ANTIMICROBIAL ACTIVITIES OF LACTIC ACID BACTERIA FROM GUT OF *CATLA CATLA* (HAMILTON, 1822)

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Abstract

This research aims at assessing and determining the antimicrobial activity of Lactic acid bacteria (LAB) from the gut of *Catla catla* (Catla). Samples of fish were collected from fishery pond near Hinthada Township, Ayeyarwaddy Region. Isolation of LAB was undertaken by serial dilution method on deMan Rogosa Sharpe (MRS) medium, supplemented with 0.5% $CaCO_3$. They were identified based on colony morphology, cell morphology, gram staining, acid fast staining, MR, VP, indole, catalase and motility tests. Total of five strains were isolated. Most isolated strains were gram positive, catalase negative and non- motile, and all were non-acid fast. Colony forming unit (CFU/mL) of isolated bacteria were counted by spread plate technique. CFU were ranging from $24x10^8$ to $30x10^{10}$ CFU/mL. Isolated bacteria were also tested for antimicrobial activities using paper diffusion assay against *Saccharomyces cerevisiae, Argobacterium tumefaciens, Pseudomonas fluorescens* and *Escherichia coli*. The diameters of the inhibition zones were ranged from 8.4 to 14.7 mm. Out of five isolates, two strains (Cc1 and Cc2) showed antimicrobial activity against four test organism and inhibition zones were significantly higher ($p< 0.05$) than that of other isolates. These strains demonstrated high antimicrobial properties and could be used as starters for probiotic in fishery pond feeding.

Keywords: Lactic acid bacteria, Antimicrobial activities, *Catla catla*

Introduction

Lactic acid bacteria produce many kinds of metabolites which might affect the other microbes in the gut. Lactic acid bacteria (LAB) are the most common types of microbes used as probiotics which are safely applied in medical and veterinary function (Divakara *et al.*, 2010). Many lactic acid bacteria (LAB) are proved to function as probiotics, which are benefit to host health, when ingested in sufficient quantities.

The beneficial role played by this microorganism in humans and animals, including effect on the immune system has been extensively reported (Perdigon *et al*., 1992). The Lactic acid bacteria are present in the intestine of most animals. The colonization of the gut by probiotic bacteria prevents growth of harmful bacteria by competition exclusion and by the production of organic acid and antimicrobial compounds (Buntin *et al*., 2008).

Lactic acid bacteria were not dominant population in fish, it has been well documented in several investigations that lactic acid bacteria are a part of the native microbiota of aquatic animals from temperature regions (Ringø, 2004). Lactic acid bacteria (LAB) are a group of gram positive, cocci or rods, catalase negative and fastidious organisms. Lactic acid bacteria have attained major attention for probiotic activity and have generally been considered as good probiotic organisms (O'sullivan *et al*., 2002).

Yasuds and Taga (1980) suggested that probiotic bacteria would be found to be useful not only as food but also biological controllers of fish disease and activators of nutrient regeneration. The optimization of the use of probiotic *Lactobacilli* for the gastrointestinal disorders requires the knowledge of their antibiotic resistance to reinforce the concomitant antibiotic therapy (Salminen *et al.*, 1998).

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Probiotic bacteria would be found to be useful not only as food but also serves as biological controllers of fish disease and activators of nutrient regeneration. Nowadays the focus is not merely on bacteria collected from fermented dairy products but also bacteria collected from the intestine (Sanders, 1999). Fish intestinal tract is considered to be valuable waste and a good source for LAB isolation. LABs are a group of Gram-positive, non-motile, cocci or rods, catalase negative. Various authors have shown that lactic acid bacteria are also part of the normal intestinal flora of fish (Ringø *et al*, 1998).

There are increased understanding of probiotics use of LAB would lead to the development of natural antibiotic and reduce the dependency on chemical or drug uses in aquaculture (Subasinghe, 1997). In addition to the numerous investigations demonstrating the presence of LAB in the digest tract of several different fish species several studies have reported on the isolation of LAB from cold-smoked and fermented fish. Based on that this research was conducted to obtain Lactic acid bacteria, and then to select and characterize its ability.

In the future, lactic acid bacteria can be used as a probiotics to support the feeding of fishes. Keeping this in view, the present study was undertaken with the following objectives: to isolate the lactic acid bacteria from gut of *Catla catla* (Nga gaung pwa), to study the colony morphology of isolated LAB and investigate the cell characters and staining reactions of isolated bacteria species and to investigate the antimicrobial activities of isolated lactic acid bacteria from fish gut.

