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Bioactivity of *Alcaligenes* spp. isolated from cow dung against certain human pathogens

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Abstract

Present work is focused on the antagonistic potential of *Alcaligenes* strains isolated from cow dung against certain human pathogens. Seven bacterial strains were isolated from cow dung sample by serial dilution method. Based on morphological and biochemical investigations isolate, KD109 and KD110 were probably identified as *Alcaligenes fecalis* and *Alcaligenes latus* and screened for their antagonistic activity against 14 test organisms using cross-streak method. Both the isolates *Alcaligenes fecalis* and *Alcaligenes latus* demonstrated broad-spectrum antimicrobial activity against test organisms with maximum inhibition (10 mm) showed by KD109 against *Salmonella typhi* (MTCC 3216). The study indicates the potential of *Alcaligenes spp*. in the control of certain human pathogens.

Key words: Alcaligenes* spp.*, Cow dung, Cross-streak method, Antimicrobial activity, Human Pathogens

Introduction

Secondary metabolites are produced by the microorganisms to ensure their survival in a competing environment. These naturally produced chemical compounds have played a key role in the development of antimicrobial drugs (Kleinkauf and Dohren, 1990; Esikova *et al.*, 2002; Ilic *et al.*, 2007; Gupta and Rana 2016a) with many drugs present in the market now days are of microbial origin (Ilic *et al.*, 2007; Harvey 2008; Butler *et al.*, 2014; Gupta and Rana 2016b). Different habitat have been explored, for instance, *Lactobacillus* strains isolated from camel milk showed antibacterial activity against *Bacillus cereus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Salmonella typhimurium* Abbas and Mahasneh (2014), *Bacillus lentus, Micrococcus roseus, Enterobacter aerogenes, Bacillus pumilus, Bacillus alvei* were isolated from the soil showed bioactivity against *Shigella* spp., *Staphylococcus aureus, Pseudomonas* spp., *Proteus* spp. Abdulkadir and Waliyu (2012), Marine microorganisms such as *Brevibacterium* sp., *Moraxella* sp. were also found effective against *Bacillus subtilis* and *Staphylococcus aureus* (Al-Zereini, 2014), *Enterococcus faecium* isolated from fermented Spanish sausage was found effective against *Listeria innocua* BL86/26 (Aymerich *et al.*, 1996), Sponge associated symbiotic bacterias were also explored, *Micrococcus luteus,* a symbiont to *Xestospongia testudinaria* was found effective against *Bacillus subtilis, Escherichia coli* and *Klebsiella pneumonia* (Cita *et al.*, 2016). But in spite of that, even at present, pace of approved antimicrobial agents against the rate of resistance is lagging, hence emergence of drug resistant bacteria is an alarming situation worldwide (Pelaez, 2006; Fischbach and Walsh, 2009; Bhatta and Kapadnis, 2010; Dandekar and Dandekar, 2010; Eggleston *et al.*, 2010; Donadio *et al.*, 2010; Davies and Davies, 2010; Ascencio *et al.*, 2014; Kapley *et al.*, 2016). Researchers around the world are continuing their effort to isolate bacteria capable of producing bioactive compounds from a range of different habitats like soil, marine environment and even from the gut of animals and insects (Gupta and Rana, 2016a). Cow dung, is the waste generated after the digestion of consumed food material, harbour wide variety of microorganisms (Nene, 1999; Morgavi *et al.*, 2010; Randhawa and Kullar, 2011; Waziri and Suleiman, 2013; Adeniyi *et al.*, 2015). Antifungal substances isolated from cow

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dung inhibits the growth of coprophilous fungi (Dhama *et al.,* 2005; Joseph and Sankarganesh, 2011; Dhama *et al.,* 2013). *Eupenicillium bovifimosum,* that produces patulodine-like compounds viz. CK2108A and CK2801B was also isolated from cow dung (Dorothy and Frisvad 2002; Lehr *et al.,* 2005. *Enterococcus faecalis* V24, isolated form cow dung, produces a heat stable, largely hydrophobic antimicrobial substance possessing antimicrobial activity against pathogenic Gram-negative bacteria (Laukova *et al.,* 1998; Teo and Teoh, 2011) isolate a strain from cow dung i.e. K4 showing antibacterial activity against *E. coli*. (Shrivastava *et al.,* 2014) evaluated water, ethanol and n-Hexane extract of whole cow dung against *Candida* sp., *E. coli*, *Pseudomonas* and *Staphylococcus aureus* revealing their antimicrobial properties.

