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Fermenation Products of β-Mannanase Producing Bacteria Inhibit Selected Poultry Borne Pathogens

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Authors' contributions

This work was carried out in collaboration between both authors. Author OOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author DJA managed the analyses of the study and the literature searches. The both authors read and approved the first manuscript.

Research Article

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ABSTRACT

Aim: The study evaluated the inhibitory effect of fermentation products of β-mannanase-producing bacteria on selected poultry borne pathogens.
Study Design: The first experiment, bacterial isolates previously confirmed positive for mannanase by plate assay technique were further screened for mannanase production in submerged state fermentation. In the second experiment, inhibitory effect of fermentation products of mannanase-producing bacteria on selected poultry pathogens was evaluated.
Place and Duration of Study: Microbiology Research Laboratory, Federal University of Technology, Akure Nigeria between September 2011 and March 2012.
Methodology: Bacterial isolates from agricultural wastes previously confirmed positive for mannanase activity by plate assay were further screened for their potential performance under submerged state fermentation and enzyme activity determined by dinitrosalicylic acid method. The inhibitory action of β-mannanase-producing bacteria was determined by supplementation of supernatant and plating method.
Results: Isolate 1A showed highest mannanase activity (13.430 U/ml), displayed broad inhibition to selected poultry borne pathogens; *Klebsiella oxytoca*, *Shigella alkalescens*, *Escherichia coli, Salmonella typhii, Staphylococcus aureus* and *Streptococcus* sp. Apart from isolate 1A, fermentation products of other isolates generated from the mannolytic action of β-mannanase on mannan containing substrate displayed different percentage

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inhibition on selected poultry borne pathogens.
Conclusion: The results suggested that fermentation products from β-mannanase-producing bacteria might possess antibacterial properties which could be applied in poultry farms.

Keywords: β-mannanase; pathogens; percentage inhibition; fermentation products.

1. INTRODUCTION

Endo-β-1,4-mannanases (EC.3.2.1.78) randomly hydrolyze the main chain of hetero mannans, the major softwood hemicellulose [1]. Mannanases have been tested in several industrial processes, such as extraction of vegetable oils from leguminous seeds, viscosity reduction of extracts during the manufacture of instant coffee and manufacture of oligosaccharide [2,3,4] as well as applications in the textile industry [1]. In paper industry, mannanases have synergistic action in the biobleaching of the wood pulp, significantly reducing the amount of chemicals used [5,6].

The growing interest in mannanase production for industrial applications is due to its importance in the bioconversion of agro-industrial residues. Various mannanases from fungi, yeasts and bacteria as well as from germinating seeds of terrestrial plants have been produced [7,8,9,10,11]. Production of β-mannanase by microorganisms is more promising due to its low cost, high production rate and readily controlled conditions [4]. Mannanases could be used in prebiotic preparation which is expected to improve the growth performance of animal. Manno-oligosaccharides, product of gaur gum galactomannan by β-mannanase was found to be a substrate which could prevent the colonization of *Escherichia coli* and *Salmonella* sp, leading to an improvement of animal growth performance [12]. Antibiotics have been utilized in the farm environment as therapeutic agents and growth promoters for over 50 years [13]. But the emergence of multidrug-resistant pathogens and imposed restrictions on the use of antibiotic feed additives have intensified the search for novel possible alternatives [14,15]. In this study, inhibitory action of fermentation products of β-mannanase-producing bacteria on selected poultry borne pathogens was investigated.

2. MATERIALS AND METHODS

2.1 Materials and Chemicals

The coconut residual cakes were collected from farm field in Akure, Ondo State, Nigeria and used as carbon source for medium formulation. The residual were treated with petroleum ether and dried at 60ºC for 2 hrs. After that, the residual were blended, milled and sieved to obtain uniform particle size of 0.5 mm. Locust bean gum was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2 Bacterial Strains

Twenty three bacterial isolates from agro-wastes previously confirmed positive for mannanase activity by plate assay were used in this study. The plate assay was conducted in Microbiology Research Laboratory, Federal University of Technology, Akure, Ondo State, Nigeria.