Materials and Methods

Study Period

This research was conducted at the laboratory of Zoology Department, Pathein University during July to November, 2019.

Collection of samples

Fish sample of *Catla catla* was collected from the fishery pond near Hinthada Township, Ayeyarwady Region (Site-I, 17°41′ 46″ N and 95°25′ 37″ E) and (Site -II, 17°41′ 46″ N and 95°25′ 38″ E) (Fig. 1). Fish samples were put into sterilized polythene bag and carried to the laboratory of Zoology Department, Pathein University by using ice-box. Samples were stored in the refrigerator at 4℃ for further study.

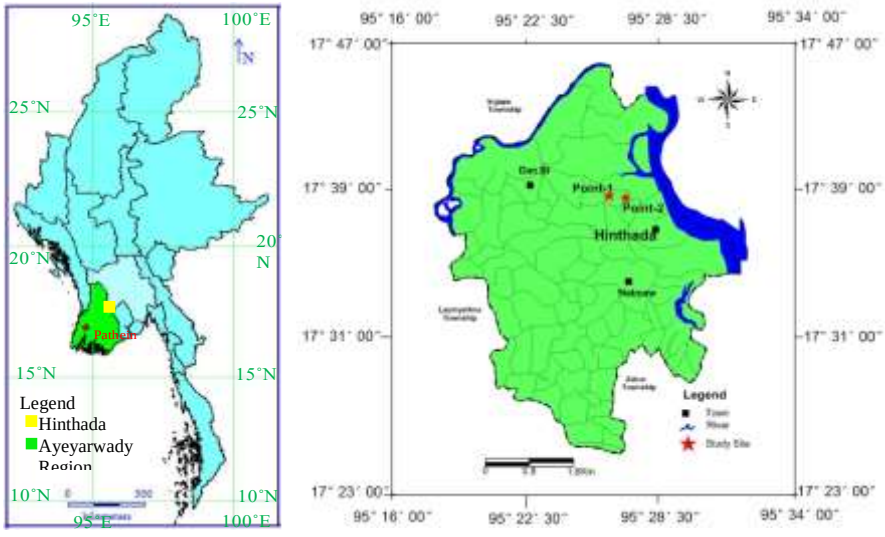


Figure 1 Map of sample collected area (Source: DIVA-

Culture of bacteria

The length and weight of the fish were measured before dissection. The skin was washed with 70% ethanol before opening the ventral surface with sterile scissors. The fish were dissected to remove the gut, one gram of intestine was taken from each fish sample.

Isolation of pure culture

Then the gut surface was sterilized with ethanol and washed with distilled water for three time and cut into small pieces with sterilized scissors. These pieces were mixed 9 mL of sterile saline diluent (0.85% NaCl). This mixture was shaking agitatedly. Then serially diluted to 10 fold dilutions and plated on deMan Rogosa Sharpe (MRS) media (Himedia, India) containing 0.5% $CaCO_3$. Then, 20 µL of bacterial suspensions from each dilution was inoculated onto MRS agar and incubated at 31℃ for three days. Clear zone forming colonies on MRS agar were selected as Lactic acid bacteria. Streak plate method was used to purify these selected bacteria.

For pure culture from plate to test tube, about 100mL of culture medium were separately distributed into test tubes. The test tube were plugged with cotton wool and sterilized by autoclaving at 1.05 kg per cm^2 (15 lb per in^2) for 15 minutes at 121℃. The sterilized media were cooled down. Each of separate colonies on petridishes was taken out to streak on the slant medium to obtain pure culture (Atlas, 1997).

Study of morphology

Colony and cells characteristics were studied that is size, shape, colour, opacity, elevation, margin, morphology, gram staining, acid fast staining nature.

Gram staining

A drop of normal saline was placed on clean grease free slide. A small loop of isolated bacteria was smeared on the slide and allows it to dry. The smear was fixed by passing the dried slide three or four times rapidly over a flame. Covered the slide with crystal violet stain and allow it to act for one minute. Then, the slide was rinsed with distilled water for a few seconds. The slide was covered with fresh iodine solution and allowed it to act for about one minute. Add the alcohol drop by drop and stop adding alcohol when no more colour flows out from the smear. As a counter stain, the smear was covered with safranin for about 20-30 seconds and washed with distilled water. Then the slide was dried. The stained slide was examined under the oil immersion objective of the microscope.

