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Research Article

ISOLATION AND EVALUATION OF COW DUNG BACTERIA FOR THEIR ANTIMICROBIAL POTENTIAL

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ABSTRACT

Antibiotic resistance among human pathogens still remains a major concern for public health. The aim of this work is to investigate the bacterial isolates of cow dung for their antimicrobial properties. Twenty-six bacterial isolates were screened for their antimicrobial potential against 6 test organisms namely *Bacillus cereus* (MTCC 6728), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 7443), *Vibrio cholerae* (MTCC 3904), *Salmonella typhi* (MTCC 3216) and one clinical isolate *Escherichia coli* using cross-streak method. The preliminary screening revealed significant antimicrobial activity of isolates I4 and I5 against all the test bacterial strains. The morphological and biochemical investigations indicated that I4 and I5 belong to *Escherichia* sp. and *Citrobacter* sp. respectively.

Key words: Cow dung, Cross-streak method, Broad-spectrum activity, Antimicrobial metabolite.

INTRODUCTION

Bioactive compounds being produced by bacteria to compete with other organisms in their natural environment have played a major role in the development of antimicrobial drugs in the past few decades (Esikova *et al.*, 2002; Ilic *et al.*, 2007). Bacteria capable of producing bioactive compounds have been isolated from a range of different habitats like soil, marine environment and even from the gut of animals and insects. Today researchers around the world are continuing their effort to isolate bacteria producing antimicrobial metabolites against microbes of medical importance.

Overuse of antimicrobial drugs has resulted in the emergence and re-emergence of the infectious diseases caused by resistant microorganisms (Fleming, 1929; Schatz *et al.*, 1944; Kardos & Demain, 2011; Kaaria *et al.*, 2015; Ling *et al.*, 2015). The bacterial resistance is of great concern as it not only increase the substantial morbidity and

mortality but also the cost of treatment (Naiemi *et al.*, 2006; Sikarwar & Batra, 2011; Abo-State *et al.*, 2012; Aly *et al.*, 2012; Jeyasanta *et al.*, 2012; Ullah *et al.*, 2012; Desriac *et al.*, 2013; Sharif *et al.*, 2013). According to WHO, infectious diseases are the second most imported causes of death globally (WHO, 2014; Ravnikar *et al.*, 2015).

It has been suggested that novel microorganisms and their products continuously originate from poorly investigated sources like, soil, water, marine ecosystem and places like, Jordan, Antarctica and certian biotype of Manipur. (Saadoun & Gharaibeh, 2003; Nedialkova & Naidenova, 2005; Singh *et al*., 2009; Singh *et al*., 2014).

Cow dung can be considered as gold mine of microorganisms with over 60 different bacterial species and 100 species of protozoa and yeast such as *Citrobacter koseri, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Kluyvera* spp., *Morgarella morganii, Pasteurella* spp., *Providencia alcaligenes, Providencia stuartii, Pseudomonas* spp., *Nocardia, Mucor* and *Rhizopus* sp. have been identified from cow dung. (Nene, 1999; Sawant *et al*., 2007; Randhawa & Kullar, 2011). Cow dung has the ability to inhibit disease causing microorganisms, for example, antifungal agents inhibiting the growth of coprophilous fungi have successfully been extracted from cow dung (Muhammad & Amusa, 2003, Dhama *et al.*, 2005; Joseph & Sankarganesh, 2011; Dhama *et al.,* 2013). Solvent extract of cow dung has also been tested against *Candida* sp., *Escherichia coli, Pseudomonas* sp. and *Staphylococcus aureus* by Shrivastava *et al*. (2014) revealing the antimicrobial property of cow dung.

In view of this, the present study was undertaken to find potential bacterial isolate from cow dung having ability to produce bioactive compounds.

