatic **[3].** Antibiotics are defined according to their mechanism for targeting and identifying microorganism broad spectrum antibiotics are active against a wide range of microorganisms; narrow –spectrum antibiotics target a specific group of microorganism by interfering with the metabolic process specific to those particular organisms.

Intrinsic resistance - Natural resistance of bacteria to certain antibiotics.

Acquried resistance - Normally susceptible have become resistance as a result of adaption through genetic change **[4]**.

Multidrug resistance - corresponds to resistance of a bacterium to multiple antibiotics **[5, 9]**.

Bacteria can acquire resistance by genetic mutation and by accepting gene coding for resistance from other bacteria resulting in MRD bacteria that are resistance to many different classes of antibiotic. Drugs with new mechanisms of action are needed to be effective against MRD bacteria. Bacteria resistant to currently available antibiotic becoming increasing frequent in both hospital and community settings.

Resistance to entire antibiotic classes (e.g. Beta-lactams, Quinolones, Tetracycline, Glycopeptides and Macrolides) is emerging rapidly coupled with insufficient investment in new antibiotic treatment; this issue is becoming a pressing public health concern **[6]**.

In order to curd the growth of antibiotic resistance and prevent major morbidity and mortality from bacterial infection. We must both address over use and actively promote R&D for antibiotic medicines with novel mechanisms of action (MoA) **[11]**.

Resistance to antibiotic present a major challenges in health care as resistant bacteria dramatically decrease the change of effectively treating infection and increases the risk of complication and death **[7].** Within the EU alone it is estimated that 2 million patients acquire nosocomial infection 3 each year **[7],** over half of which are drug resistant antibiotic resistant infection are associated with a 1.3 to 4 fold increase in mortality compared to susceptible infection **[8]**.

The development of entirely new drug dose implies a significant amount of risk which should be shared by developer and the funder.

Medicine products that kill or stop the growth of living microorganisms and included to as antibacterial agents (more commonly referred to as antibiotics) which are active against bacterial infection **[9]**.

Antimicrobials differ of antibiotics in they can we either natural or synthetics substances which kill the growth of viruses, fungi and parasite in addition to bacteria **[10].**

METHODOLOGY

Sample collection:

The soil samples were collected from different places of Lucknow. These soil samples were designated as sample MRDNS-MB (Maqui berry) and MRDNS-FS (forest soil).

Sample 1: Soil sample MRDNS-MB was collected from near MRD lab Maqui berry.

Sample 2: Soil sample MRDNS-FS was collected from village Rewa (MP)

These samples were isolated for bacteriological analysis by serial dilution and then agar plate culture techniques **[12, 13]**.

Isolation of bacteria:

The soil sample was serially diluted in normal saline solution and then spread on sterilized nutrient agar plates. The colonies were selected on the basis of their morphological parameters and pure cultures were prepared **[14, 15]**.

Screening of antimicrobial metabolites of bacteria:

The screening were carried out by antibiotic sensitivity test using agar well diffusion method **[16,17]**.

Media optimization:

The media components were optimized on the basis of one factor at a time rule for enhancing the oil degradation potential and then the percentage degradation was calculated from the optimization **[18]**.

Production and purification of metabolites from MRDNS-FS05

The production of antimicrobial metabolites was performed by using shake flask method and submerged fermentation. Further the purification were carried out by solvent extraction method **[19]**.

The estimation of the activity of antibacterial metabolite was done by performing antibiotic sensitivity test against bacterial pathogens **[20]**.

RESULTS

Collection of samples

Soil sample was collected from the area near kukrail, lucknow and marked as mrdns-mb and from village rewa, mp and was marked as mrdns-fs and was subjected to serial dilution procedure

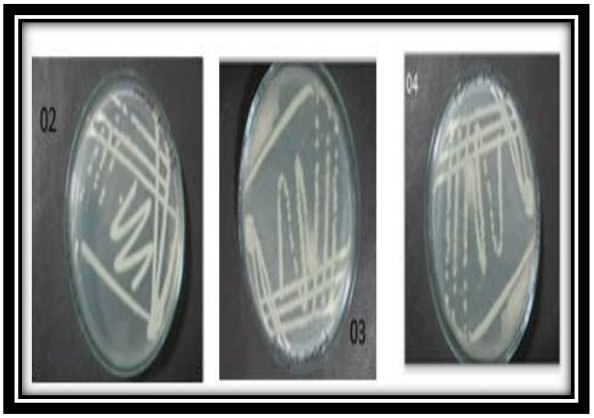
Figure 1: MRDNS-MB **Figure 2:** MRDNS-FS

Isolation of microbes by serial dilution method

Bacterial colonies were isolated and marked as mrdns-mb01 to mrdns-mb05 and mrdns-fs01 to mrdns-fs09.