Acid fast staining

Bacterial was smeared on clean and grease free slide. Allow smear to air dry and then heat fix. A generous amount of carbol fuchsin stain was applied over the smear. Heat the stain until vapour. Allow the heated stain to remain on the slide for 5 minutes. After five minutes, the slide was cooled and gently washed with distilled water. Acid alcohol was used to decolorize the stain and then methylene blue was used to flood the smear for 2 minutes and air dry. Then, observed under oil immersion lens.

Catalase test

Two to three drops of 3% H_2O_2 were placed on a clean dry glass slide. A bacterial colony was picked using a clean sterile straight wire loop and mixed into the 3% H_2O_2. Production of bubbles in 5-10 second is a positive test. No appearance of bubbles or appearance after 30 seconds is considered negative.

Motility

Motility of the isolated bacteria can be detected in semi-solid agar medium. Ten milliliter of semi-solid agar was dispensed in test tubes. The tubes containing the medium were inoculated by stabbing with straight wire to about half the depth of the medium. After incubation, motile bacteria will spread into the medium and non- motile will confine to the stab.

Biochemical characterization

Methyl red test, Voges-Proskauer test and Indole test were performed.

Methyl red test

Sterilized MR broth was inoculated by a loopful of pure bacterial isolate and incubated at 32℃ for 24 hour. 5mL broth cultured was aseptically transferred into test tube and three drops of methyl red was added. Red colour formation within 15minutes is a positive result. No red colour formation after 15 minutes is a negative result.

Voges-Proskauer test

Sterilized VP broth was inoculated by a loopful of pure bacterial isolate and incubated at 32℃ for 24 hour. The broth cultured 5mL was dispensed into test tube to perform Voges-Proskauer test. Three drops of Barritt's reagent A was added to broth cultured and shaken slightly. And then equal amount of Barritt's reagent B was added into broth cultured. The test tube was kept still for 15 minutes. Appearance of red colour on the reagent layer signified positive. No change colour is negative.

Indole test

Test tubes containing 5mL of sterilized tryptone broth were inoculated with isolated bacteria. After 24h incubation, three drops of Kovac's reagent was added. A positive result is shown by presence of red colour in the surface alcohol layer of the broth. A negative result appears yellow.

Enumeration of lactic acid bacteria by standard plate count method

All isolated bacteria species associated from fish gut of *Catla catla* were enumerated. The isolated of lactic acid bacteria from slant cultures were placed and grow in peptone water about 24h and then streaked on de Man Rogosa Sharpe (MRS) agar medium plates. After growing, few bacteria are picked out with sterile loop and inoculated into the test tubes containing of peptone water. These tubes were incubated at 32℃ for 24h. Ten fold dilution were then prepared with sterile distilled water and 20µL of each dilution was spread on the surface of plate count agar with three replicates. The agar plates were incubated at 32℃ for 24h. After incubation the number of colonies was counted and the broth culture of viable cell per milliliter was calculated as suggested by Reynolds and Perez-Ramos (2009).

$$\text{Colony forming unit per milliter or gram} = \frac{\text{Number of colonies}}{\text{Dilution factor x amount plated}}$$

Antimicrobial assay

Antagonistic assay was done on assay medium. *Saccharomyces cerevisiae* NITE52847, *Agrobacterium tumefaciens* IFO5431, *Pseudomonas fluorescens* IFO94307 and *Escherichia coli* AHU5436 were sub cultured in nutrient agar medium. These test organisms were supported by

NITE Japan in 2004 and maintained at PBDC, Pathein University. Each test organism culture was suspended in peptone water. 0.2 mL suspension of test organisms was cultured on assay medium.

Isolated LAB strains were inoculated into 10 mL of seed medium at 32℃ for 3 days. One mL of seed medium culture was inoculated into fermentation medium at 32℃ for 3 days. And then sterilized paper discs were dipped into each LAB broth cultured (10^8 CFU/mL) of fermentation medium. And then paper discs were dried in the Biosafety cabinet for three hours and these discs were placed on assay medium with test organism. Control paper discs were also dipped into fermentation medium and placed on assay medium. Tests and control plates were incubated at 32℃ for 3 days and then measured the inhibition zone by using digital callipers. Five replicate were carried out to test the antimicrobial activity of isolated bacteria.

