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ISOLATION AND PRODUCTION OF ANTIMICROBIAL METABOLITES FROM BACTERIAL SOURCE

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ABSTRACT

Most of the antibiotics used was purified and derived from microbial sources. The appearance antibiotic resistant and the need for new broader variety of antibiotics are still in high demand. The current analysis the antibiotic-producing bacteria have been isolated from the hospital sample. Culture AKSJ 11 was isolated after screening and showing maximum antibacterial by expressing preeminent zones of inhibition activity against *E. coli, P. aeruginosa* and *S. aureus.* For identification of the AKSJ 11, biochemical tests were performed and it was found that the strain belongs to the Bacillus species.

Key words: *Bacillus*, Metabolites, Zone of inhibitions, Antibiotics, Resistant.

INTRODUCTION

Bacteria present in soil or water can include cocci (spherical), rods (bacilli) and spirilla (spiral) or which bacillus is more widespread in number than others. They are one of the main soil bacteria communities and are widely spread **[1, 2].**

Although several antibiotics are known to exist, attempts are still being made to find new antibiotics **[3]**. As a rule, number of species, Streptomyces, penicillium and bacillus have been actively researched for their ability to produce antibiotics **[4]**. Additionally, bacillus species have produced insoluble antibiotics and that such antibiotics have been found to be cheaper and more efficient and preferred to commercial production. The goal is currently to develop antibiotics such as polymyxin and bacitracin **[5].** Members of the genus bacillus have been reported to have commonly developed polypeptide type bacteriocins, and these have been reported **[6]**. Antibiotics usually affect Gram positive bacteria **[7].** The key antibiotic sources in this group are *B. cereus* (e.g., zwittermicin, cerexin), *B. subtilis* (e.g., mycobacillin, subtilin, bacitracin, polymyxin, difficidin), *B. brevis* (e.g., tyrothricin , gramicidin), *B. laterosporus* (e.g., laterosporin), *B.licheniformis* (e.g., bacitracin), *B. pumilus* (e.g.,pumulin), *B. circulans* (e.g., circulin), *B. polymyxa* (e.g., polymyxin, colistin) **[8]**.

It is commonly believed, these are antibiotics which are mainly polypeptides **[9-11].** Pathogenic bacteria are becoming more resistant to current antibiotics, most of which are costly and have been used. It combined with side effects such as nephrotoxicity etc. **[12, 13]**. Bacteria have evolved a variety of techniques to get resistant against antibiotics and antibacterial metabolites **[14]**. It is especially true of the bacteria that are the sources of antibiotics. Bacteria produce antibiotics in order to achieve a competitive advantage over other opposing species in their natural world **[15]**. Unless they were prone to their own metabolic goods, even a comparative advantage would have been missed. Drugs sensitivity pattern tests have been performed with other antibiotics. The purpose of this research was to isolate and classify microorganisms that are capable of producing antibiotics.

MATERIALS AND METHODS

Collection of samples:

Areas in which microorganisms survive under stressful conditions have been chosen for the isolation of bacteria-producing antibiotics **[16]**. Area belongs to a) hospital / clinical soil sample b) river water sample c) waste water sample d) soil sample near to medical stores chosen for sample collection.

Isolation of antibiotic producing bacteria:

The bacteria were isolated from the water as well soil samples by using serial dilution and spread plate technique. Pure colonies, which were collected after streaking, were then screened for the antibiotic production by performing antibiotic susceptibility test using agar well diffusion method **[17,18]**.

Strain identification:

The strain was described following the analysis of colony morphology, staining and biochemical tests as specified in the Bergy's manual **[19]**.

Table 1: Compositions of production media for the selection and optimization.

S no.	Media / Factors	Components	Quantity
1	Production media 1	Nutrient broth	13g/L
2	Production media 2	$MgSO_4$	0.2g/L
		KH_2PO_4	3g/L
		NaCl	5g/L
		Dextrose	8g/L
		Yeast	5g/L
3	Production media 3	Dextrose	8g/L
		Peptone	5g/L
		NaCl	5g/L
4	Effect of Carbon sources		
	MM 1	Dextrose	8g/L
	MM 2	Sucrose	8g/L
	MM 3	Maltose	8g/L
5	Effect of Nitrogen sources		
	MM 4	Peptone	5g/L
	MM 5	NH_4Cl	5g/L
	MM 6	Yeast	5g/L
6	Effect of salt		
	MM 7	NaCl	5g/L
	MM 8	$CaCl_2$	5g/L
	MM 9	$FeCl_3$	5g/L
7	Effect of pH		
	MM 10	4	-
	MM 11	7	-
	MM 12	9	-
	MM 13	11	-
8	Effect of temperature		
	MM 14	4°C	-
	MM 15	22°C	-
	MM 16	37°C	-
	MM 17	50°C	-

Fermentation and purification or antibacterial metabolites:

The synthesis of antibacterial metabolites was conducted using a shake flask process. The colony was inoculated in controlled media under sterilized conditions and the purification of metabolites was done using solvent extraction methods with polar (acetone, methanol) and non-polar (chloroform, petroleum ether) solvents **[21]**.

