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Research Article

ANTIMICROBIAL ACTIVITY OF CERTAIN BACTERIAL ISOLATES – A SCREENING STUDY

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ABSTRACT

The aim of this work is to investigate the bacterial isolates of cow dung for the production of bioactive compounds. Eleven 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 cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216) and *Escherichia coli* using cross-streak method. The preliminary screening revealed significant antimicrobial activity of isolates I1, I2, I3, I4, I6, I7, I10 & I11 against all the test bacterial strains.I7, I10 & I11 showed broad spectrum antimicrobial activity against test organisms.

Keywords: cow dung, resistance, cross-streak, antimicrobial potential

INTRODUCTION

Bacteria synthesizing secondary metabolites of various structures and chemical compounds possess antibacterial and antifungal activity (Kleinkauf and Von Döhren, 1990; Esikova *et al.*, 2002). These secondary metabolites represent an opulent source (Berdy, 2005; Kumari *et al.*, 2013) and played a key role in the discovery and development of many antibiotics. About 4000 antibiotics discovered and approved for marketing nowadays are of microbial origin (Ilic *et al.*, 2007; Harvey, 2008; Butler *et al.*, 2014).

Since the discovery of antibiotics, resistance of microorganisms towards them is continuing to evolve and this problem is further aggravated by the increasing use and misuse of the antimicrobial agents which pose serious public health problems (Maataoui *et al.*, 2014; Balachandran *et al.*, 2015). Due to the development of resistance (Esikova *et al.*, 2002) there is an alarming scarcity of new antibiotics (Singh *et al.*, 2014). Thus screening microorganisms from unexplored habitats is one of the promising ways to isolate a candidate with new antimicrobial properties (Watve *et al.*, 2001; Zitouni *et al.*, 2005; Hozzein *et al.*, 2011; Khanna *et al.*, 2011; Wadetwar and Patil, 2013; Maataoui *et al.*, 2014).

Livestock sustain the livelihood of millions of people in the world in both

developing and developed countries (Morgavi *et al.*, 2010; Adeniyi *et al.*, 2015). Cow dung, also known as cow chips, cow pit or cow pie (Waziri and Suleiman, 2013) is defined as the waste which contains undigested residue of consumed food passed through the gastrointestinal system of cow. Its colour ranges from greenish to black. Cow dung has been broadly studied for its use as organic agriculture fertiliser and as a source for alternative fuel such as biogas for generation of electricity and heat. Cow dung has also been studied for its bioremedial properties (Abdulkareem, 2005; Teo and Teoh, 2011; Waziri and Suleiman, 2013). However, there is lack of research on the antimicrobial potential of cow dung bacteria (Yokoyama *et al.*, 2007; Teo and Teoh, 2011).

Therefore, the objective of present study is to evaluate the antimicrobial potential of cow dung bacteria.

MATERIALS AND METHODS

Sample collection

Dung sample of American cow breed was collected from the campus of Gurukula Kangri Vishwavidyalaya, Haridwar. The dung sample was collected aseptically and analysed immediately after transporting to the laboratory.

Isolation of bacteria

Bacteria were isolated by serial dilution method (Hayakawa, 2008). Stock solution was prepared by diluting 1g of cow dung in 9ml of sterile saline water and shaking well by using a vortex mixer. From the stock solution, 1ml was used to prepare the final volume of 10^{-2} to 10^{-8} by serial dilution method. Samples were inoculated on Beef Peptone Agar (BPA) media. Plates were incubated at 37ºC for 24h. After incubation, different bacterial colony were selected and streaked on BPA plates. Until further use, the slants were kept in cold room at 4°C (Das *et al.*, 2010; Mohseni *et al.*, 2013).

Antimicrobial activity by cross-streak method

Isolated bacterial strains were inoculated onto BPA plates by streak in the centre and incubated at 37ºC for 24h. Six bacteria i.e., *Bacillus cereus* (MTCC 6728), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 7443), *Vibrio cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216) and *Escherichia coli* were used as test organisms. A pure culture of test bacteria was transferred into fresh nutrient broth and incubated at 37°C for 24h. The test bacterial suspension was streaked perpendicular to the cow dung bacterial isolate on the BPA medium. The plates were incubated at 37°C for 24 hours. The microbial inhibitions were observed by determining the diameter of the inhibition zone (Mohseni *et al.*, 2013).