Identification of Bacteria and Plants

Bacterial species identification was followed after Breed *et al.,* (1957) Buchanan, Gibbons (1974) and Holt *et al*. (1994). Identification of the fish species was made according to Jayaram (2013).

Statistical analysis

The antimicrobial activities were subjected to analysis of variance and means of sample were compared by least significance difference (LSD) using IBM-SPSS software (version 25).

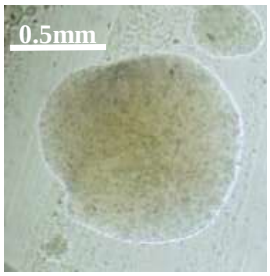
Results

Totally five strains of lactic acid bacteria were isolated from the gut of *Catla catla*. Isolated strains from fish gut of *Catla catla* were designated as Cc-1 to 5. Most isolated strains were gram positive, catalase negative, non- motile, rod and cocci bacteria and all were non-acid fast. Methyl test, Voges-Proskauer test and Indole test were performed and all LAB strains showed negative in these tests (Table 1 and 2). Colony forming units of Lactic acid bacteria were ranging from 24.38 x 10^8 to 30.31 x 10^{10} CFU/mL. Lactic acid bacteria from gut of *Catla catla* Cc-1 (30.31 x 10^{10} CFU/mL), Cc-2 (32.81 x 10^9 CFU/mL) and Cc-3 (24.38 x 10^8 CFU/mL) were the good growth rate strains. The antimicrobial activity of LAB was tested against four test organisms by using paper disc diffusion method. The result showed that LAB inhibited *Saccharomyces cerevisiae, Agrobacterium tumefaciens, Pseudomonas fluoresence,* and *Escherichia coli*. The diameters of the inhibition zones were varied and ranged between 8.4 to 14.7 mm.

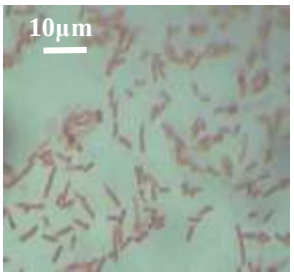
Lactic acid bacteria from gut of *Catla catla*

The colony morphology of Cc-1 was circular, Creamy-White, entire and convex. The single colony on MRS medium was 1.0-1.6 mm in diameter and on nutrient was 1.0-1.6 mm in diameter. Cells were rod shape with 2.25-3.6 μm in width, gram positive, non-acid fast, singly and pair, non-motile and catalase negative. The colony morphology of Cc-2 was circular, yellow, entire and convex. The single colony on MRS medium was 1.3-1.9 mm in diameter and on nutrient was 1.0-1.4 mm in diameter. Cells were rod shape with 1.8-2.7 μm in width, gram positive, non-acid fast, singly and pair, non-motile, and catalase negative. The colony morphology of Cc-3 was circular, yellow, entire and convex. The single colony on MRS medium was 1.0-1.6 mm in diameter and on nutrient was 1.0-1.4 mm in diameter. Cells were short rod shape with 1.8-2.7 μm in width, gram positive, non-acid fast, singly and pair, non-motile, and catalase negative. The colony morphology of Cc-4 was circular, yellow, entire and convex. The single colony on MRS medium was 1.0-1.5 mm in diameter and on nutrient was 1.0-1.5 mm in diameter. Cells were rod shape with 2.25 -3.6 μm in width, gram negative, non-acid fast, singly and pair, slightly motile and catalase

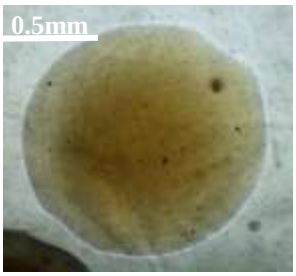
positive. The colony morphology of Cc-5 was circular, yellow, entire and flat. The single colony on MRS medium was 1.0-1.4 mm in diameter and on nutrient was 1.0-1.5 mm in diameter. Cells were cocci shape with 1.8-2.7 µm in diameter, gram positive, non-acid fast, singly and chain, non-motile, and catalase negative (Fig. 2).