2.3 Enzyme Production and Assay

The positive bacterial isolates were further screened for mannanase production under submerged state fermentation. Enzyme production was performed in 250 ml Erlenmeyer flask containing 50 ml of enzyme producing medium (PM) (modified from [2]). The composition was as followed: 1% Copra meal, 0.1% peptone, 0.1% yeast extract, 1.4% KH_2PO_4, 0.06 % $MgSO_4.7H_2O$, and 1% inoculums, pH 6.8. The flasks were incubated at 37^{o}C for 24 hr with agitation speed of 150 rpm. Then, the cultured broth was centrifuged at 6,000 rpm, 4^{o}C for 15 min. The supernatant was collected and kept at -20ºC for further study. Mannanase activity was assayed in the reaction mixture composing of 0.5 ml of 50 mM potassium phosphate buffer pH 7.0 and 1% Locust Bean Gum (LBG) with 0.5 ml of supernatant at 45ºC for 60 min (modified from [2]). Amount of reducing sugar released was determined by the dinitrosalicylic acid reagent (DNS) [16]. One unit of mannanase activity was defined as amount of enzyme producing 1 micromole of mannose per minute under the experimental conditions.

2.4 Effect of Fermentation Products on Poultry Borne Pathogens

The selected poultry borne pathogens (*Klebsiella oxytoca*, *Shigella alkalescens*, *Escherichia coli, Salmonella typii, Staphylococcus aureus* and *Streptococcus* sp.) were cultivated in 50 ml of nutrient broth (NB) under aerobic condition for 15-18 hrs at 37ºC. After that, 1% of the inoculum with the same absorbance was transferred into 5 ml NB supplemented with or without 1% supernatant obtained from mannanase production medium and grown at 37ºC for 4 hrs. The cells numbers were determined by plating in on nutrient agar and incubated overnight at 37ºC. The supernatant of each of the isolate obtained from mannanase production medium showing inhibition on pathogens were selected [2].

Percentage (%) inhibition was determined as:

$$(CB - SB)/CB \times 100$$

Where SB is the number of cells in NB with supernatant from mannanase production medium (cfu/ml) and CB is the number of cells in NB without supernatant from mannanase production medium (cfu/ml).

2.5 Bacterial Identification

The bacterial isolates were presumptively identified by means of morphological examination and some biochemical characterizations. The parameters investigated include colonial morphology, Gram reactions, endospore formation, catalase production, Voges-Proskauer (V-P) reaction, Indole production, starch hydrolysis, citrate utilization and gelatine hydrolysis. The results were compared with Bergey's Manual of Determinative Bacteria [17].

3. RESULTS

3.1 Survey of Bacterial Isolates for the Production of Extracellular β-Mannanase

The results indicated the potential differences of bacterial isolates to produce an extracellular mannanase to degrade mannan containing substrate under submerged state fermentation in

shaken condition (Fig. 1). Bacterial isolate 1A displayed the highest extracellular mannanase activity (13.430 U/ml) followed by isolate X3, while the lowest activity was recorded for isolate 1B (0.560 U/ml).

3.2 Protein Content Determination from Fermentation Medium

The protein content of each of the tested culture from the fermentation media for mannanase production was investigated (Fig. 2). The highest protein content was recorded for isolate 8B (3.472mg/ml), while the lowest was obtained for isolate 1B (1.528 mg/ml) although, there was no relationship between the protein content of the tested cultures and the production of mannanase.

3.3 Inhibitory Action of Fermentation Products of β-Mannanase-Producing Bacteria on Selected Poultry Borne Pathogens

Percentage inhibition of some selected poultry borne pathogens by fermentation products generated by mannanase producing bacteria isolates is evaluated (Figs. 3-8). The percentage inhibition on *Klebsiella oxytoca* of the tested cultures compare to the control was represented in Fig. 3. Thirteen isolates (1A, 2B, 2C, 3A, 5A1, 5D, 10B, 11B, BS, 5A11, X2, BP & X5), (+y axis) out of the 23 tested cultures showed different % inhibition, while the rest of the isolates exhibited % promotion (-y axis). The highest % inhibition was recorded for isolate 1A (61.18 %) while the least was observed with 5A11 (4.64 %). In Fig. 4, 75% (1A, 1B, 2B, 2C, 3A, 4B, 5A1, 5D, 9B, 9E, 10B, 11B, BS, 5A11, X2, BP, X5 & X1) of the tested cultures had inhibitory effect on *Shigella alkalescens* with highest % inhibition occurred in 1A (93.62%) and lowest in 9B (4.40%). Fig. 5 revealed the % inhibition of some tested cultures against *Esherichia coli*. All the tested cultures had inhibitory effect on *Esherichia coli*, although with differences in % inhibition. In Fig. 6, above 95% of the tested cultures had inhibitory effect on *Salmonella typii*, while in Fig. 7, 70.83% of the tested cultures was observed to exhibit different percentage inhibition on *Staphylococcus aureus.* The % inhibition of *Streptococcus* sp is shown in Fig. 8. All the tested cultures exhibited different % inhibition except for the isolates 9E, X2 and X5 where % promotion was observed.