Figure 2: mrdns-fs01-09

Purification of obtained mixed culture



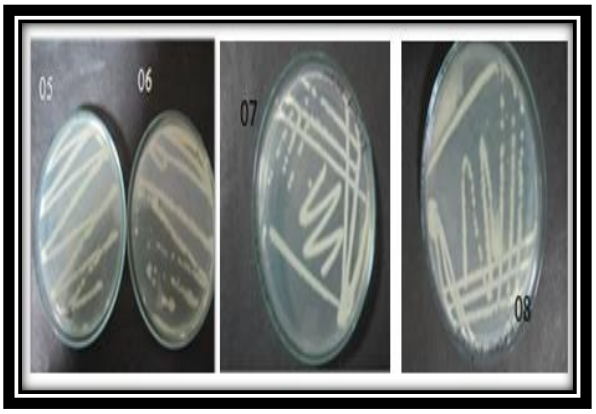


Figure 3: mrdns-fs05/06 fig12: mrdns-fs07 fig13: mrdns-fs08

Test pathogen:

Test pathogens (*eschrechia coli, pseudomonas aeruginosa,, staphylococcus aureus)* were collected from imtech, chandigarh and was subcultured.

below show the picture of culturing of pathogens namely *pseudomonas aeruginosa, eschrechia coli, staphylococcus aureus* in nutrient brothand **figure 15 (a,b,c)** below show the picture of streaking of pathogen namely *pseudomonas aeruginosa, eschrechia coli, staphylococcus aureus* on nutrient agar plates.

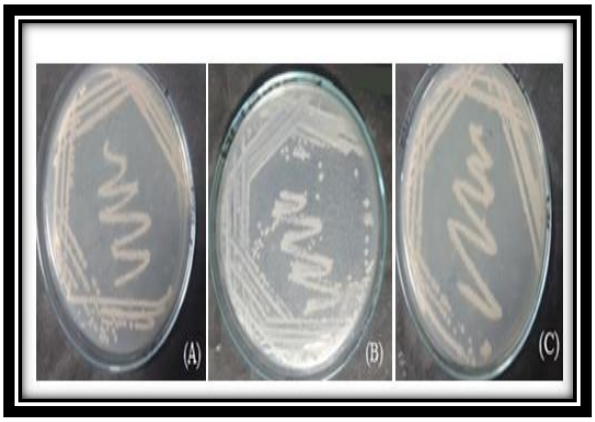


Figure 4: (a) *escherichia coli*, (b) *staphylococcus aureus*, (c)*pseudomonas aeruginosa.*

Antibiogram analysis

Antibiogram analysis was done to know the antibacterial activity of the mrdns-mb 01-05 crude antibioitic extracts. Zone of inhibition was marked and calculated in milimeter. Tetracyclin was used as positive control and dmso as negative control. It can be seen from the result below in **table 1,** in which mrdns-mb cultures gave no zone of inhibition against any of the pathogen used.

Table1:- antibiogram analysis of mrdns-mb cultures

Pathogens	Zoi by 01 (mm)	Zoi 02 (mm)	Zoi 03 (mm)	Zoi 04 (mm)	Zoi 05 (mm)
Escherichia coli	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0

Antibiogram analysis was done to know the antibacterial activity of the mrdns-fs 01-09 crude antibioitic extracts. Zone of inhibition was marked and calculated in milimeter. Tetracyclin (c) was used as positive control and dmso (d) as negative control. It can be seen from the result below in **table 2 and fig 16**, show zone of inhibition of mrdns-fs01,02 and 03 cultures.

Table 2:- antibiogram analysis of mrdns-fs cultures

Pathogens	Zoi by 01 (mm)	Zoi 02 (mm)	Zoi 03 (mm)	Zoi by tetracycline in(mm)	Zoi by dmso in (mm)
Escherichia coli	15	17.5	16.5	0	0
Pseudomonas aeruginosa	12	11.5	0	10.2	0
Staphylococcus aureus	0	0	0	10	0

Table 3:- antibiogram analysis of mrdns-fs cultures

Pathogens	Zoi by 04 (mm)	Zoi 05 (mm)	Zoi 06 (mm)	Zoi by tetracycline in(mm)	Zoi by dmso in (mm)
Escherichia coli	14	19.7	10.5	0	0
Pseudomonas aeruginosa	11	14	0	0	0
Staphylococcus aureus	0	0	0	0	0

Table 4:- antibiogram analysis of mrdns-fs cultures

Pathogens	Zoi by 07 (mm)	Zoi 08 (mm)	Zoi 09 (mm)	Zoi by tetracycline in(mm)	Zoi by dmso in (mm)
Escherichia coli	13	11	15	0	0
Pseudomonas aeruginosa	10	0	0	0	0
Staphylococcus aureus	10	0	0	12	0

Mrdns-fs05 showed best zone of inhibition against mdr pathogen and was taken for further analysis.