A. Colony of Cc-1 on MRS medium

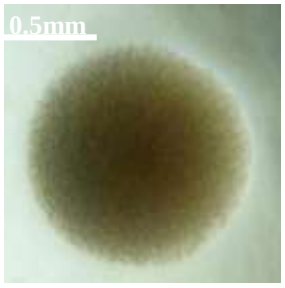


B. Gram staining of Cc-1

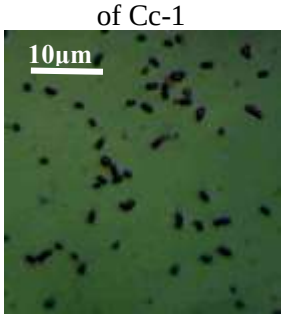


C. Colony of Cc-1 nutrient medium

D. Acid fact staining of Cc-1

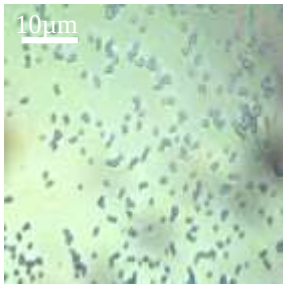


A. Colony of Cc-2 on MRS medium

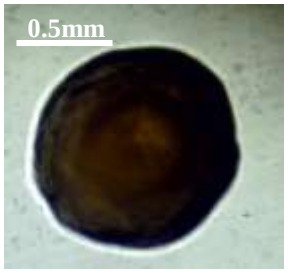


B. Gram staining of Cc-2

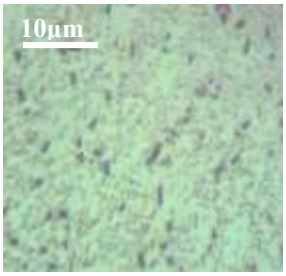
C. Colony of Cc-2 on nutrient medium



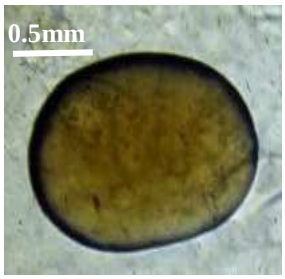
D. Acid fact staining of Cc-2



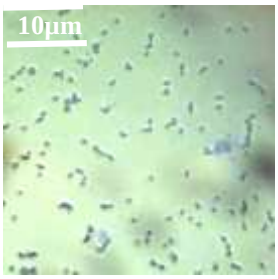
A. Colony of Cc-3 on MRS medium



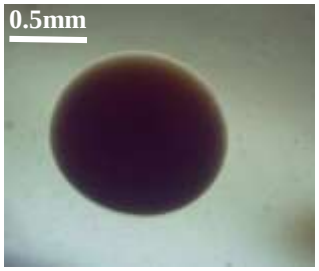
B. Gram staining of Cc-3



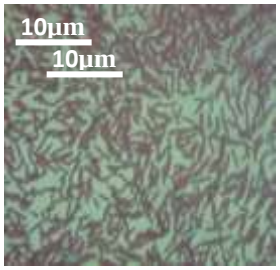
C. Colony of Cc-3 on nutrient medium



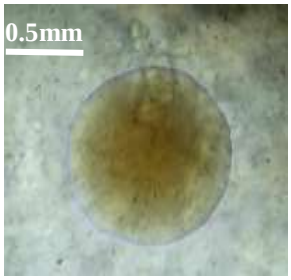
D. Acid fact staining of Cc-3



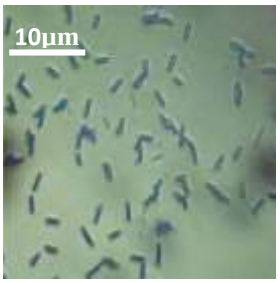
A. Colony of Cc-4 on MRS medium



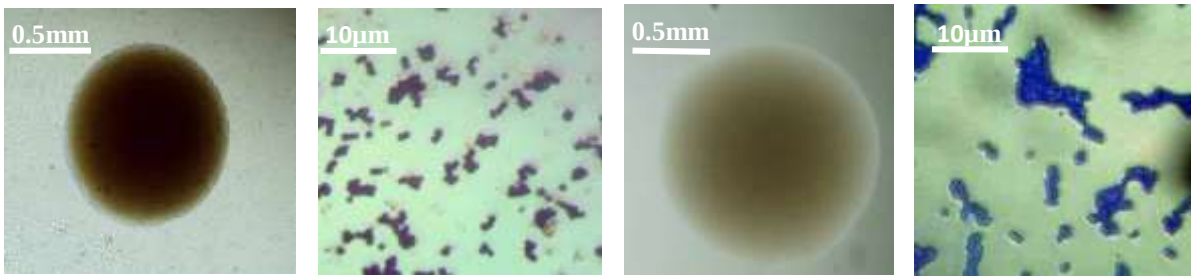
B. Gram staining of Cc-4



C. Colony of Cc-4 on nutrient medium



D. Acid fact staining of Cc-4

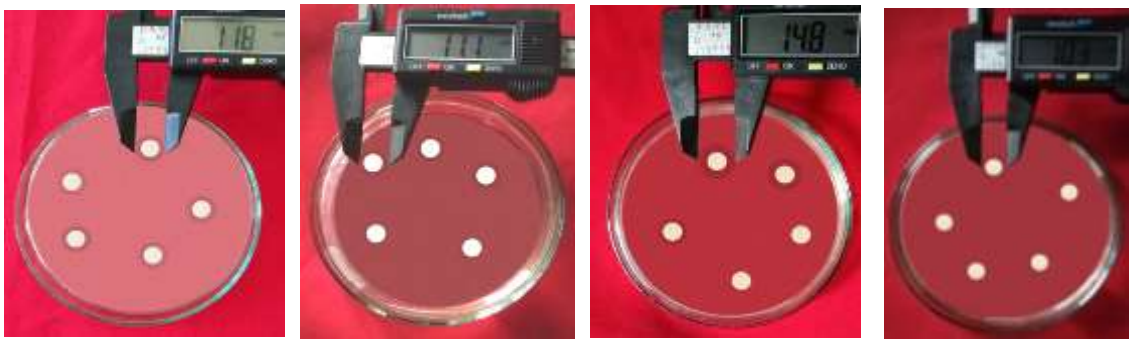


A. Colony of Cc-5 on MRS medium | B. Gram staining of Cc-5 | C. Colony of Cc-5 on nutrient medium | D. Acid fact staining of Cc-5

Figure 2 Colony and cell morphology of isolated bacteria

Antimicrobial activities of isolated lactic acid bacteria

The antimicrobial activity of LAB was tested against four test organisms by using paper disc diffusion method. The result showed that LAB inhibited *Saccharomyces cerevisiae*, *Agrobacterium tumefaciens*, *Pseudomonas fluoresence*, and *Escherichia coli*. For *S. cerevisiae* (11.44 