Alcaligenes spp. have also been evaluated for their antimicrobial properties against *Staphylococcus aureus Staphylococcus epidermidis*, *Bacillus subtilis*, *Salmonella enteritidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Kamigiri *et al.,* 1996). However, *Alcaligenes* spp. from cow dung exhibiting bioactivity has not been reported. Hence, the present study was undertaken to explore cow dung as a source for the isolation of *Alcaligenes* spp. exhibiting antimicrobial properties.

Material and Methods

Sample collection: Dung sample of desi cow breed (Krishna) was collected in sterile container from Saharanpur, transported to laboratory and stored at. 4°C for further studies Gupta and Rana (2016a).

Isolation of Bacterial Species: Bacteria were isolated on nutrient agar medium (NAM) by standard serial dilution method. Stock solution was prepared by mixing 1g of cow dung sample with 9ml of normal saline. The stock solution was diluted up to 10^{-8}. 100 µl of solution from each dilution was spread on nutrient agar medium followed by incubation in upright position at 37°C for 24h (Das, 2010; Gupta *et al.,* 2016; Gupta and Rana, 2016a; Gupta and Rana, 2016b).

Morphological characterization of *Alcaligenes* spp: Morphological characterization of bacteria was done in accordance with standard Gram-staining technique (Beveridge, 2001; Gupta *et al.,* 2016).

Biochemical characterization of *Alcaligenes* spp.: All the isolates were subjected for carbohydrate fermentation test, gelatine liquefaction, starch hydrolysis, citrate utilisation, catalase production, indole and MR-VP for biochemical characterization as described by Holt *et al.,* (1994) and Das (2011).

Bioactivity of *Alcaligenes* spp. by cross-streak method: Cross streak method Mohseni *et al.,* (2013) was used to evaluate the bioactivity of *Alcaligenes* spp. against 14 test organisms i.e., *Vibrio cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 6728), *Proteus vulgaris* (MTCC 426), *Enterococcus faecalis* (MTCC 439), *Pseudomonas aeruginosa* (MTCC 424), *Escherichria coli* (MTCC 118), *Shigella flexneri* (MTCC 1457), *Salmonella typhimurium* (MTCC 3231), *Streptococcus pyogenes* (MTCC 442), *Staphylococcus aureus* (MTCC 3160) and *Escherichia coli*. *Alcaligenes fecalis* and *Alcaligenes latus* were streaked onto NAM plates as a single streak in the centre and incubated at 37°C for 24h. The test bacterial strains were streaked perpendicular to the isolates on the NAM plates and incubated further at 37°C for 24 hours. The microbial inhibitions were observed by determining the distance of the inhibition zone between bacterial strain and test organisms.

Results and Discussion

Isolation of bacterial species: In the present study, cow dung samples of desi cow breed was collected from a cow shed in Saharanpur (India) located at latitude of (29°5'04 N) and longitude of (77° 33' 04 E). Bacteria were isolated from dung sample by serial dilution method and total of 7 isolates namely KD104, KD105, KD106, KD107, KD108, KD109, KD110 were obtained.

Morphological and Biochemical characterization of *Alcaligenes* spp.:Morphological characterization of seven isolates revealed that isolate KD109 & KD110 were Gram-negative rods while isolate

KD104 and KD106 are Gram-positive cocci and KD105, KD107 and KD108 were Gram-positive rod. Hence both the isolates (KD109 & KD110) were subjected for biochemical characterisation for probable identification of *Alcaligenes* spp. Isolate KD109 was not able to utilise glucose, lactose and sucrose, while KD110 was found to be positive for glucose utilisation and gelatine hydrolysation. Therefore, both the isolates i.e., KD109 and KD110 were identified as *A. fecalis* and *A. Latus* respectively (Holt *et al.,* 1994; Das, 2011). Detailed result of morphological and biochemical test are summarised in Table 1.

Table 1. Morphological and biochemical analysis of the bacterial isolates.

Test/Isolates	KD109	KD110
Gram Reaction	-	-
Shape	Rod	Rod
Glucose Fermentation	-	+
Lactose Fermentation	-	+
Sucrose Fermentation	-	-
Manitol Fermentation	+	-
Gelatine Liquification	+	+
Starch Hydrolysis	+	-
Indole	-	-
Methyl-Red	+	+
Vogeus-Proskeur	-	-
Citrate Utilisation	-	-
Catalase Production	+	+