Biochemical characterization of isolated bacterial culture using bergey's manual

Table 5: biochemical characterization

S .no	Test	Result
1 .	Gram staining	Positive+rods
2 .	Endospore test	Positive
3 .	Catalase test	Positive
4 .	Mannitol test	Negative

Bacteria identified via bergey's manual:

Bacillus cereus

Optimization of culture condition:

Temperature mrdns-fs05

Spreading was performed of the isolated strain mrdns-fs05 on sterile na plates and was incubated at different temperature (4^0c, 37^0c, 40^0c and 60^0c). The table and images below shows the results of this test:

Table 6: effect of temperature on culture mrdns-fs05

Temperature	Growth	Remarks
50^0c	Growth observed	-
37^0c	Growth observed	++
25^0c	Growth observed	++
4^0c	No growth	-

Effect of pH

The isolate mrdns-fs05 was in four flask with different ph as 5,7 and 9 after inoculation the o.d was taken which is given in the table. The result showed that the culture had maximum growth at ph7.

Table 7: effect of pH on culture mrdns-fs05

S .no	PH	O.d (at 620nm)
1.	Ph-5	0.14
2.	Ph-7	0.16
3.	Ph-9	0.00
4.	Ph-11	0.08

Effect of carbon source:

Table 8: effect of carbon sources on culture mrdns-fs05

Carbon sources	O.d (at 600nm)
Starch	0.26
Sucrose	0.15
Dextrose	0.20
Maltose	0.26

Effect of nitrogen sources:-

Table 9: effect of nitrogen sources on culture mrdns-fs05

Nitrogen sources	O.d (at 600nm)
Potassium nitrite	0.14
Glycine	0.10
Ammonium sulphate	0.06
Urea	0.10

Antibiogram analysis of purified antibiotic produced and extracted from *bacillus cereus*.

Antibiogram analysis was done to know the antibacterial activity of purified antibiotic extracted from ***bacillus cereus*** **(** mrdns-fs 05) using solvent e(ethanol), ea(ethyl acetate) and c(choloform). Zone of inhibition was marked and calculated in milimeter. It can be seen from the result below in **table 10 and fig 25**, show zone of inhibition of antibiotic e, ea and c extracts.

Table 10:- antibiogram analysis of mrdns-fs cultures

Pathogens	Zoi by e(mm)	Zoi by ea (mm)	Zoi by c (mm)
Escherichia coli	0	17	0
Pseudomonas aeruginosa	0	16.2	14
Staphylococcus aureus	0	18.5	15

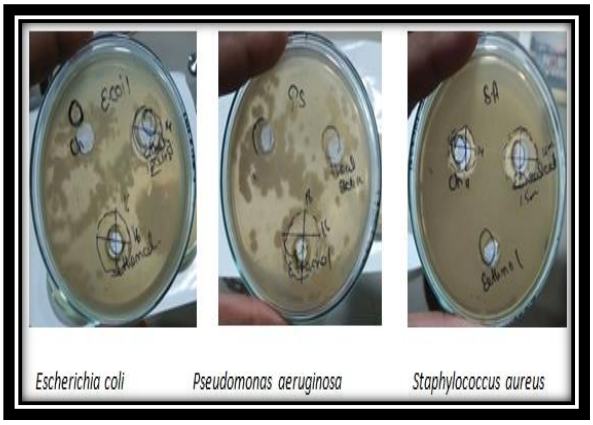


Figure 5: antibiogram analysis of purified antibiotic extracts e, ea & c against *escherichia coli, pseudomonas aeruginosa, staphylococcus aureus*

Antibiotic extracts from ethyl acetate inhibited the maximum growth of *escherichia coli pseudomonas aeruginosa and staphylococcus aureus.*

DISCUSSION

In present time there are so many drugs which are used for curing chronic disease but pathogens are getting resistant to them.

In this study there were total of 9 cultures isolated from mrdns-fs01-09 out of which mrdns-fs05 cultures were identified as *bacillus cereus* and further used for experiment.

Primary and secondary screening was performed using crowded plate and agar well diffusion method as done earlier by **khan, j.a. and tabassum, m. 2011**.

Production of antimicrobial components was done on production media for the production of antimicrobials optimized by **jeffrey (2008)**.

Antibiotic was produced and extracted from *bacillus cereus* and further antibiogram analysis was done to check antimicrobial activity of antibiotic produced from *bacillus cereus.* Antibiotics with solvents like ethanol, ethyl acetate, chloroform among which antibiotic produced from ethyl acetate gave positive result in antibiogram analysis with zone of inhibition of 17mm against *escherichia coli,* 16.2mm of zone of inhibition against *pseudomonas aeruginosa and* 18.5 mm of zone of inhibition against *staphylococcus aureus*.

CONCLUSION

Although many potent strains are on market for antibiotic production, scientists prefer studying on new isolates because they could be alternative for commercial use. In our study the isolated new source of antibiotic producing bacteria like *bacillus cereus.*

From the local soil sample may be alternative source also for the potential industrial applications. Further future work includes optimization with modified production media, identification and characterization of drug.

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