



Original Research Article

Isolation, identification and functional characterization of *Escherichia coli* as probiotic against *Shigella* in BangladeshA M Masudul Azad Chowdhury¹, Sanjida Akter¹, Sohana Akter Mina^{1,*}¹Dept. of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong, Bangladesh

ARTICLE INFO

Article history:

Received 13-10-2020

Accepted 28-11-2020

Available online 25-12-2020

Keywords:

Antibiotic resistance

Probiotic

*Escherichia coli**Shigella*

PCR

ABSTRACT

Background: Antibiotic resistance due to misuses of antibiotics is currently became potent threat for treating patients suffering from infectious diseases in Bangladesh. It is the high time to develop some alternative placebo for the treatment of infectious diseases especially in Bangladesh. Probiotic especially *E. coli* could be that alternative choice of treatment against *Shigella* infection.

Aims: The present study was undertaken to examine the potentiality of *Escherichia coli* as probiotic against *Shigella*.

Materials and Methods: During this study, four poor hygienic areas around Chattogram City, Bangladesh were selected through survey and then human stool samples were collected. Isolation and identification of *Escherichia coli* were done with a combination of microbiological and PCR analysis. Probiotic activity of isolated *E. coli* against *Shigella* was determined by co-culture test.

Results: Total eight isolates were identified as *Escherichia coli* by microbiological and biochemical test. All the isolates were also confirmed as bacteria, coliform as well as fecal coliform through PCR. In the probiotic activity test, all the identified isolates except one showed significant result as probiotic.

Conclusion: This research identified the potentiality of *Escherichia coli* as probiotic to treat shigellosis in Bangladesh. The outcomes of this study might function as a strong background to develop *Escherichia coli* as probiotic against *Shigella* infection in Bangladesh.

© This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. Introduction

E. coli is a gram negative, rod shaped, non-spore forming, and motile bacteria. This is a commensal bacterium that resides as the most common and predominant inhabitant in the intestinal microflora of human and other mammal.¹ It is transmitted via fecal–oral route. Its versatility and adaptability makes it very commonly found in water, soil, and food.² *E. coli* are usually nonpathogenic and may serve as indicators of faecal contamination of food and water.³

Probiotic is a Latin derived word that means ‘for life’.⁴ Nonpathogenic micro-organisms, used to control the growth of other micro-organisms are called probiotics and worldwide studies have shown that they constitute a

significant group of bio therapeutics.⁵ Humans live in close association with vast numbers of micro-organisms that can be present on the skin, in the mouth and in the gastro-intestinal tract where gastro-intestinal tract consists of a rich flora of more than 500 different bacterial species, some of which have important health functions.⁶ These nonpathogenic microorganisms known to have beneficial effects on the digestive ecosystem as they colonize in the bowel and confer resistance to infections as demonstrated by studies in animal models as well as by clinical trials.⁷ Bacteria and yeast are the most common microorganisms used as probiotics. They differ in their mechanisms of action, metabolism and resistance to antibiotics. Essentially, four bacterial genera and one yeast genus are the basis for most preparations: *Enterococcus*, *Bifidobacterium*, *Escherichia*, *Lactobacillus* and *Saccharomyces*. *E. coli*

* Corresponding author.

E-mail address: sohanageb@cu.ac.bd (S. A. Mina).

Nissle 1917 (Mutaflor) is one of the most broadly studied and used probiotic bacteria worldwide.⁸

Species of the genus *Shigella* are among the bacterial pathogens that are most frequently isolated from patients suffering diarrhea. Every year around 165 million *Shigella* induced diarrheal cases are reported and among that 99% of cases occur in Lower to middle income countries mainly in children (69%) because of overcrowding and poor sanitation.⁹ Antibiotic is prescribed in shigellosis to speed recovery, reduce the seriousness of disease and reduce the length of time patients are infective,¹⁰ however, some antimicrobial drugs can have serious side effects while others may not be effective against the *Shigella* bacteria. With the continuing use of antibiotics, the bacterial population will soon amplify the resistant type replacing the sensitive predecessor and thereby abolishing clinical value of the antibiotic.¹¹ Resistance to antimicrobials is creating hindrance to the healthcare system worldwide. This complicate treatment results, increases treatment cost, and limits the therapeutic options that contribute to the loss of efficiency of antimicrobial drugs.¹² Therefore, to overcome this antibiotic associated problems, probiotics especially *E. coli* might be a suitable choice of treatment against *Shigella* infection in developing countries.

This research will try to understand and hypothesize that some people who live in poor hygienic environmental condition may remain unaffected from shigellosis because they might contain *E. coli* in their gut which inhibits *Shigella* infection. In addition, in poor sanitation condition there should be competition among microorganisms. To establish this phenomenon, this study was designed to isolate novel *E. coli* strain as probiotic from the poor sanitation areas of Chattogram City, Bangladesh.