3.4 Bacterial Identification

Biochemical reactions and characteristics of the isolate 1A is reveal in Table 1. The isolate 1A was identified to species level using conventional method based on the fact that it had the highest mannanase activity and % percentage inhibition on all the selected pathogens. The colonies of isolate 1A appeared creamy on mannan-agar medium containing LBG. A microscopic examination of the isolate revealed that it was a Gram-negative bacterium with long rod and produced catalase enzyme. Furthermore, the isolate 1A displayed positive reaction on VP and nitrate reduction test, while negative reaction was displayed towards citrate utilization, MR and sulphide indole motility.

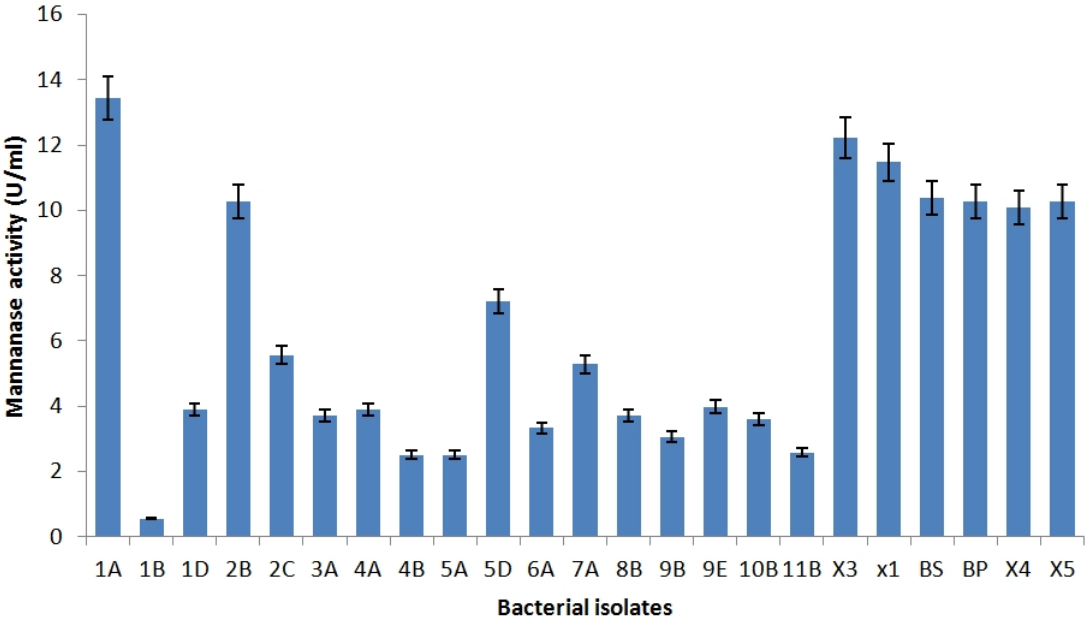

Fig. 1. Mannanase activity of bacterial isolates using copra meal as substrate

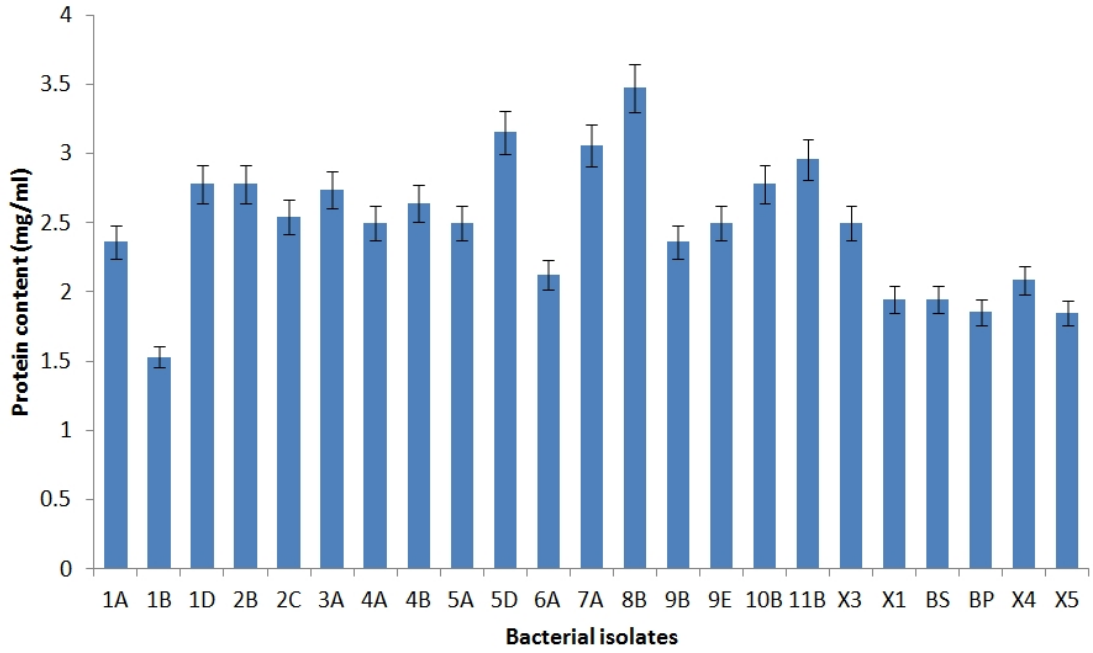

Fig. 2. Protein content of bacterial isolates using copra meal as substrate

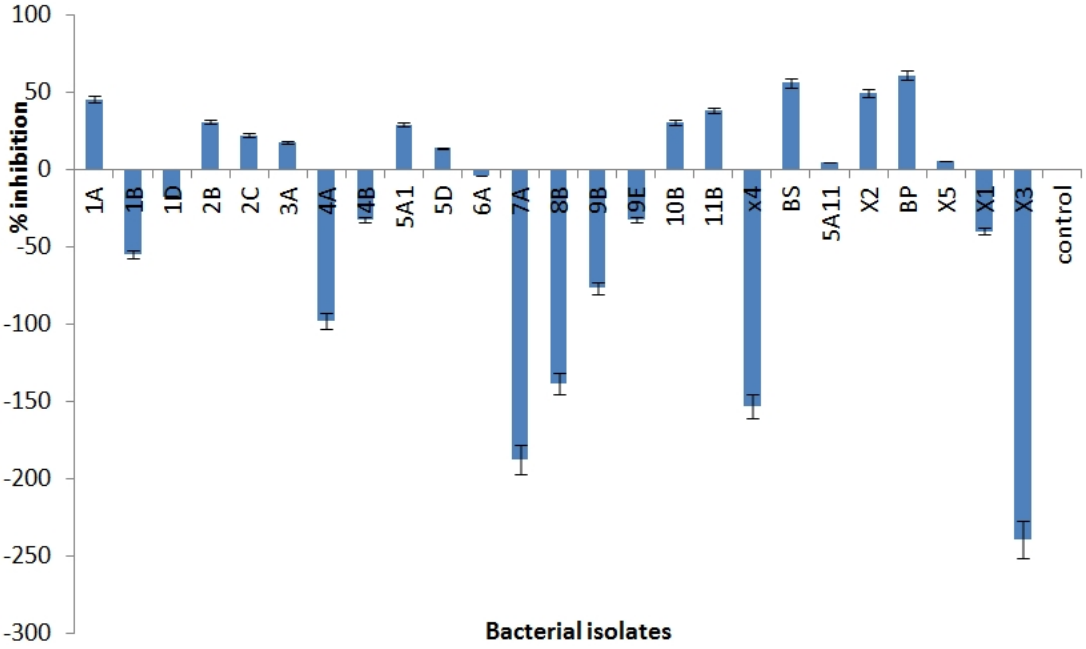

Fig. 3. Percentage inhibition of *Klebsiella oxytoca* by fermentation products of mannanase producing bacteria