mm, 12.38 mm, 10.8 mm, 0 mm and 10.94 mm respectively), for *A. tumefaciens* (12.06 mm, 13.72 mm, 0 mm, 10.16 and 0 mm respectively), for *P. fluoresence* (13.6 mm, 13.78 mm, 11.22 mm, 8.4 mm and 8.4 mm respectively), and for *E. coli* (10.3 mm, 14.7 mm, 10.84 mm, 0 mm and 8.4 mm respectively) (Fig. 3). Inhibition zone of isolated strains of LAB against test organisms were significantly different ($p < 0.05$) from each other. Cc-2 showed the highest zone of inhibition against four test organisms and significantly inhibited ($p<0.05$) test organisms than other LAB strains. Cc-1 showed second largest zone and significantly inhibited ($p<0.05$) the test organisms than Cc-3, Cc-4 and Cc-5. Cc-3 can inhibit only three test organisms while Cc-4 and Cc-5 can against only one test organism (Table 3, 4 and Fig. 4).



(i) *S. cerevisiae* (ii) *A. tumefaciens* (iii) *P. fluorescens* (iv) *E. coli*

A. Antimicrobial activity of *Cc1* against four test organisms

(i) *S. cerevisiae* (ii) *A. tumefaciens* (iii) *P. fluorescens* (iv) *E. coli*

B. Antimicrobial activity of *Cc2* against four test organisms

Figure 2 Antimicrobial activity of isolated bacteria against four test organisms

Table 1 Colony morphology of isolated LAB strains

LAB strains	Size (mm)	Shape	Colour	Margin	Elevation
Cc-1	1.0-1.6	Circular	Creamy-White	Entire	Convex
Cc-2	1.3-1.9	Circular	Yellow	Entire	Convex
Cc-3	1.0-1.6	Circular	Yellow	Entire	Convex
Cc-4	1.0-1.5	Circular	Yellow	Entire	Convex
Cc-5	1.0-1.4	Circular	Yellow	Entire	Flat

Table 2 Cells morphology, staining reactions and motility test of isolated LAB strains