2. Materials and Methods

2.1. Sample survey

This study focused on poor hygienic regions firstly for the isolation of *E. coli* as probiotic strains against common pathogenic organism and secondly to evaluate misuse of antibiotics in a vastly populated city of Bangladesh. One of the hypothesis of this study presume that in the unhygienic areas *E. coli* from human sources might has the capacity to inhibit pathogenic organism like *Shigella* by which most of the people remain protected though their living and sanitation condition is very poor. So, sample that is associated with human gut such human stool was our primary target. To select sample location a survey was carried out for choosing appropriate sample location in the Chattogram city area. The survey took almost two weeks. The study population was drawn from four poor areas of Chattogram Figure 1. The locations were visited and the situation was observed. These locations are very crowded. Huge number of people lives in these small areas. Most

of them are day laborer. Most of the people use common latrines and the sanitation system is not developed. These areas are: Chittagong railway station, JhautolaTpp colony, Shahid lane Akbarshah, and Karnaphuli market Figure 1. Several points in these areas were chosen for sample collection where collective stool samples of these people can be found. We targeted conduits that came out from latrines of common people.

2.2. Sample collection and processing

Aseptic condition was maintained while collecting sewage samples and after collection the samples were brought to molecular biology lab of the Department of Genetic Engineering and Biotechnology at University of Chittagong, Bnagladesh. The EMB agar media was prepared into a sterile conical flask and sterilized by autoclaving. After that the media was cooled to 45°C and then was poured into sterile petridishes. The dishes were allowed to solidify. After solidification, a sterile micro wire loop for the semi-quantitative method was used for the plating and it has a 4.0 mm diameter designed to deliver 0.01 ml. A loopful of the uniformly mixed stool sample was inoculated into EMB agar plate (EMB contains dyes that are toxic to gram positive bacteria. It is a specialized media for gram negative bacteria. In EMB agar plate typical *E. coli* colony is usually characterized by green metallic sheen). The loop was sterilized using bunsen burner. After inoculation, all the plates were kept in the incubator in an inverted position at 37°C for 24 hours. All the steps were done in laminar air flow that was previously swiped with 70% ethanol. There is always a bunsen burner light up while working in the laminar air flow. To maintain aseptic condition, hands were washed with 70% ethanol. From each sample spot 3 stool samples were collected (with in close proximity) in 3 separate tubes. Three stool samples instead of one stool sample were collected from same position to avoid experimental error as well as to increase the probability getting positive result for the target organism. After collection, samples carried to the lab in ice bag on the same day and perform streak plating (as explain above) in 3 separate EMB plate to get the single colonies of *E. coli*. The next day EMB agar plates were prepared and the previous plates were observed for bacterial growth. From the previous 3 plates the plate that shown perfect streaking pattern with characteristics single *E. coli* colony was selected for next step. Only one single colony that showed green metallic sheen were picked up with sterile inoculating loop and further inoculated in EMB agar plates. After inoculation, the plates were kept in the incubator in an inverted position at 37°C for 24 hours. At 24 hours after incubation the plates were observed. On the same day, MacConkey agar media was prepared and sterilized and cooled to 45°C-50°C and poured into sterile petri plates. After solidification, these plates were also streaked by single



Fig. 1: Location of samples in Bangladesh. As mention in the above section, four poor hygienic areas were selected around Chattogram city, Chittagong, Bangladesh. Samples were collected from eight spots from those locations. Each red mark indicates the position of sample spot in the map

colonies from previous EMB agar plates and then kept in the incubator for 24 hours. MacConkey is an indicator, a selective and differential culture medium that is used for the isolation of gram negative enteric bacteria and the differentiation of lactose fermenting gram negative bacteria. Lactose fermenting strains grow as pink colony and lactose non fermenting strains are colorless and transparent. The sample that showed positive results *E. coli* both in EMB and MacConkey agar was selected, coded and stored in slant for biochemical and molecular identification. According to

above protocol, Eight single colonies were picked from 24 stool samples ((3 samples x 8 sample spots = 24 samples).of different sample spots and coded as Ec-CRS1 (*E. coli* Chittagong Railway Station 1), Ec-RS2 (*E. coli* Chittagong Railway Station 2), Ec-JHT3 (*E. coli* Jhautola station 3), Ec-AKS4 (*E. coli* Akbarshah 4), Ec-AKS5 (*E. coli* Akbarshah 5). Ec-AKS6 (*E. coli* Akbarshah 6), Ec-KPM7 (*E. coli* Karnaphuli market 7), Ec-KPM8 (*E. coli* Karnaphuli market 8).

2.3. Biochemical tests

Primarily selected *E.coli* colonies were confirmed by performing Catalase test, Indole test, Methylene blue test, Voges-Proskauer test and citrate utilization test according to the procedure describe in Cowan and steel, 2004 and also by gram staining.^{13,14}

2.4. Molecular identification

2.4.1. DNA extraction

Extraction of the genomic DNA from the isolated *E. coli* strains were conducted by boiling method.¹⁵ DNA concentration was measured using Nanodrop 2000 spectrophotometer (Thermo Scientific, USA).