RESULT

Sampling and isolation of bacteria

In the present study, total of 11 isolates namely I1, I2, I3, I4, I5, I6, I7, I8, I9, I10, and I11 were obtained from the cow dung sample.

Screening of bacterial isolates for antibacterial activity

All the 11 isolates were screened for their antibacterial activity by cross-streak method against a panel of three Gram-negative (*Vibrio cholera* MTCC 3904), *Salmonella typhi* (MTCC 3216) and *Escherichia coli*) and three Gram-positive bacteria (*Bacillus cereus* MTCC 6728), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* MTCC 7443). Out of these, 8 bacterial isolates showed antibacterial activity against the test organisms. However, 3 isolates were able to show inhibition against all the test

organisms. Isolate I10 demonstrated the maximum inhibition zone of 13mm,10mm and 10 mm against *E. coli, V. Cholerae* (MTCC 3904) and *B. Subtilis* (MTCC 441) respectively (Table 1).

Table 1. Zone of inhibition (in mm) of bacterial isolate against test bacteria.

Isolates/ Test organisms	*V. cholera* (MTCC 3904)	*S. typhi* (MTCC 3216)	*E. coli*	*S. aureus* (MTCC 7443)	*B. subtilis* (MTCC 441)	*B. cereus* (MTCC 6728)
I1	**3.0**	**-**	**3.0**	**4.0**	**3.0**	**3.0**
I2	**3.0**	**-**	**-**	**6.0**	**4.0**	**-**
I3	**-**	**-**	**-**	**2.0**	**3.0**	**-**
I4	**1.0**	**-**	**-**	**-**	**-**	**-**
I5	**-**	**-**	**-**	**-**	**-**	**-**
I6	**7.0**	**-**	**1.0**	**9.0**	**9.0**	**6.0**
I7	**1.0**	**5.0**	**5.0**	**3.0**	**5.0**	**7.0**
I8	**-**	**-**	**-**	**-**	**-**	**-**
I9	**-**	**-**	**-**	**-**	**-**	**-**
I10	**10.0**	**9.0**	**13.0**	**7.0**	**10.0**	**8.0**
I11	**5.0**	**3.0**	**5.0**	**7.0**	**1.0**	**1.0**

Discussion

The incidence of multidrug resistant organisms is increasing which ultimately affect the treatment of infectious diseases. Consequently, there is an urgent need for developing new drugs which are effective against current antibiotic resistant pathogens. Cow dung contains abundant number of bacilli, lactobacilli and cocci and some identified and unidentified fungi and yeasts (Muhammad and Amusa, 2003). Various microorganisms including *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bacillus subtilis, Enterococcus diacetylactis*, etc. have been identified from the lower part of the gut of the cow (Ware *et al.*,1988).Fresh cow dung has antifungal and antiseptic properties (Nene, 1999), which might be due to secretion of antimicrobial metabolites by cow dung microflora. Cow dung also inhibited postharvest rot pathogens of yams (Naskar *et al.*, 2003). Antagonistic activity of cow dung microflora has also been reported against *Fusarium oxysporum*, probably due to the production of antifungal metabolites (Swain and Ray, 2009).The present study was carried out to evaluate the ability of cow dung microflora for the production of antimicrobial metabolite. In this study, 8 isolates showed antimicrobial activity against all the indicator organisms. Out of these 8 isolates, 3 isolates (I7, I10 & I11) showed the broad spectrum antimicrobial activity suggesting that cow dung microflora can be explored for its therapeutic effect and for development of new antimicrobial agents.

Conclusion

The present study shows that bacterial strains isolated from cow dung possess ability to produce antimicrobial metabolite against bacteria of medical importance. Therefore, the intensive efforts must be initiated for screening of cow dung as 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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