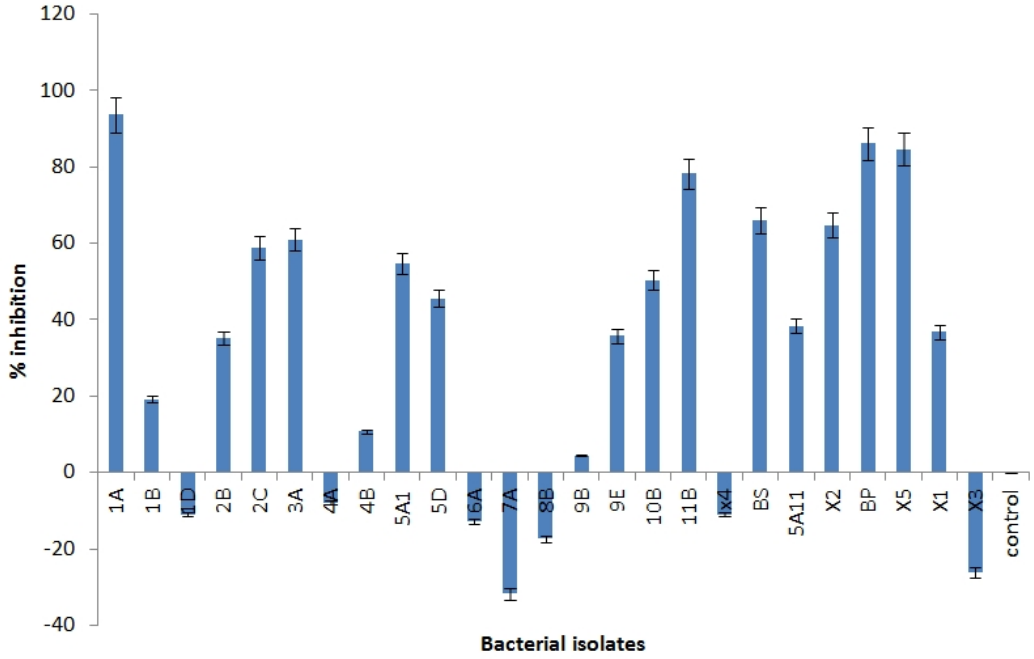

Fig. 4. Percentage inhibition of *Shigella alkalescens* by fermentation products of mannanase producing bacteria

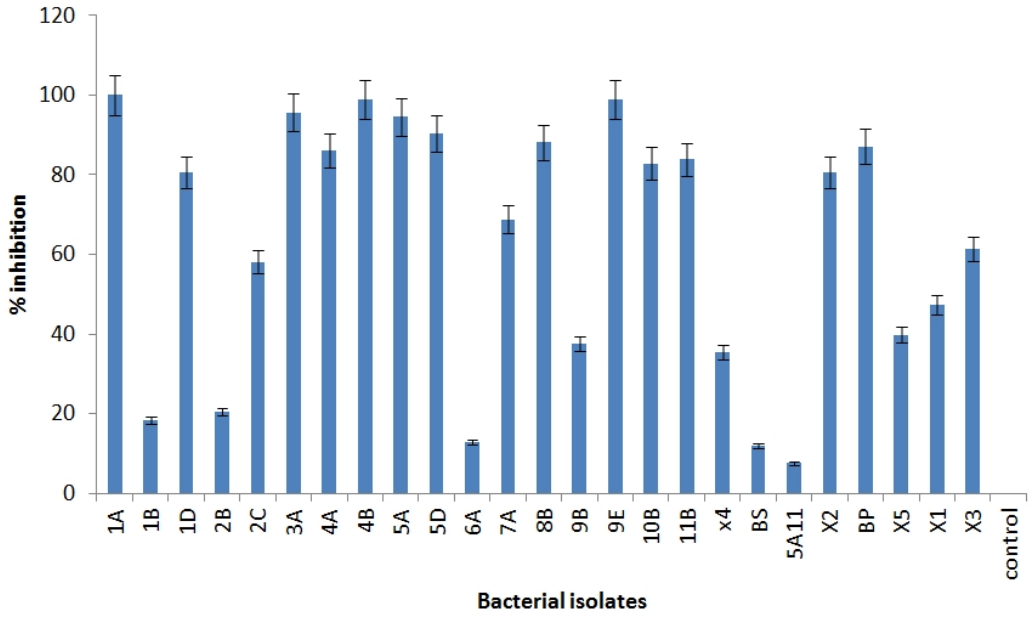

Fig. 5. Percentage inhibition of *Escherichia coli* by fermentation products of mannanase producing bacteria