2.4.2. PCR assay for the identification of bacteria, coliform and faecal coliform

In this study, molecular detection of organism was carried out by PCR using the previously published primers and targeted gene.¹⁶ Primer specificity was determined by searching for similar sequences in microbial genome using the Basic Local Alignment Search Tool (BLAST). A PCR thermal cycler (NyxTechnik) was used for amplification and the PCR products were analyzed by 1.5% agarose gel electrophoresis. In each experiment, positive control (Previously identified *E. coli*,¹⁶ was carried out as the standard genomic DNA along with negative control (PCR mixtures except genomic DNA). Target gene, primer sequence, cycling parameters, amplicon size are shown in the supplementary Table 1.

2.5. Probiotic activity test

Probiotic activity test was performed by co culturing *Shigella* and *E. coli* on the same plate. *Shigella* strain was provided from Microbiology lab of Department of Microbiology, University of Chittagong, Bangladesh. Nutrient broth was taken in eight tubes and was inoculated with freshly prepared *E. coli* culture. Eight test tubes were inoculated with both *Shigella* and *E. coli* culture. One test tube was inoculated with *Shigella* culture. The tubes were incubated at 37°C for overnight at shaking condition. The next day, each culture was serially diluted with in test tubes containing sterile distilled water up to 10⁻⁷ times. From each diluted solution, 1ml of solution was transferred into plate containing MacConkey agar media. The plates were stirred with hand gently clockwise and anti-clockwise so that sample was mixed thoroughly with the media. The plates were allowed to stand steady to solidify the media. After solidification, the plates were incubated in inverted position for 24 hours at 37°C. The next day, colony counting was done by total viable count (TVC) method.

2.6. Statistical analysis

Statistical significance was evaluated with Student's t-test for repeated measurements. All values are represented as the means ± standard deviation for three-independent experiments.

3. Results

3.1. Selective plating

As mention in the methodology part, all the stool samples from different sample location were streaked on EMB agar plates for characteristic *E. coli* colony (metallic green sheen). EMB positive *E. coli* were further culture on MacConkey agar to observe characteristic *E. coli* colony (pink) on MacConkey agar. Finally, 8 single colonies from 24 stool samples that were collected from eight sample points of four different locations around Chattogram city were selected and coded (explained in detail in the methodology section) as *E. coli* according to selective plating result Table 2.

3.2. Biochemical test

First Gram staining experiment were done for each selected isolate. Gram staining result revealed that all the isolates were Gram negative. Five biochemical tests were carried out for the identification of selected *E. coli*. All of them found positive to Indole test, Methyl red test, Catalase test and negative to Voges-Proskauer test, Citrate utilization test. The biochemical test results are summarized in the Table 2. The biochemical test results confirmed the identification of all the primarily selected isolates as *E. coli* Table 2.

3.3. Molecular identification

Molecular identification *E. coli* as a bacteria, coliform and faecal coliform was done by amplifying *16srDNA*, *lacZ* and *uidA* gene, respectively. The PCR analysis of these genes resulted in 100% positive for all the eight selected isolates Figure 2. These were identified by observing the band size with respect to DNA marker on 1.5% agarose gel on the basis of 800 bp, 874 bp and 147bp for the genes *16srDNA*, *LacZ* and *uidA*, respectively (Figure 2 B, C and D).

3.4. Probiotic activity test

The antagonism of *E. coli* for *Shigella* was perceived by co-culturing *Shigella* with *E. coli* and observing their growth on the same plate. The result showed that in the co-culture *E. coli* effectively decreased the number of *Shigella* colony. On MacConkey agar plates, all the isolates of *E. coli* except Ec-AKS6 inhibited *Shigella* growth in co-culture Figure 3. *Shigella* and *E. coli* colonies were also counted separately on MacConkey agar plates for comparison as control to evaluate culture condition Figure 3. The test was done in

Table 1: Target genes, primers, cyclic condition, composition of PCR mixture and amplicon size

Target genes	Primer sequence 5'-3'	Cycling parameters	Composition of PCR mixtures	Amplicon Size (bp)
Bacterial: 16srDNA	AGAGTTGATCCTGGCTCAG ^a GACTACCAGGGTATCTAAT ^b	5 min at 95°C, 35 cycles of 95°C for 40s, 57°C for 72°C for 1 min	For 20 µl: 10 µl master mix, 4µl template, 2 µl ^a , 2µl ^b , 3µl water	800
Coliform: lacZ	ATGAAAGGCTGGCTACAGGAAGGCC ^a CACCATGCCGTGGGTTTCAATATT ^b	5 min at 95°C, 25 cycles of 95°C for 1 min and 72°C for 1 min	For 20 µl: 10 µl master mix, 4µl template, 2 µl ^a , 2µl ^b , 3µl water	874
Faecalcoliform: uidA	TGGTAATTACCGACGAAAACGG ^a ACGCGTGGTTACAGTCTTGCG ^b	5 min at 95°C, 30 cycles of 95°C for 50s, 62°C for 50s and 72°C for 1 min	For 20 µl: 10 µl master mix, 4µl template, 2 µl ^a , 2µl ^b , 3µl water	147