Estimation of antibacterial metabolites:

The antibacterial activity of metabolites was carried out by conducting the agar well diffusion method suggested by Sudha et al., (2015) **[22]**.

RESULTS AND DISCUSSIONS

Sample collection:

The table 2 elaborate the place from where the respective samples were collected for the isolation of antibiotic producing bacteria.

Table 2: the collection of the samples from different locations in Lucknow, UP.

S no.	Samples	Location	pH
1	Soil sample	Ram Manohar Lohia Hospital, Lucknow	5.2
2	Soil sample	Medical shop, Chawk, Lucknow	6.1
3	Water sample	Gomti river, River front, Lucknow	5.9
4	Water sample	Sewage, Khuram nagar, Lucknow	7.5

Isolation of antibiotic producing bacteria:

By serial dilution method 16 different bacterial colonies from four different samples were obtained, these bacterial colonies are chosen on the basis of clear zone shown around them i.e. Primary Screening.

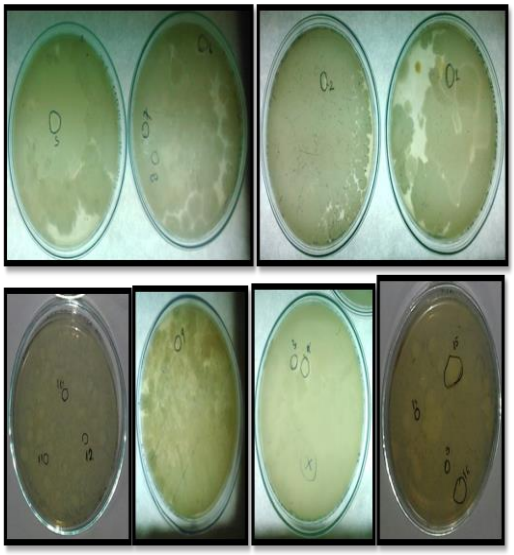

Figure 1: Above figures are of spreading plates of diluted soil samples in which mark circle around different bacterial colonies that are producing antibiotics on the basis of Primary Screening.

Screening for antibacterial metabolites producing bacteria

After primary screening and purification of bacterial colonies we were check on all of these bacterial colonies which one is better antibiotic producer, for this Antibiotic sensitivity test was performed against pathogens *E. coli, P. aeruginosa,* and *S. aureus*. We found that culture AKSJ 01, 09, 11, and 13 are best antibiotic producers as compare to rest of bacterial colonies. AKSJ 09, 13 & 01 are shown the partial zone of inhibition against different pathogens so we choose AKSJ 11 bacterial culture that give complete zone of inhibition against *E. coli, P. aeruginosa, and S. aureus*.

Table 3: - Shown result of Antibiotic Sensitivity test of AKSJ 01 to 08 against different pathogens.

Zone of inhibition (mm)			
	Ec	***Pa***	***Sa***
1	14	14	15.5
2	0	0	15
3	0	0	0
4	0	0	0
5	0	0	14.5
6	0	14	15
7	0	0	0
8	0	0	0
9	10	12	13
10	0	14	0
11	16	18	16
12	0	13	0
13	12	0	0
14	0	11	0
15	0	0	15
16	0	0	0

Table 5: Colony morphology and biochemical characterization of AKSJ 11

S no.	NAME OF TEST	RESULT OF TEST
1.	**Colony morphology**	
A	Shape	Spindle
B	Margin	Discrete
C	Elevation	Raised
D	Texture	Gummy
E	Pigmentation	Cream
F	Surface	Smooth
G	Opacity	Opaque
2.	**Biochemical characterization**	
A	Gram staining	+ & *Bacillus*
B	Endospore test	+
C	Mannitol test	-
D	Catalase test	+
E	VP test	+

Where, *Ec: E. coli, Pa: P. aeruginosa, and Sa: S. aureus*.

Media selection and otimization for the AKSJ 11

On the basis of the antibacterial activity of the culture AKSJ 11 against pathogens by showing the zone of inhibiton in the presence of different media.

PM 3 was selected best fot the production of anbacterial metabolite and proceeded for the optimization of its components. Where Maltose and NH_4Cl (MM 3 and MM 5) selected as best carbon and nitrogen source. The effective pH was 7 (MM 11) for the enhancing the antibacterial activity of the AKSJ 11.