LAB Strains	Cell size (µm)	Shape	Arrangement	Gram reaction	Acid fast reaction	Motility	Catalase test
Cc-1	2.25-3.6	Rod	Singly and pair	Positive	Non-acid fast	Non motile	Negative
Cc-2	1.8-2.7	Rod	Singly and pair	Positive	Non-acid fast	Non motile	Negative
Cc-3	1.8-2.7	Short rod	Singly and pair	Positive	Non-acid fast	Non motile	Negative
Cc-4	2.25-3.6	Rod	Singly and pair	Negative	Non-acid fast	Slightly motile	Positive
Cc-5	1.8-2.7	Cocci	Singly and chain	Positive	Non-acid fast	Non motile	Negative

Table 3 Analysis of antimicrobial activities of isolated LAB strains

		Sum of Squares	df	Mean Square	F	Sig.
S. cerevisiae	Between Groups	526.594	4	131.649	898.012	.000
	Within Groups	2.932	20	.147		
	Total	529.526	24			
A. tumefaciens	Between Groups	892.854	4	223.214	681.360	.000
	Within Groups	6.552	20	.328		
	Total	899.406	24			
P. fluorescens	Between Groups	140.124	4	35.031	63.600	.000
	Within Groups	11.016	20	.551		
	Total	151.140	24			
E. coli	Between Groups	593.314	4	148.329	879.766	.000
	Within Groups	3.372	20	.169		
	Total	596.686	24			

Table 4 Comparison of antimicrobial activities of isolated LAB strains (n=5)

LAB Strains	Inhibition Zone against Test Organisms (mean ± SD mm)			
	S. cerevisiae	***A. tumefaciens***	***P. fluorescens***	***E. coli***
Cc1	11.44 ± 0.38^{b}	12.06 ± 0.63^{b}	13.6 ± 0.90^{b}	10.3 ± 0.19^{a}
Cc2	12.38 ± 0.36^{c}	13.72 ± 1.10^{c}	13.78 ± 1.13^{b}	14.7 ± 0.53^{b}
Cc3	10.8 ± 0.52^{a}	-	11.22 ± 0.27^{a}	10.84 ± 0.48^{a}
Cc4	-	10.16±0.15	+	-
Cc5	10.94 ± 0.44^{a}	-	+	+

+ = inhibition zone <10, - = no inhibition zone, Paper disc = 7mm

Means with different superscript within same column were significantly different at $p< 0.05$

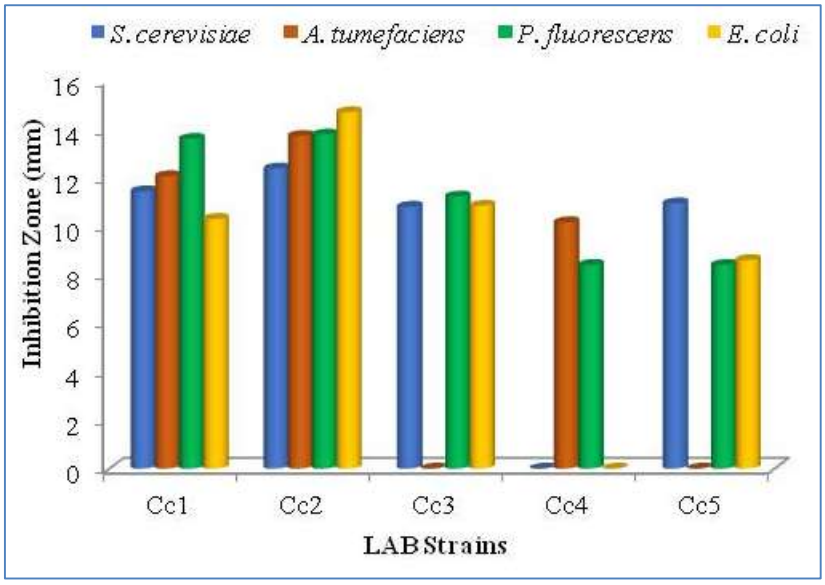


Figure 3 Antimicrobial activity of LAB strains against test organisms

Discussion

In this research, LAB strains were isolated by the formation of clear zone on the MRS medium. The clear zone appearance is due to the dissolution of $CaCO_3$ on MRS medium by acid agent (Panthavee *et al.*, 2007). The clear zone around colonies indicated that the bacteria were able to produce acid substances. *Bacillus sp*. as non-lactic acid bacteria was competent in producing acids (Prihanto *et al*., 2020). These selected isolates have similar characteristic which are gram positive bacteria, rod shaped, catalase negative, non-motile. These results were also in line with the finding of Lawalata *et al.* (2011) who reported that the LAB isolates are gram positive, rod shape, catalase negative and non-motile. LAB colonies were circular, flat, low convex with entire margin. All member of lactic acid bacteria were gram positive rods or cocci found in chain, singly and in pairs (Bukola and Onilude, 2008). The nature of lactic acid bacteria was positive in gram character, non-motile, and negative calalyze (Axelsson, 2004).