^aForward primer; ^bReverse primer

Table 2: Summarized results of microbiological analysis

Sample ID	Colony character		Gram staining	IT	Biochemical tests				Comments
	EMB	MCK			MRT	CUT	VPT	CT	
Ec-CRS1	GMS	Pink	G ⁻	+	+	-	-	+	E. coli
Ec-CRS2	GMS	Pink	G ⁻	+	+	-	-	+	E. coli
Ec-JHT3	GMS	Pink	G ⁻	+	+	-	-	+	E. coli
Ec-AKS4	GMS	Pink	G ⁻	+	+	-	-	+	E. coli
Ec-AKS5	GMS	Pink	G ⁻	+	+	-	-	+	E. coli
Ec-AKS6	GMS	Pink	G ⁻	+	+	-	-	+	E. coli
Ec-KPM7	GMS	Pink	G ⁻	+	+	-	-	+	E. coli
Ec-KPM8	GMS	Pink	G ⁻	+	+	-	-	+	E. coli

GMS = Green metallic sheen, G⁻ = Gram negative, IT = Indole test, MRT = Methyl red test, CUT = Citrate utilization test, VPT = Voges Praskauer Test, CT = Catalase test.

Ec-CRS1 (*E. coli* Chittagong Railway Station 1), Ec-RS2 (*E. coli* Chittagong Railway Station 2), Ec-JHS3 (*E. coli* Jhautola station 3), Ec-AKS4 (*E. coli* Akbarshah 4), Ec-AKS5 (*E. coli* Akbarshah 5), Ec-AKS6 (*E. coli* Akbarshah 6), Ec-KPM7 (*E. coli* Karnaphuli market 7), Ec-KPM8 (*E. coli* Karnaphuli market 8).

triplicate for statistical analysis.

4. Discussion

The normal resident gastrointestinal microbiotas are the major factor protecting animals and humans against intestinal colonization by pathogenic bacteria.¹⁷ Microbes being such a large physical part of the gastrointestinal tract, so it is vitally important that specialists appreciate their existence, and consider what role they might have in health and disease. Recent understanding of the functions of intestinal microflora and the use of probiotic microorganisms is a novel concept for the improvement of human health and an innovative approach for new food product development in functional foods for specific diseases.¹⁸ *E. coli* and its human host usually coexist with mutual benefit for decades.¹ The probiotic effect of *E. coli* is well established and proven by several in vitro and in vivo experiments¹⁹ reported that exoproducts of the *E. coli* strain H22 inhibits some enteric pathogens both in vitro and in vivo by agar overlay method (in vitro) and

observing inhibition zone (ex vivo)²⁰ published that *E. coli* strain Nissle 1971 combat lamdoid bacteriophage stx and λ thus opening a new window for the treatment of infections caused by shiga toxin producing pathogens.

In this study, eight *E. coli* strains were isolated and identified through conventional microbiological analysis in order to examine probiotic activity against Shigella one of the causing agent of diarrhea in Bangladesh. The results of microbiological detection Table 2 of *E. coli* were similar as.^{21,22} Along with culture based detection, molecular identification of the selected isolates were done by PCR amplification of the three genes; *16s rDNA* for bacterial identification Figure 2 B, *lacZ* gene for coliform identification Figure 2 C and *uidA* gene for fecal coliform identification¹⁶ Figure 3. All the amplified products showed bands on agarose gel electrophoresis showing positive results for identification. Bands of around 800 bp, 874 bp, and 147 bp were found respectively for the three genes in all sample isolates. Through molecular identification it was confirm that all the samples are coliform bacteria and they are from the intestines of warm blood animal. As the study