MATERIALS & METHODS

SAMPLE COLLECTION

Total 3 cow dung samples; two of desi and one of crossbreed were collected aseptically from 3 different locations of Haridwar. All the samples were labelled and transported to the microbiology laboratory, Department of Botany & Microbiology, Gurukul Kangri University for further processing. The dung samples were processed on same day.

ISOLATION OF BACTERIA

For isolation of bacteria, 1 g of fresh cow dung sample was added in a test tube containing 9 ml of distilled water and shaken well with vortex mixer for 2h. This solution was serving as stock culture. From stock culture a volume of 1ml was transferred aseptically in the tube containing 9ml of distilled water and mixed well. From this tube 1ml of aliquot was again transferred in another tube containing 9ml of distilled water to make 10^{-2} dilution factor. Similarly dilutions up to 10^{-8} were made using serial dilution technique for the cow dung samples. 0.1 ml of aliquot from each dilution was added on Beef Peptone Agar (BPA) plates and spreaded evenly with the help of L-shaped glass spreader for enumerating bacteria. Three replicates of each dilution were maintained and incubated at 37^0C for 24 h. After incubation, different bacterial colony were selected and streaked on BPA plates.

These colonies were purified by repeated streak plate method.

ANTIBACTERIAL ACTIVITY OF ISOLATES

All the cow dung isolates were screened for their antimicrobial potential against test bacteria namely *Bacillus cereus* (MTCC 6728), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 7443), *Vibrio cholerae* (MTCC 3904), *Salmonella typhi* (MTCC 3216) procured from IMTECH (Chandigarh) while *Escherichia coli* was collected from SGPGI, Lucknow using Cross-streak method (Kumari *et al.,* 2013). Each isolate was streaked in the centre of the BPA plate and incubated at 37^{o}C for 24 h. Then fresh sub-cultured test organisms were streaked perpendicular to the bacterial isolates and plates were incubated for 24h at 37^{0}C. After incubation, zone of inhibition (in mm) between bacterial strain and test organisms was measured and recorded.

CHARACTERISATION OF BACTERIAL ISOLATE

Bacterial isolates showing antibacterial activity were characterised by morphological and biochemical methods according to the criteria given in Bergeyôs Manual of Determinative Bacteriology (Holt *et al.*, 1994).

RESULTS

SAMPLING AND ISOLATION OF BACTERIA

In the present study, a total of 26 bacterial isolates from 3 cow dung samples were obtained (Table 1).

Table 1. Sample Collection sites and isolated bacteria

S.No.	Location	Cow Breed	Isolates
1	Avdoot Mandal Cow Sheed	Desi	I1,I2,I3,I4,I5,I6,I7,I8,I9
2	BHEL Sector-4	Cross-breed	I10, I11, I12, I13, I14,I15,I16,I17, I18
3	Cow Shed in Bahadrabad	Desi	I19, I20, I21, I22, I23,I24,I25,I26

Where, I is isolate

SCREENING OF BACTERIAL ISOLATES FOR ANTIBACTERIAL ACTIVITY

All the 26 isolates were screened for their antibacterial activity by cross-streak method against a panel of three Gram-negative (*V. cholerae, E. coli* and *S. typhi*) and three Gram-positive bacteria (*S. aureus, B. Cereus* and *B. subtilis*). Out of these, only 2 bacterial isolates i.e., I4 and I5 obtained from dung sample of desi cow showed antibacterial activity against all the test organisms. Isolates I4 and I5 demonstrated maximum zone of inhibition against *B. Cereus* and *B. Subtilis* respectively (Table 2).

Table 2. Zone inhibition (in mm) of bacterial isolate against test bacteria.