They can live singly in pairs or irregular cluster and sometimes in chains of varying length. Isolated bacteria in this work are similar with the above statements for lactic acid bacteria. Five strains were isolated of which micromorphologically one was coccus, four were bacilli. Jack *et al*. (1995) reported that the isolated LAB species conformed by the methyl red and catalyzed negative test. In this work isolation of lactic acid bacteria are methyl red and catalyzed negative test. Reynolds and Perez-Ramos (2009) used standard plate count method by spreading 100µL of a given dilution on the entire surface of nutrient agar plates. Only the dilutions producing 30 to 300 colony forming units were enumerated. In this study counting methods were the same above work. From the five isolates Cc-1 (30.31 $x10^{10}$ CFU/mL), Cc-2(32.81 x 10^9 CFU/mL) and Cc-3 (24.38 x 10^8 CFU/mL) were the best grown bacteria.

Davidson and Paris (1989) screened the antimicrobial activity of LAB from fish aganist *Escherichia coli* ATCC 35218, *Stapylococcus aureus* ATCC 25923, *Pseudomonas fluorescens* FNCC 0070 was performed. Five isolates of Lactic acid bacteria in this work were tested for the antimicrobial activity of LAB aganist *Saccharomyces cerevisiae, Agrobacterium tumefaciens, Pseudomonas fluorescens* and *Escherichia coli.*

Kim and Austin (2008) reported that antibiotic sensitivity test was carried out in aquaculture by disc diffusion technique. The plates were incubated at 32°C for 24 hour to observe and measure the inhibition zone. Hamid *et al.* (2012) reported that the antagonistic activity of the

isolated LAB against *Salmonella typhimrium* and *Escherichia coli* were determined by using agar disc diffusion.

In this research antibiotic sensitivity test was carried out by using disc diffusion method and bacteria were incubated at 32℃ for 24 hours. The diameters of the inhibition zones of isolated bacteria were varied and ranged between 8.4 to 14.7 mm. This result was very close to the results of Lawalata *et al.* (2011) who studied the antimicrobial activity of LAB from fish and they stated that the diameters of the inhibition zones were ranged between 3.0 mm to 15.0 mm.

From the five isolates, two bacterial isolates Cc-1 and Cc-2 were the best LAB against on four test organisms; *S. cerevisiae, A. tumefaciens, P. fluorescens* and *E. coli*, and inhibition zones were significantly higher ($p< 0.05$) than other isolated LAB strains.

The report of these findings is similar to the work reported by Rodríguez *et al*. (2012) who reported that LAB were effective against many pathogenic bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The antagonistic activity of LAB metabolites against the spoilage bacteria also agrees with the findings of Vasiee *et al*. (2018).

The inhibitory effect caused by LAB strains can be considered that the LAB produced organic acids, especially lactic and acetic acids, exerting a strong inhibitory effect on Gram-negative and positive bacteria (Muruzović *et al*., 2018).

LAB strains are effective against a variety of bacterial pathogens and some food borne microorganisms; they can serve as alternative antimicrobial agents and food preservatives. This potential can be harnessed by the food and animal feeding industries on a large scale as bio-preservatives and probiotic instead of the chemical preservatives and antibiotic that we use which have some side effects (Adeyemo *et al*., 2018).

Conclusion

In conclusion, among five, two strains were also active more against *S. cerevisiae, A. tumefaciens, P. fluorescens* and *E. coli* than other strains. Cc-1 and Cc-2 showed maximum inhibition zone against all tested microorganisms and inhibition zones were significantly higher ($p< 0.05$) than that of other isolated LAB strains. Vine *et al.* (2004) reported that many studies on probiotics in aquaculture have used *in vitro* models of specific bacteria as antagonists of pathogens. Using the culture as probiotics could be expected to reduce antibiotics usage for animal feeding. Therefore, isolated bacteria have antimicrobial activity and they could be used as starter culture for antibiotic feed of animals.

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