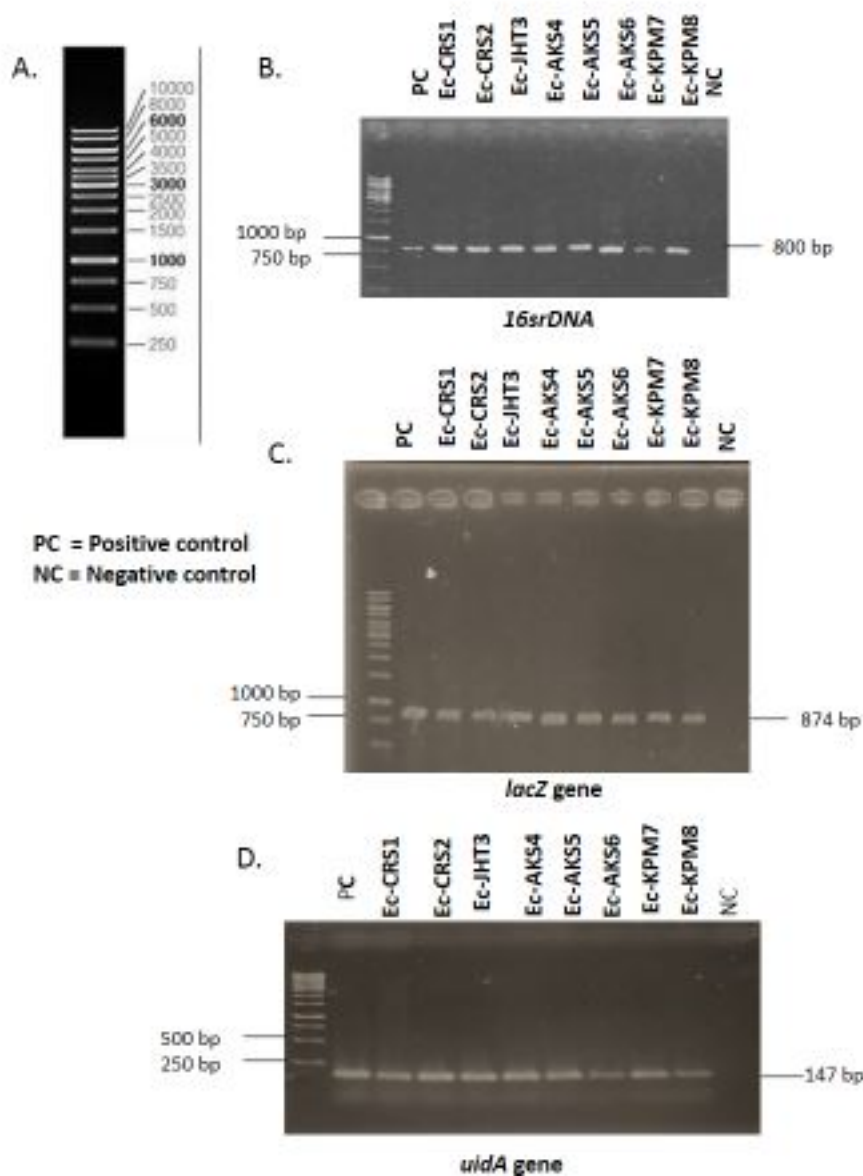


Fig. 2: Molecular identification isolates as *E. coli*. through electrophoretic (1.5% agarose) separation of *16 srDNA*, *LacZ* and *uidA* gene. (A) Thermo Scientific 1 kb DNA ladder. (B) Detection *16srDNA* gene to confirm the isolates as bacteria. (C) Detection of coliform by *lacZ* gene. (D) Detection of faecal coliform by *uidA* gene. Here, EC-CRS1, EC-CRS2, EC-CRS1JHT3, EC-AKS4, EC-AKS5, EC-AKS6, EC-KPM7 and EC-KPM8 are isolates; PC: Positive Control; NC: Negative Control

areas of this research project were mainly the slum areas, it can be assumed that all the isolates are from human gut.²³

The antagonism of *E. coli* for *Shigella* was perceived by co-culturing *Shigella* with *E. coli* and observing their growth on same plate. The statistical analysis of the result showed that in the co-culture, all the *E. coli* samples except Ec-AKS6 (*E. coli* Akbarshah 6), caused significant inhibition of *Shigella* Figure 3. According to this result Ec-CRS1 (*E. coli* Chittagong Railway Station 1), Ec-RS2 (*E. coli* Chittagong Railway Station 2), Ec-JHS3 (*E. coli* Jhautola station 3), Ec-AKS4 (*E. coli* Akbarshah 4), Ec-

AKS5 (*E. coli* Akbar Shah5), Ec-KPM7(*E. coli* Karnaphuli Market 7), Ec-KPM8 (*E. coli* Karnaphuli market 8) are considered as effective probiotic strain Figure 3. The exact mechanism how *E. coli* inhibit intestinal pathogen is still not clearly understood. The inhibition activity might be due to the production of specific antimicrobial substances, such as microcins. However, the microcin negative isogenic mutant of *E. coli* has been shown to be as effective as the wild strain in competing with pathogenic bacteria. In fact, because of the narrow spectrum of bacteriocin activity, it is unlikely to be responsible for the inhibitory