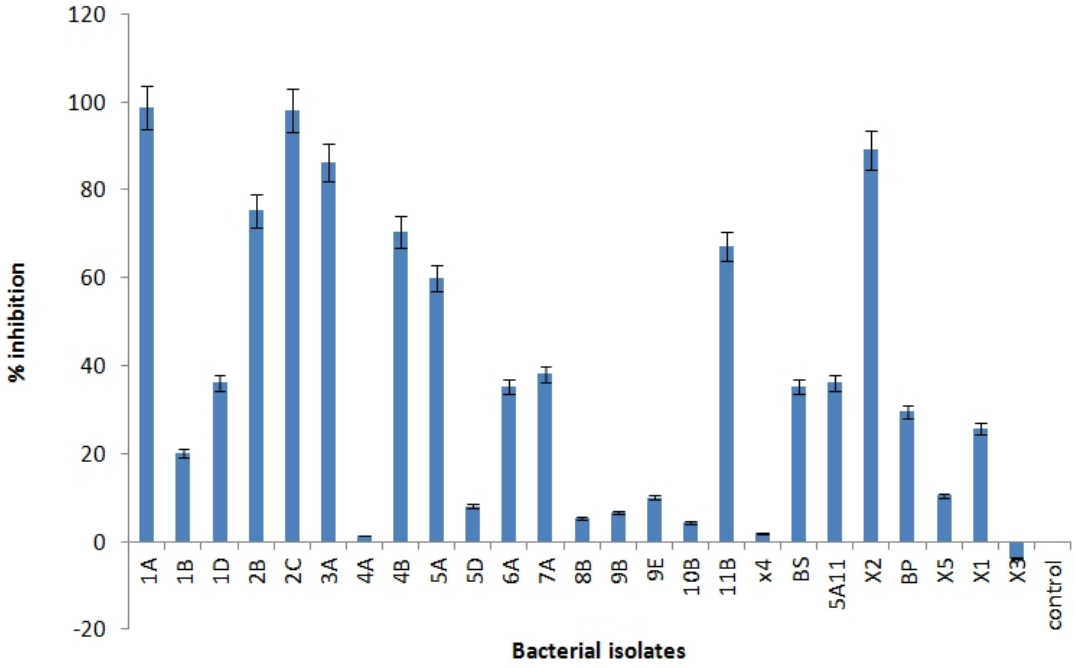

Fig. 6. Percentage inhibition of *Salmonella typii* by fermentation products of mannanase producing bacteria

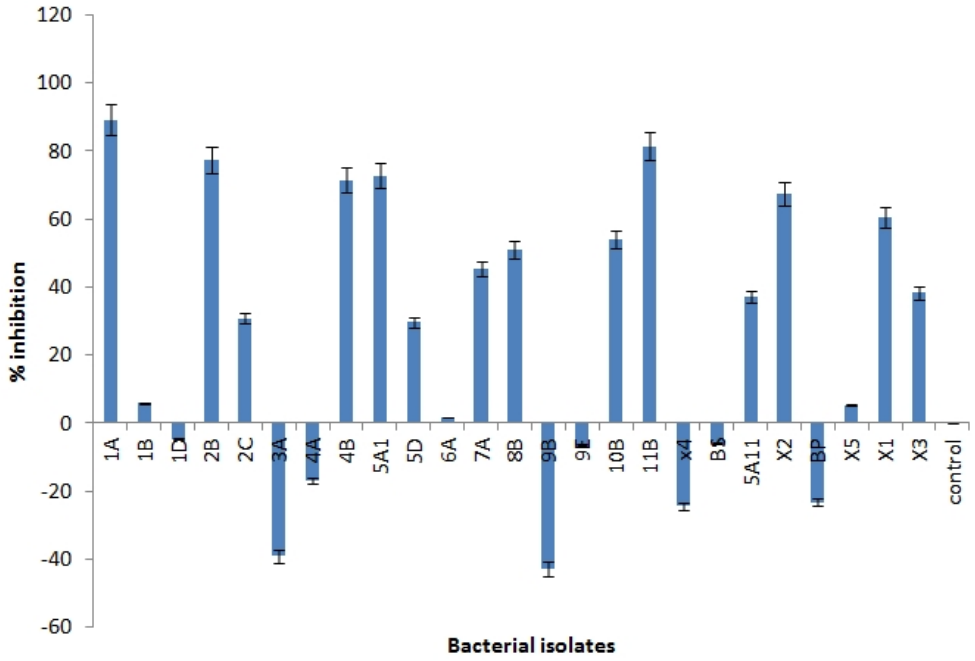

Fig. 7. Percentage inhibition of *Staphylococcus aureus* by fermentation products of mannanase producing bacteria