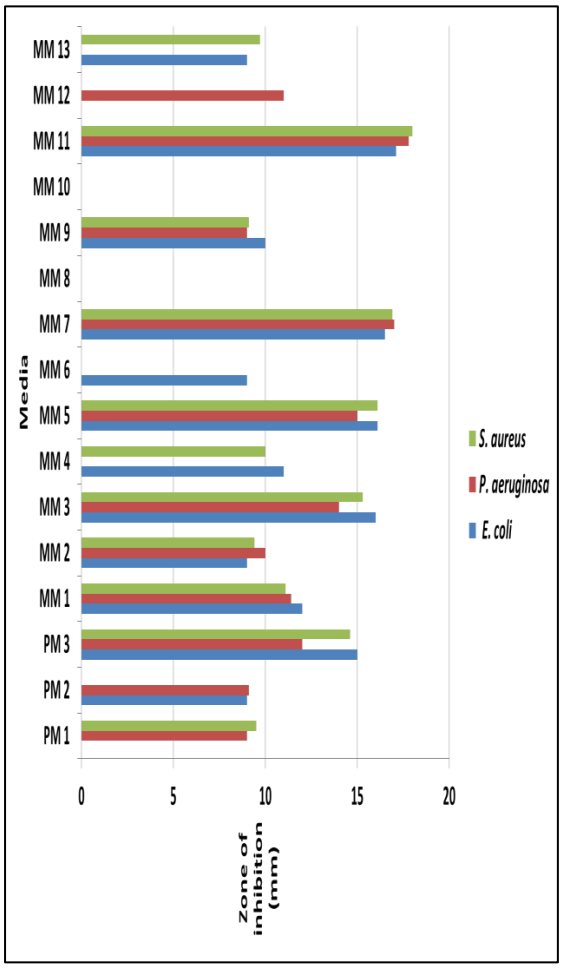

Figure 2: Graphical analysis of the antibacterial activity of media against pathogens.

Effect of temperature on growth of bacteria AKSJ 11:

Each bacterium grown on different temperature to check bacterial culture growth and it was found that 37^0C best for the maximum growth.

Table 6: The observation of the bacterial culture at different temperature.

S no.	Temperature	Remarks
1	4^0C	-
2	22^0C	+
3	37^0C	+++
4	50^0C	-

Assessment of the antibacterial metabolites:

The achieved pure antibacterial metabolites after the fermentation were screened for their antibacterial activity and compared with the crude and the positive control i.e., tetracycline. Acetone extracts showed maximum inhibitory activity as compared to other extracts similarly the methanol extract also express the activity but the extracts of nonpolar solvent doesn't possess effective results.

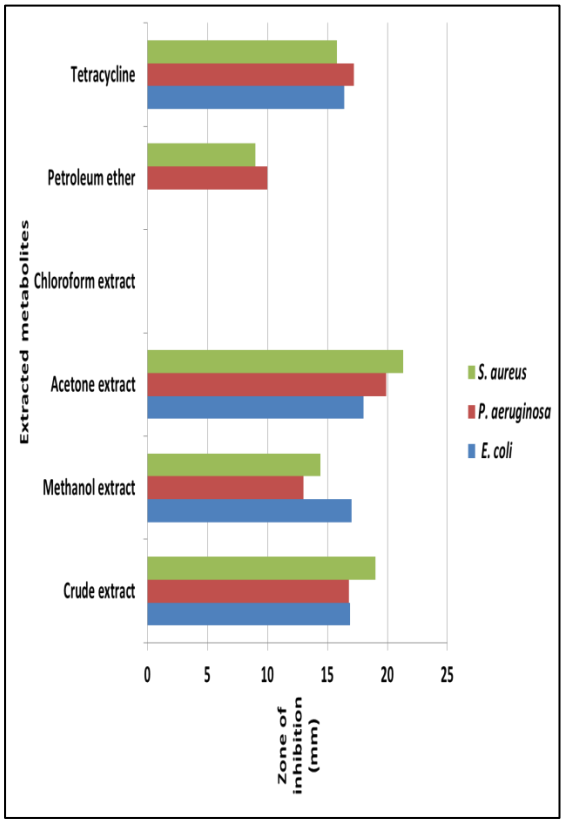

Figure 4: Graph expressing the activity of the extracted metabolites of crude and purified methanol, acetone, chloroform and petroleum ether extracts against the pathogens.

Conclusion:

The extractions from polar solvents are far better than nonpolar extraction for the selected culture AKSJ 11. Hence it can be concluded that the extracted active metabolites are polar in nature. Further the purification and identification of the metabolites will be done by using various chromatographic methods, spectrum and NMR analysis.

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