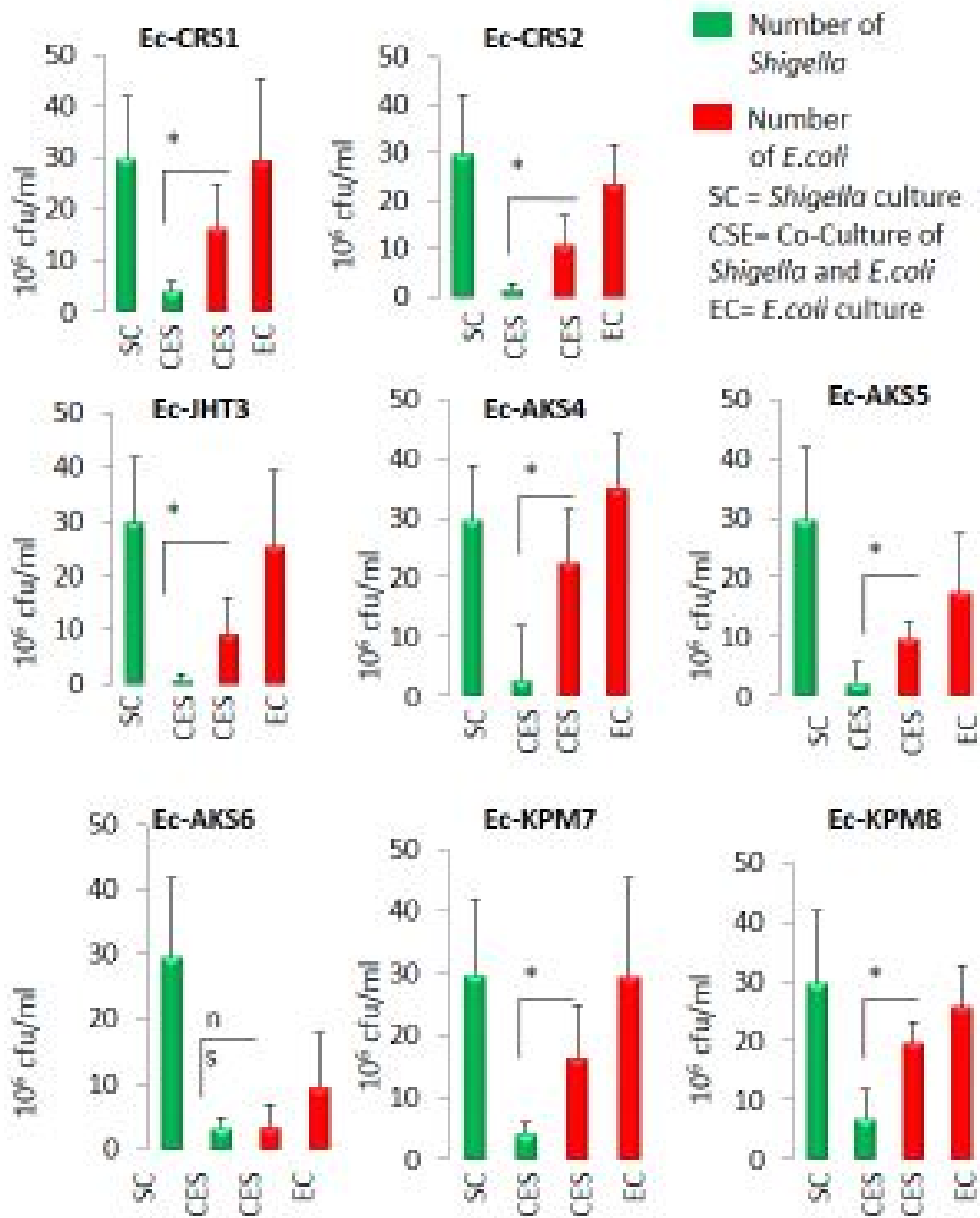


Fig. 3: Probiotic activity of *E. coli* against *Shigella*., *E. coli* with *Shigella* were cultivated in test tube containing equal volume of Nutrient broth. At 18 hours after incubation probiotic activity of *E. coli* against *Shigella* were measured by Total viable count experiment through pour plate method on MacConkey agar plates. The error bars represent data from three independent experiments (mean \pm standard deviation). The two-tailed Student's t-test was used for the statistical analysis. * $P < 0.05$, ns: No significance