Bioactivity of *Alcaligenes* spp. by cross-streak method: Out of the seven isolated strain, both the strains i.e. *Alcaligenes fecalis* and *Alcaligenes latus* were subjected to antimicrobial activity by cross-streak method against a panel of 14 test organisms having medical importance. Both the isolated strains *Alcaligenes fecalis* and *Alcaligenes latus* showed antibacterial activity against most of the test organisms. An important observation was recorded when *Alcaligenes fecalis* (KD109) inhibited around 50% of the test organisms. Maximum inhibition (10 mm) by KD109 was recorded against *Salmonella typhi* while minimum (6 mm) was recorded against *Escherichia coli* and *Bacillus subtilis* (MTCC 441). Antimicrobial activity of *A. latus* (KD110) was recorded against two gram-negative (*Salmonella typhi and Escherichia coli*) and one gram-positive (*Bacillus cereus*) bacteria (Table 2). *Alcaligenes fecalis* showed maximum inhibition (10 mm) against *Salmonella typhi* which is the best described cause of typhoid fever (TF) and paratyphoid fever (Hsiao *et al.,* 2016) and minimum inhibition (6 mm) against *Bacillus subtilis* and *Escherichia coli. Escherichia coli* is one of the major medically important organisms that cause urinary tract infection as well as food poisoning and diarrhoea (Alteri *et al.*, 2009; Teo and Teoh, 2011). Both isolates were active against Gram-positive and Gram-negative test organisms, thereby indicating the secretion of broad spectrum antimicrobial agents. The difference in the sensitivity of Gram positive and Gram negative bacteria against *Alcaligenes* spp. may be due to the morphological differences in their outer membrane (Gebreyohannes *et al.,* 2013). KD109 showed better result than KD110 for the reason that it inhibited more number of test organism to a greater extent (Zahir *et al.,* 2013) isolate *Alcaligenes* spp. from tannery waste and reported antimicrobial activity of ethyl acetate extract against *Escherichia coli, Bacillus subtilis*

Table 2. Inhibitory activity spectra of *Alcaligenes* spp. against test bacteria.

Isolates/ Test organisms	*V. cholera*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*B. cereus*	*P. vulgaris*	*E. fecalis*	*P. aeruginosa*	*E. coli*	*S. fexneri*	*S. typhimurium*	*S. pyogenes*	*S. aureus*
KD109	7mm	10mm	6mm	8mm	6mm	7mm	-	-	-	-	-	-	-	-
KD110	-	5mm	5mm	-	-	5mm	-	-	-	-	-	-	-	-

and *Staphylococcus aureus* with inhibition zone of 18mm, 16mm and 16mm respectively which is greater in comparison to the present study. This difference in activity may be due to the reason that crude extract were used by Zahir *et al.,* (2013). Although antimicrobial activity of *Alcaligenes* spp. has been reported earlier against many organisms such as *Bacillus subtilis* (Li *et al.,* 2007), *Staphylococcus aureus* (Li *et al.,* 2008), *Pseudomonas aeruginosa*, *Mycobacterium avium*, *Mycobacterium tuberculosis* (Basic *et al.,* 2001), *Mycobacterium smegmatis*, *Pseudomonas aeruginosa* (Zahir *et al.* 2013), *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 10876, *Shigella flexneri* ATCC 9199, *Enterobacter* (MDR strain) and *Serratia* sp. GMX1 (MDR strain) (Kapley *et al.,* 2016). However, *Alcaligenes* spp. showing inhibition against *Salmonella typhi* is not well reported in the literature. Other authors showed that this species also produced compounds that can be of industrial use in the production of D-aminocyclases, semi-synthetic antibiotics (penicillin, cephalosporin, B-1015), hormones (lutein), bioactive peptides with immunostimulatory activities (cyclo-(l-Pro-Gly)5) and chemical pesticides (Isono *et al.,* 1993; Tripathi *et al*., 2000); Liaw *et al.,* 2003; Gayen *et al.,* 2007; Wang *et al.,* 2011). Besides, strains of *Alcaligenes faecalis* type N.C.T.C. 8764 and A.T.C.C. 9220 were antagonistic against *Escherichia* sp. (Puah *et al.,* 2016). Antibiotic resistance among clinical pathogens i.e. *Salmonella typhi* , *Vibrio cholerae, Staphylococcus aureus* , *Escherichia coli* and *Bacillus cereus* has been reported recently against not only 1st and 2nd generation antibiotic but also against 4th generation of antibiotics; (Mare and Coetzee, 1964; Alteri *et al.,* 2009; Puah *et al.,* 2016; Uppal *et al.,* 2017). Taking into consideration of these facts, the results of present study indicates that the isolated strains *Alcaligenes fecalis* (KD109*)* and *Alcaligenes latus* (KD110) from cow dung have the potential of producing antimicrobial substances and therefore might be explored for their effectiveness against the bacteria of medical importance.

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