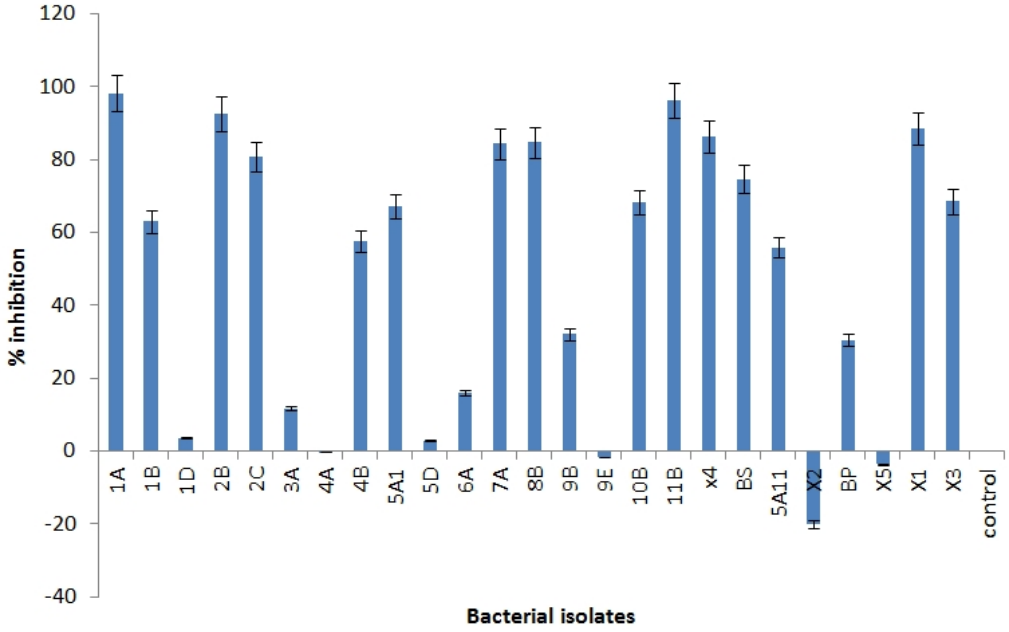

Fig. 8. Percentage inhibition of *Streptococcus* sp by mannolytic products of mannanase producing bacteria

Table 1. Biochemical reaction and characteristics of the isolate 1A

Characteristics/biochemical tests	Result
Cell shape	LR
Gram reaction	-
Spore formation	-
Methyl red test	+
Sulphide indole motility test	-
Oxidation-Fermentation test	F
Triple sugar iron test	Y
Nitrate reduction test	+
Catalase	+
V-P reaction	+
Citrate utilization	-
Fermentation of	
Maltose	NC
Glucose	Y
Mannitol	NC
Sucrose	NC
Lactose	NC

+= positive, -=negative reaction, F=fermentative, Y=Acid production, LR=Long rod NC=No change

4. DISCUSSION

In the present work, the production of extracellular mannanase by some bacterial isolates and its inhibitory fermentation products on selected pathogens was investigated. The production of mannanase on mannan containing medium and its inhibitory effect had been reported for *Bacillus circulans* [2]. Extracellular mannanase is very important for the digestion of hemicelluloses, one of the most abundant groups of polymers in nature. This enzyme hydrolyses mannan yielding mannotriose and mannobiose [18]. In the screening programme conducted on all the isolates in submerged state fermentation, isolate 1A showed the highest mannanase activity. The highest mannanase activity exhibited by isolate 1A could be attributed to the fact that its genes could code for mannanase with high diffusion rate [19].

In this study, it was observed that there was no direct relationship between the protein content of the tested cultures and the production of mannanase. Protein concentration does not really indicate an increase in the production of an enzyme. Different bacterial isolates had been reported to produce varieties of enzymes apart from the enzyme been examined for in this study. Besides that, the protein from bacterial cells might also interfere with mannanase causing variation in protein content, since the protein assay could only identify accumulated protein in solutions. This view was supported by the findings of [20] and [21].

The percentage inhibition showed by the fermentation products of the tested isolates might be due to the accumulation of toxic metabolites produced as a result of mannolytic action of mannanase on substrate containing mannan during the cause of fermentation and the failure of the pathogens to convert these metabolites to digestible nutrients. Another factor that might responsible for inhibitory action on pathogens could be attributed to the fact that mannanase will hydrolyse pathogens cell walls containing mannan or *Klebsiella edwardsii* can produce some bacteriocins that can kill the pathogens. Apart from this, organic acids might be produced in the course of fermentation that may negatively affect the metabolic

activity of pathogens. The growth promoting effect exhibited by few of the isolates could be attributed to fact that the pathogens could convert the toxic metabolites to a form that could be utilized for growth and metabolic functions.

Carbon catabolite repression is another regulatory mechanism which could cause inhibitory effect on some of these pathogens. In this case, the end product of mannan hydrolysis might interacts with a cellular protein and form a complex which interacts with a particular gene at the transcription level and represses metabolic pathways which in turns inhibits the growth of pathogens [22,23].

A microscopic examination of the isolate 1A revealed that it was a Gram negative bacterium with long rod and produced catalase enzyme. Furthermore, the isolate 1A displayed positive reaction on VP and nitrate reduction test, while negative reaction was displayed towards citrate utilization, MR and sulphide indole motility. From these morphological and biochemical reactions, the isolate was presumptively identified as *Klebsiella edwardsii* [17].

5. CONCLUSION AND RECOMMENDATION

This work collectively suggests a possible use of fermentation products obtained from mannolytic activity of mannanase-producing bacteria might possess antibacterial properties which could be used to combat bacterial pathogens implicated in poultry farming. Therefore, it is recommended that much research be done on this topic to elucidate specific fermentation products responsible for the inhibitory action using this work as template.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1 Mabrouk MEM, El Ahwany AMD. Production of β-mannanase by *Bacillus amylolequifaciens* 10A1 cultured on potato peels. Afri. J. Biotechnol. 2008;7(8):1123-1128.