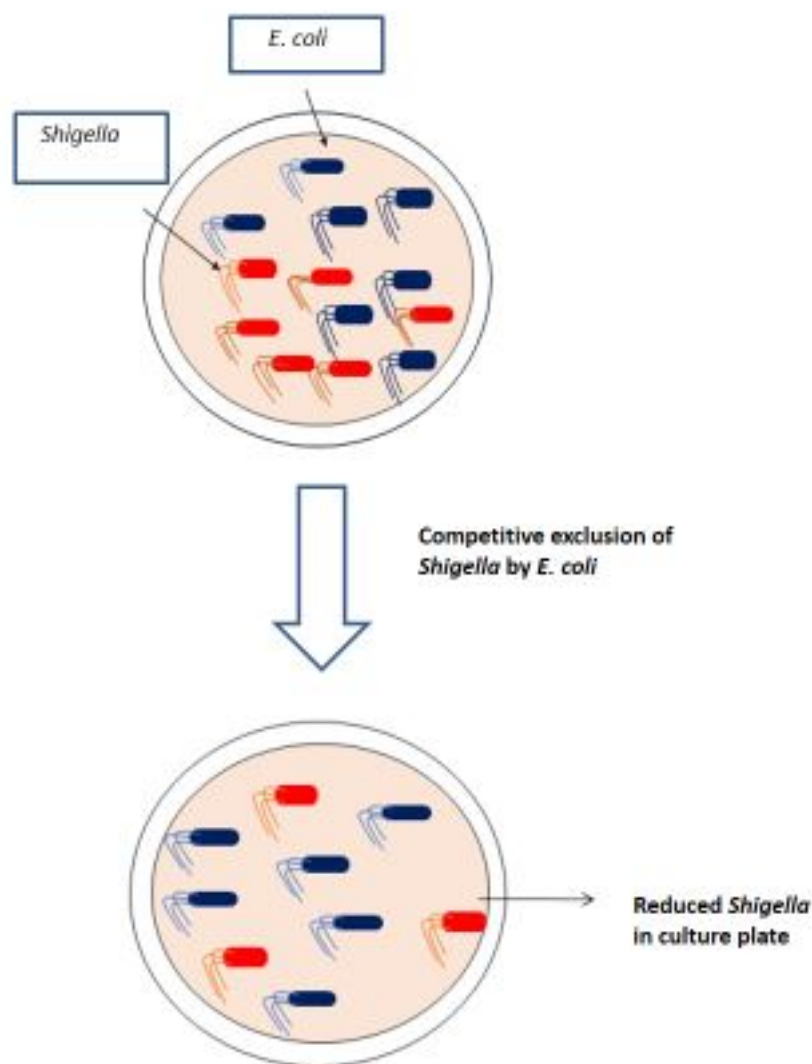


Fig. 4: Schematic model of probiotic activity of *E. coli*. In this study, the *E. coli* isolates inhibiting growth of the pathogenic *Shigella* strain. This inhibition might be the result of competitive exclusion of the pathogen by *E. coli* strain

effect of *E. coli*.²⁴ Effective adherence of *E. coli* to intestinal epithelial cells may block necessary receptors for attachment of invasive bacteria thereby inhibiting them. *E. coli* adheres strongly to the intestinal cell wall that results in a biofilm formation of nonpathogenic bacteria thus restricts the pathogenic bacteria. According to some other studies, *E. coli* Nissle 1917 and other probiotic strains may stimulate the synthesis of endogenous epithelial antimicrobial peptides such as human Beta Defensin-2 which helps to exert the beneficial effects of the probiotic strain. The growth and metabolic activity of *E. coli* may also cause changes in the pH or chemical composition of the colonic lumen that make the surface unfavorable to the pathogenic bacteria.²⁵ In this study, the sample isolates showed positive results by inhibiting growth of the

pathogenic *Shigella* strain. This inhibition might be the result of competitive exclusion of the pathogen by *E. coli* by creating hostile micro ecology and competitive reduction of essential nutrients Figure 4.

Production and secretion of antimicrobial substances and selective metabolites can also be the reason of inhibition. To check this, toxicity test has been done to check whether *E. coli* supernatant has any effect in *Shigella* inhibition.²⁶ This experiment was performed but due to lack of proper equipment facilities *E. coli* supernatant could not properly separated (result not shown).

5. Conclusion

Probiotics may help not only in disease reduction but also in health improvement and can be included in other sectors

like poultry, agriculture where extensive antibiotic use is harmful because of emergence of multi drug resistance bacteria. This study demonstrated that *E. coli* strains from environmental source can act as a potent antagonist against enteric pathogen *Shigella*. This study will serve as a background to find a friendly alternative to this massacre. In future, this study can be done on a bigger platform targeting larger regions and sample numbers for the isolation of novel probiotic strains. There is an urge to think of a substitute of the conventional treatment of infectious disease in Bangladesh, so more studies on probiotic should be enrolled.

6. Source of Funding

Research and Publication Offices, University of Chittagong, Bangladesh, for research grant. Research Grant number; Memo no- 6429/2018.

7. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

- Kaper JB, Nataro JP, Mobley HLT. Pathogenic *Escherichia coli*. *Nat Rev Microbiol*. 2004;2(2):123–40. doi:10.1038/nrmicro818.
- Waturangi DE, Hudiono F, Aliwarga E. Prevalence of pathogenic *Escherichia coli* from salad vegetable and fruits sold in Jakarta. *BMC Res Notes*. 2019;12(1):247. doi:10.1186/s13104-019-4284-2.
- Azad MA, Rahman A, Rahman M, Amin R, Begum M, Ara I, et al. Susceptibility and multidrug resistance patterns of *Escherichia coli* isolated from cloacal Swabs of Live Broiler Chickens in Bangladesh. *Pathog*. 2019;8(3):118.
- Ozen M, Dinleyici EC. The history of probiotics: the untold story. *Beneficial Microbes*. 2015;6(2):159–65. doi:10.3920/bm2014.0103.
- Fuller R, Gibson GR. Probiotics and prebiotics: microflora management for improved gut health. *Clin Microbiol Infect*. 1998;4(9):477–80. doi:10.1111/j.1469-0691.1998.tb00401.x.
- McGhee JR. Mucosal immune responses. An overview. *Mucosal Immunol*. 1999;p. 485–505.
- Cukrowska B, Lođňnová-Žádníková R, Enders C, Sonnenborn U, Schulze J, Tlaskalová-Hogenová H. Specific Proliferative and Antibody Responses of Premature Infants to Intestinal Colonization with Nonpathogenic Probiotic *E. coli* Strain Nissle 1917. *Scand J Immunol*. 2002;55(2):204–9. doi:10.1046/j.1365-3083.2002.01005.x.
- Nissle A. Old and new experiences on therapeutic successes by restoration of the colonic flora with mutaflo in gastrointestinal diseases. *Die Med Welt*. 1961;29:1519.
- Williams PC, Berkley JA. Guidelines for the treatment of dysentery (shigellosis): a systematic review of the evidence. *Paediatr Int Child Health*. 2018;38:50–65.
- Christopher PR, David KV, John SM, Sankarapandian V. Antibiotic therapy for *Shigella* dysentery. *Cochrane Database Syst Rev*. 2010;8:CD006784.
- Begum J, Mir NA, Dev K, Khan IA. Dynamics of antibiotic resistance with special reference to Shiga toxin-producing *Escherichia coli* infections. *J Appl Microbiol*. 2018;125(5):1228–37. doi:10.1111/jam.14034.
- Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrob Resist Infect Control*. 2017;6(1):47. doi:10.1186/s13756-017-0208-x.
- Cowan ST. Cowan and Steel's manual for the identification of medical bacteria. Cambridge University Press; 2004.
- Lower SK. Bacterial Recognition of Mineral Surfaces: Nanoscale Interactions Between *Shewanella* and alpha-FeOOH. *Sci*. 2001;292(5520):1360–3. doi:10.1126/science.1059567.
- no MIQO, Colmenero JDD, Macias M, Bravo MJ, Morata P. Preparation of Bacterial DNA Template by Boiling and Effect of Immunoglobulin G as an Inhibitor in Real-Time PCR for Serum Samples from Patients with Brucellosis. *Clin Vaccine Immunol*. 2008;15(2):293–6. doi:10.1128/cvi.00270-07.
- Mina SA, Marzan LW, Sultana T, Akter Y. Quality assessment of commercially supplied drinking jar water in Chittagong City, Bangladesh. *Appl Water Sci*. 2018;8(1):24. doi:10.1007/s13201-018-0673-2.
- Hentges DJ. Role of the intestinal microflora in host defense against infection. In: Human intestinal microflora in health and disease. New York: Academic Press; 1983. p. 311–31.
- Rahman SM. Probiotic properties analysis of isolated lactic acid bacteria from buffalo milk. *Arch Clin Microbiol*. 2015;7(1):6.
- Cursino L, Smajs D, Smarda J, Nardi RMD, Nicoli JR, Chartone-Souza E, et al. Exoproducts of the *Escherichia coli* strain H22 inhibiting some enteric pathogens both in vitro and in vivo. *J Appl Microbiol*. 2006;100(4):821–9. doi:10.1111/j.1365-2672.2006.02834.x.
- Bury S, Soundararajan M, Bharti R, von Bünau R, Förstner KU, Oelschlaeger TA. The Probiotic *Escherichia coli* Strain Nissle 1917 Combats Lambdaoid Bacteriophages stx and λ. *Front Microbiol*. 2018;9:929. doi:10.3389/fmicb.2018.00929.
- Sarker MS, Mannan MS, Ali MY, Bayzid M, Ahad A, Bupasha Z. Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh. *J Adv Vet Anim Res*. 2019;6(3):272. doi:10.5455/javar.2019.f344.
- Ghosh S, Bupasha Z, Nine H, Sen A, Ahad A, Sarker MS. Antibiotic resistance of *Escherichia coli* isolated from captive Bengal tigers at Safari parks in Bangladesh. *J Adv Vet Ani Res*. 2019;6(3):341. doi:10.5455/javar.2019.f352.
- Odonkor ST, Ampofo JK. *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiol Res*. 2013;4(1):2. doi:10.4081/mr.2013.e2.
- Hoover DG, Steerson LR. Bacteriocins of lactic acid bacteria. 1st ed.; 1993.
- Dezfulian A, Zadeh AM, Aslani MM, Dabiri H, Zali MR. Influence of the probiotic *Escherichia coli* strain Nissle 1917 on the growth of different pathogenic bacteria isolated from patients with diarrhea. *Gastroenterol Hepatol Bed Bench*. 2009;1(3):113–7.
- Jiang Y, Kong Q, Roland KL, Wolf A, Curtiss R. Multiple effects of *Escherichia coli* Nissle 1917 on growth, biofilm formation, and inflammation cytokines profile of *Clostridium perfringens* type A strain CP4. *Pathog Dis*. 2014;70:390–400. doi:10.1111/2049-632x.12153.

Author biography

A M Masudul Azad Chowdhury, Associate Professor

Sanjida Akter, MS

Sohana Akter Mina, Assistant Professor

Cite this article: Chowdhury AMMA, Akter S, Mina SA. Isolation, identification and functional characterization of *Escherichia coli* as probiotic against *Shigella* in Bangladesh. *Indian J Microbiol Res* 2020;7(4):313-321.