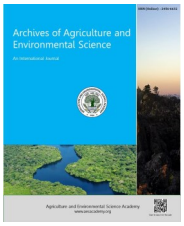
Archives of Agriculture and Environmental Science 2(4): 353-356 (2017)
https://doi.org/10.26832/24566632.2017.020419

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: www.aesacademy.org



ORIGINAL RESEARCH ARTICLE

Evaluation of antagonistic activities of *Bacillus* spp. against certain bacteria of medical importance

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ARTICLE HISTORY

Received: 25 September 2017
Revised received: 28 October 2017
Accepted: 15 November 2017

Keywords

Antagonistic activity
Bacillus spp.
Cross-streak method
Cow dung

ABSTRACT

In this work we focused on the antagonistic potential of *Bacillus* spp. isolates from cow dung. Out of fourteen bacterial strains, isolate KD104 and KD117 were probably identified as *Bacillus* spp. These two isolates were screened for their antagonistic activity against 14 test organisms *viz., Vibrio Cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI), *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*), Streptoccus pyogenes* (MTCC 442) and *Staphylocccus aureus* (MTCC 3160) using cross-streak method. The preliminary screening revealed significant antimicrobial effect of both isolates against *Vibrio Cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI), and *Bacillus cereus* (MTCC 6728). Therefore, this study indicates that these *Bacillus* species may be up-hold to industrial level for production of antimicrobial agent, which should be further analyzed for its possibility to be used as therapeutic agent.

Citation of this article: Gupta, K.K. and Rana, D. (2017). Evaluation of antagonistic activities of *Bacillus* spp. against certain bacteria of medical importance. *Archives of Agriculture and Environmental Science*, 2(4): 353-356, DOI: 10.26832/24566632.2017.020419

INTRODUCTION

Control of disease causing organisms through the use of natural antagonistics has emerged as a promising alternative in the field of medical science and secondary metabolites from microorganisms are most promising in this context (Mohseni *et al.,* 2013; Amin *et al.,* 2015). Bacterial secondary metabolites are one of the adaptation mechanisms, which give advantage in competition for available nutrients and living space. Secondary metabolites are highly diverse in relation with structure and only some species can produce them (Karlovsky, 2008). They also serve as competitive weapons against other organisms, as metal transporting agents, as sexual hormones and as differentiation effectors (Demain and Fang, 2000). These metabolites have various applications in human activities, such as medicine, agriculture, pharmaceuticals, food processing, chemical industries and many others (Alekseevna *et al.,* 2012).

Bacillus species from family *Bacillacese* is one of the major bacteria having potential to produce secondary metabolite. They are gram-positive, endospore forming, chemohetrotropic rods usually motile with peritrichous flagella, aerobic and catalase positive (Waites *et al.,* 2008). Endospores produced by *Bacillus* species are highly resistant to unfavourable environment conditions (Claus and Berkeley, 1986). Different secondary metabolites such as antibiotics, antifungals and siderophores are produced by these bacteria (Sansinenea and Ortiz, 2011). Antibiotics like bacitracin, pumulin and gramicidin are one of the major secondary metabolites produced by *Bacillus* species (Waites *et al.,* 2008). Many gram positive organisms such as *Staphylococci, Streptococci,* Anaerobic *cocci, Corneybacter* and *Clostridia* were inhibited by Bacitracin produced from *Bacillus licheniformis* (Mc Evoy, 1993; Biswas *et al.,* 2016). Gramicidin is produced by *Bacillus brevis*, which is a linear polypeptide antibiotic mixture of gramicidin A, B, C and D (Waites *et al.,* 2008; Abdulkadir and Waliyu, 2012). Iturins are antifungal antibiotics produced by *Bacillus subtilis* (Peypoux *et al.,* 1986; Eshita *et al.,* 1995; Tamehiro *et al.,* 2002). *Bacillus* species such as *B. subtilis* and *B. amyloliquefaciens* may dedicate up to 8% of their genetic equipment to the synthesis of a wide array of antimicrobial compounds (Chen *et al.,* 2009; Rückert *et al.,* 2011; Cawoy *et al.,* 2014). Keeping in view of this perspective, the objective of present study was to isolate *Bacillus* species from cow dung having ability to produce bioactive compounds.

MATERIALS AND METHODS

Collection of sample: Dung sample of Desi cow breed was collected aseptically from cow shed located in Saharanpur, Uttar Pradesh. The sample was analysed immediately after transporting to the laboratory (Gupta and Rana, 2016a).

Isolation of *Bacillus* spp.: 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 homogenise by using a vortex mixer. From the stock solution, dilutions up to 10^{-8} were made and inoculated on Nutrient Agar media (NAM). Plates were incubated at 37°C for 24h. After incubation, assumed bacteria were gram stained. The Gram-positive, rod shaped bacteria were selected. Until further use, the slants were kept at 4°C (Das *et al.,* 2010; Mohseni *et al.,* 2013; Amin *et al.,* 2015; Gupta and Rana, 2016b).

Biochemical investigation: Subsequent identification test such as carbohydrate fermentation, gelatine liquification, starch hydrolysis, Indole, MR-VP, citrate utilisation & catalyse production of selected Bacterial strains was performed according to the criteria given in Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

Antagonistic activities of *Bacillus* spp. by cross-streak method: Isolated *Bacillus* species were evaluated for their antagonistic activity by cross-streak method against 14 test organisms i.e., *Vibrio Cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI), *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*), Streptoccus pyogenes* (MTCC 442) and *Staphylocccus aureus* (MTCC 3160). *Bacillus* spp. were streaked onto NAM plates as a single streak in the centre and incubated at 37°C for 24h.Then test bacterial strains were streaked perpendicular to the isolates on the NAM plates which were 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 (Mohseni *et al.,* 2013).