Isolates	*V. cholera* (MTCC 3904)	*S. typhi* (MTCC 3216)	*E. coli*	*S. aureus* (MTCC 7443)	*B. subtilis* (MTCC 441)	*B. cereus* (MTCC 6728)
I1	-	-	-	-	-	-
I2	-	-	-	-	-	-
I3	-	-	-	-	-	-
I4	3.0	4.0	3.0	3.0	4.0	6.0
I5	3.0	6.0	5.0	4.0	7.0	6.0
I6	-	-	-	-	-	-
I7	-	-	-	-	-	-

I8	-	-	-	-	-	-
I9	-	-	-	-	-	-
I10	-	-	-	-	-	-
I11	-	-	-	-	-	-
I12	-	-	-	-	-	-
I13	-	-	-	-	-	-
I14	-	-	-	-	-	-
I15	-	-	-	-	-	-
I16	-	-	-	-	-	-
I17	-	-	-	-	-	-
I18	-	-	-	-	-	-
I19	-	-	-	-	-	-
I20	-	-	-	-	-	-
I21	-	-	-	-	-	-
I22	-	-	-	-	-	-
I23	-	-	-	-	-	-
I24	-	-	-	-	-	-
I25	-	-	-	-	-	-
I26	-	-	-	-	-	-

CHARACTERISATION OF BACTERIAL ISOLATE

Both the isolate (I4 and I5) were found to be Gram-negative, rod shaped and motile as revealed by morphological examination. Biochemical examination showed that they were positive for carbohydrate fermentation and catalase while negative for getaline liquefaction and amylase production. However isolate I4 was citrate negative while isolate I5 was citrate positive. Result of biochemical and morphological test have been summarised in Table 3. By considering the above results it was preliminary identified that isolate I4 belongs to *Escherichia* sp. and isolate I5 belongs to *Citrobacter* sp.

Table 3. Morphology and biochemical characterization of the bacterial isolates.

Test/Isolates	I4	I5
Gram Reaction	Negative	Negative
Shape	Rod	Rod
Motility	Motile	Motile
Glucose Fermentation	+	+
Lactose Fermentation	+	+
Sucrose Fermentation	+	+
Gelatine Liquification	-	-
Starch Hydrolysis	-	-
Indole	+	+
Methyl-Red	+	+
Vogeus-Proskeur	-	-
Citrate Utilisation	-	+
Catalase Production	+	+

DISCUSSION

Screening of microorganisms from new or less exploited source can be considered as an effective way to find novel antimicrobial producers.

Cow dung can be considered as an inexhaustible resource for microbial diversity that has not been well exploited. (Hozzein *et al*., 2007; Hozzein *et al.*, 2011). Microorganisms that reside as normal microbiota in animal gut protect the host from over colonisation by producing secondary metabolites which inhibits the settlement of other bacteria.

Cow dung can also be explored as a source of potential antimicrobial producers because of its microbial diversity (Sawant *et al*., 2007). Cow dung has been used from ancient times in ayurvedic treatments, used for biogas production and increasing crop productivity.

The present study was carried out to evaluate the ability of cow dung microflora for the production of antimicrobial metabolite. In this study, 26 isolates were evaluated for their antimicrobial potential and two isolates (I4 and I5) showed broad spectrum antimicrobial activity against all the indicator organisms. Until now, only few researchers demonstrated that cow dung

microflora exhibit antimicrobial activity. Teo & Teoh, 2011 reported the antimicrobial activity of one cow dung isolate against *E. coli*. In the present study, isolate I4 and I5 belonging to *Escherichia* sp. and *Citrobacter* sp. could be a promising antimicrobial producer as both predominantly showed the activity against *V. cholerae, S. typhi, E. coli, B.cereus, B. subtilis and S.aureus.* Thus, both the isolate can be up-hold to industrial level and produced antimicrobial agent should be further analyzed for its possibility to be used as therapeutic agent.

CONCLUSIONS

Escherichia sp. and *Citrobacter* sp. isolated from cow dung have shown broad spectrum activity against both the Gram positive and Gram negative bacteria establishing that microbial diversity related to this underexplored source have a great potential to produce novel bioactive compounds enabling the discovery of new drugs. Isolates can be further identified by phylogenetic methods and antimicrobial agent can be investigated for their possible applications in the management of human diseases.

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