2 Phothichitto K, Nitisinprasert S, Keawsompong S. Isolation, screening and identification of mannanase producing microorganisms. Kasetsart J. (Nat. Sci.). 2006;40:26-38

3 Sanchez C. Lignocellulosic residues: biodegradation and bioconversion by fungi**.** Biotechnol. Advance. 2009;27(2):185-194.

4 Meenakshi M, Singh G, Bhalla A, Hoondal GS. Solid state fermentation and characterization of partially purified thermostable mannanase from *Bacillus* sp. MG-33. Bioresource. 2010;5(3):1689-1701.

5 Ninawe S, Kuhad RC. Bleaching of wheat straw-rich soda pulp with xylanase from a thermoalkalophilic *Streptomyces cyaneus* SN32, Biores. Technol. 2006;94:2291-2295.

6 Singh C, Ahuja N, Batish M, Capalash N, Sharma P. Biobleaching of wheat wheat straw-rich-soda-pulp with alkalophilic laccase from γ-proteobacterium JB: Optimization of process parameters using response surface methodology," Bioresource Technol. 2008;99:7472-7479.

7 Ferreira HM, Filho, EX F. Purification and characterization of a mannanase from *Trichoderma harzianum* strain T4, Carbohydr Polym. 2004;57:23-29.

8 Heck JX, Soares HB, Ayub MAZ. Optimization of xylanase and mannanase production by *Bacillus circulans* strain BL53 on solid state cultivation, Enzy. Microb. Technol. 2005;37:417-423.

9 Juhasz T, Szengyel Z, Reczey K, Siika-Aho M, Viikari L. Characterization of cellulases and hemicellulases produced by *Trichoderma reesei* on various carbon sources. Proc. Biochem. 2005;40:3519-3525.

10 Jiang ZQ, Wei, Y, Li D, Li L, Chai P, Kusakabe I. High level production, purification and characterization of a thermo-stable mannanase from the newly isolated *Bacillus subtilis* WY34, Carbohydr. Polym. 2006;66:88-96.

11 Lin SS, Dou WF, Xu HY, Li HZ, Xu ZH, Ma YH. Optimization of medium composition for the production of alkaline beta-mannanase by alkaliphilic *Bacillus* sp. N16-5 using response surface methodology, Applied Microbiol. Biotechnol. 2007;75:1015-1022.

12 Ishihara N, Chu DC, Akachi S, Juneja L. Preventive effect of partially hydrolysed guar gum on infection of *Salmonella enteritidis* in young and laying hens. Poult. Sci. 2000;79:689-697.

13 Joerger RD. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poult. Sci. 2003;82:640-647.

14 Diep DB, Nes IF. Ribosomally synthesized antibacterial peptides in gram positive bacteria. Curr. Drug Therapy. 2002;3:107-122.

15 Gillor O, Nigro LM, Riley MA. Genetically engineered bacteriocins and their potential as the next generation of antimicrobials. Curr. Pharm. D. 2005;11:1067-1075.

16 Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. 1959;31:426-428.

17 Holt JG, Kneg NR, Sneath PH, Stanly JJ, Williams ST. Bergey's Manual of Determinative Bacteriology. Wilkins Publishers, Baltimore; 1994.

18 El-Naggar MY, Youssef SA, El-Assar SA, Beltagy EA. Optimization of cultural conditions for β-mannanase production by a local *Aspergillus niger* isolate. Int. J. Agric. Bio. 2006;8(4):539-545.

19 Rattanasuk S, Ketudat-Caims M. *Chryseobacterium indologenes*, novel mannanase-producing bacteria. Songklanakarin J. Sci. Technol. 2009;31(4):395-399.

20 Khan FAB, Husaini AAS. Enhancing α-amylase and cellulase *in vivo* enzyme expressions on sago pith residue using *Bacillus amyloliquefaciens* UMAS 1002. Biotechnol. 2006;5(3):391-403.

21 Akinyele BJ, Olaniyi OO, Arotupin DJ. Bioconversion of selected of selected agricultural wastes and associated enzymes by *Volvariella volvacea*: An edible mushroom. Res. J. of Microbiol. 2011;12:567-573.

22 Lewin B. Genes III. An up-to-date treatment of the molecular genetics of both eukaryotes and prokaryotes, along with related cell and molecular biology. John Wiley, New York; 1987.

23 Moussa TAA, Tharwat NA. Optimization of cellulase and β-glucosidase induction by sugarbeet pathogen *Sclerotium rolfsii*. Afri. J. Biotechnol. 2007;6(8):1048-1054.

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