RESULTS AND DISCUSSION

Sampling and isolation of bacteria: In the present study, total of 14 isolates namely KD104, KD105, KD105, KD107, KD108, KD109, KD110, KD111, KD112, KD113, KD114, KD115, KD116 and KD117 were obtained from the dung samples of two desi cows. Out of these 14 isolates two isolates namely KD104 & KD117 were found to be Gram-positive rods (Table 1).

Biochemical investigation: Biochemical identification of KD104 & KD117 showed that they were positive for carbohydrate fermentation and catalase while negative for amylase production and citrate utilisation. However isolate KD104 was gelatine positive and isolate KD117 was gelatine negative. Result of biochemical and morphological test are summarised in Table 2.

Screening of bacterial isolates for antagonistic activity: Identified *Bacillus* spp. were screened for their antibacterial activity by cross-streak method against a panel of 14 test organisms such as *Vibrio Cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI), *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*), Streptoccus pyogenes* (MTCC 442) and *Staphylocccus aureus* (MTCC 3160). Both the isolates showed the antibacterial activity against at least one gram-positive and one gram-negative bacterium. Out of 14 test organisms isolate KD104 inhibit 6 test organisms namely *Vibrio Cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI), *Bacillus cereus* (MTCC 6728), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 7443) with inhibition zone of 4mm, 3mm, 5mm, 3mm, 4mm and 2mm respectively and isolate KD117 inhibit 4 test organisms namely *Vibrio Cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI) and *Bacillus cereus* (MTCC 6728) with inhibition zone of 3mm, 5mm, 5mm, 5mm respectively (Table 3).

In search for new antibiotics, screening of microorganism from relatively new sources is an important practice now-a-days. Antibiotic is produced in idophase and may represent a survival mechanism where organisms can eliminate competition and colonize a niche (Jensen and Wright, 1997; Hanlon and Hodges, 1998; Abdulkadir and Waliyu, 2012; Amin *et al.,* 2015). Screening for new antibiotics from natural sources like, soil, water, marine ecosystem and places like, Jordan, Antarctica and certain biotype (Saadoun and Gharaibeh, 2003; Nedialkova and Naidenova, 2005; Singh *et al.,* 2009; Singh *et al.,* 2014; Gupta and Rana, 2016b) is becoming increasingly important for the Biomedical industry (Schmidt, 2004; Amin *et al.,* 2015), as disease causing bacteria are significantly developing resistant towards generally used curative agents (Coates *et al.,* 2002; Amin *et al.,* 2015). *Bacillus Lentus* and *Bacillus Alvei* isolated form soil shows antibacterial activity against *S. aureus* while *Bacillus Pumillus* only shows slight zone of inhibition on *Proteus* spp. (Abdulkadir and Waliyu, 2012). Al-Ajlani and Hasnain, 2010, also demonstrated the antagonistic activity of 54 *Bacillus* strains isolated from soil sample (Amin *et al.,* 2015). Bacitracin which is produced by *Bacillus* sp. inhibits *E. coli* and *S. aureus* (Prescott *et al.,* 2008). The present study was carried out to evaluate the antagonistic effect of *Bacillus* species isolated from cow dung. The obtained results showed that two isolated strains (KD104 & KD117) have the potential for producing antimicrobial substances which is active against certain disease causing bacteria. The above presented data also confirmed the results of our study. But according to some reports *Bacillus* strains are most active against Gram-positive bacteria than Gram-negative bacteria (Oscariz *et al.,* 1999; Aslim *et al.,* 2002; Amin *et al.,* 2015). But in our study we found that the isolated *Bacillus* spp. is active against both gram-positive and gram-negative bacteria.

Table 1. Description of sample collection sites and isolated bacteria.

S.N.	Location	Cow breed	Isolates
1	Saharanpur (Uttar Pradesh)	Desi (Khilari)	KD104, KD105, KD105, KD107, KD108, KD109 and KD110,
2	Saharanpur (Uttar Pradesh)	Desi (Gaolao)	KD111, KD112, KD113, KD114, KD115, KD116 and KD117

Table 2. Morphology and biochemical characterization of the bacterial isolates.

Test/Isolates	KD104	KD117
Motility	Motile	Motile
Glucose Fermentation	+	+
Lactose Fermentation	+	-
Sucrose Fermentation	+	+
Gelatine Liquification	+	-
Starch Hydrolysis	-	-
Indole	+	+
Methyl-Red	+	+
Vogeus-Proskeur	-	-
Citrate Utilisation	-	-
Catalase Production	+	+

Table 3. Zone inhibition (in mm) of bacterial isolate against test organisms.

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*
KD104	4mm	3mm	5mm	2mm	4mm	3mm	-	-	-	-	-	-	-	-
KD117	3mm	5mm	5mm	-	-	5mm	-	-	-	-	-	-	-	-

Conclusions

The paper describes antagonistic potential of *Bacillus* sp isolated from cow dung samples. Results indicated significant antimicrobial effect of both isolates against *Vibrio Cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI), and *Bacillus cereus* (MTCC 6728) and concluded that isolated *Bacillus* spp. were able to demonstrate broad spectrum activity against both the Gram-positive and Gram-negative test organisms. Further isolates can be identified by phylogenetic methods and purification of active metabolites from these isolates is also needed.

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