©Sierra Leone Journal of Biomedical Research ISSN 2076-6270 (Print)
Vol. 3(3) pp. 128-132, December, 2011 ISSN 2219-3170 (Online)

Short Communication

Antibacterial Activities of Lactic Acid Bacteria Isolated from Selected Vegetables Grown in Nigeria: A Preliminary Report

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ABSTRACT

Members of lactic acid bacteria (LAB) are known probiotics and have been reported to have antimicrobial properties. Although various researchers have documented the isolation of these bacteria from fruits and vegetables, studies on LAB associated with lettuce, cucumber and cabbage are limited and non-existing in Nigeria. This study was designed to assess lettuce, cucumber and cabbage as potential sources of LAB and investigate the actions of their bacterial cell supernatants (BCS) on some pathogenic bacteria. Using standard microbiological methods, isolated LAB were identified to species level with API 50 CH kits (Biomerieux, France). Cell free supernatants (CFS) from de Man Rogosa Sharpe (MRS) broth cultures of the LAB strains were used to challenge *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 12900 and *Proteus penneri* ATCC 13315 by agar well diffusion method. The control consisted of the sterile MRS broth subjected to the same growth conditions as LAB broth cultures. A total of four lactic acid bacteria were isolated as follows: *Pediococcus pentosaceus* 2 from cucumber, *Lactobacillus cellobiosus* from cabbage, *Lactobacillus salivarius* and *Lactobacillus plantarum* 1 from lettuce. *Pediococcus pentosaceus* 2 and *L. salivarius* showed inhibitory effects on all the standard strains tested while *L. plantarum* 1 showed no inhibitory activity against *E. faecalis* and *E. coli. Lactobacillus cellobiosus* showed inhibition against all except *P. penneri*. Although, the molecular characterisation and probiotic potentials of these LAB strains are being investigated in an on-going study, we presumed these vegetables are prospective sources of the bacteria in Nigeria and therefore the need to extensively investigate the vegetables and other related vegetables becomes imperative.

Keywords: Antagonistic effect, Bacterial cell supernatant, Lactic acid bacteria, Vegetables

Received 2 September 2011/ Accepted 20 December 2011

INTRODUCTION

Lactic acid bacteria (LAB) are frequently isolated from fermented foods, diary/poultry products and gastrointestinal tracts of animals and humans. They may beneficially affect the host upon ingestion by a variety of proven mechanisms (Fayol- Messaoudi *et al.,* 2005; Ljungh and Wadstrom, 2006). Some of the beneficial effects of lactic acid bacteria consumption include improving intestinal tract health (Parvez *et al*., 2006), enhancing the immune system (Agerholm-Larsen *et al*., 2000; Chang *et al*., 2000), synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance (Marteau *et al*., 2001), decreasing the prevalence of allergy in susceptible individuals and reducing risk of certain cancers (Saikali *et al*., 2004; Parvez *et al*., 2006).

In addition, these organisms possess the potentials of acting as antibacterial agents especially in their ecological environment. This is due largely to the fact that they are capable to produce inhibitory substances such as bacteriocins, lactic acid, hydrogen peroxide, diacetyl, carbon dioxide and

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low molecular weight antibacterial substances (Piard and Desmazeaud, 1992; Khay *et al*., 2011). Adeniyi *et al.* (2006) reported varied inhibitory activities of lactic acid bacteria isolated from indigenous fermented diary foods against commonly encountered bacteria implicated in urinary tract infections. The killing activity of anti-*Salmonella* enterica serovar Typhimurium produced by *Lactobacillus* and *Bifidobacterium* strains in the presence of Luria broth (LB) has also been reported (Coconnier *et al.,* 1993; Bernet-Camard *et al.,* 1997; Lievin *et al.,* 2000). Most recently, inhibition of *Neisseria gonorrheae* (NG) by the co- cultivation of LAB with NG was reported by some workers to have been due to the acidification of the medium (Graver and Wade, 2011).

Although, there is a lot of research on isolation and characterisation of LAB, only a few of them have focused on isolation from fruits and vegetables (Trias *et al*., 2008; Chen *et al*., 2010; Padmaja *et al*., 2011**;** Ravi *et al*., 2011) and information on lactic acid bacteria from lettuce, cucumber and cabbage are limited. The isolation of lactic acid bacteria from fresh vegetables appears to be interesting since they can present affordable source of the organisms. These vegetables are readily available in Nigerian markets and are often served raw or as supplements to cooked foods especially rice. This study was therefore designed to isolate LAB from lettuce, cucumber and cabbage and investigate the antibacterial activity of their bacterial cell supernatants (BCS) on some standard strains of pathogenic bacteria.

MATERIALS AND METHODS

Source and Processing of Samples

Lettuce, Cucumber and cabbage were purchased from two local markets in Lagos, Nigeria (one sample of each vegetable per market), packaged into sterile plastic containers, transported to the laboratory and processed immediately to prevent deterioration. Each sample was blended separately (2.5g lettuce, 3.5g Cucumber and 4.5g Cabbage) with a blender (NAKAI JAPAN MAGIC BLENDER, MODEL 462). The blender compartment was flooded with boiled water after each blending and allowed to cool before loading the next vegetables. The blended samples were suspended in 20 ml of sterile de Man Rogosa Sharpe (MRS) broth (Oxoid, UK), incubated in candle extinction jar at 37^{o}C for 24 hrs. (Sarkono *et al*., 2010). The control consisted of un-inoculated, sterile MRS broth incubated under the same conditions as test cultures. The test cultures and the control were used to inoculate the MRS agar and incubated at the same conditions for another 48hrs. All Gram positive rods or coccal bacteria, catalase negative colonies were further confirmed with API 50 CH systems (Biomeriux, France) and the results interpreted using the API 50 CHL V5.0 software (Apiweb).

Standard Strains

The standard strains used in this study were *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 12900 and *Proteus penneri* ATCC 13315. The strains were obtained from Molecular Biology and Biotechnology Division of Nigerian Institute of Medical Research, Lagos, Nigeria. They were subcultured from 50% glycerol broth and purified using MacConkey agar (Oxoid, UK).

Antibacterial Assay

For this assay, 48hrs broth cultures of the LAB were centrifuged at 10000 rpm for 30min at 4^{o}C (Fayol-Messaoudi *et al.,* 2005) using Labnet centrifuge (USA, Model GL-18B). The supernatants were membrane filtered (Millipore, 0.22µm) and the pH was measured using pH meter (Consort C830, Belgium). The pH of sterile MRS broth and control were also taken. The control comprised of the un-inoculated MRS broth treated the same way as the LAB culture. The cell free supernatant (100µl) was transferred into well (6mm diameter) bored in Mueller Hinton agar previously seeded with 0.5 MacFarland of standard strains, equivalent to 10^{8} CFU/ml. The culture plates were incubated at 37^{o}C for 24hrs and the zones of inhibition measured in millimeter.

RESULTS

Four (4) LAB species were isolated from the vegetables as follows: Cucumber - *Pediococcus pentosaceus* 2, Cabbage - *Lactobacillus cellobiosus*, Lettuce - *Lactobacillus salivarius* and *Lactobacillus plantarum* 1 (Table 1).

Table 1: pH Profile of Lactic Acid Bacterial Cultures from Vegetables Investigated

Vegetable	Isolated LAB	pH of CFS
Cabbage	*L. cellobiosus*	3.92
Cucumber	*Pediococcus pentosaceus* 2	3.33
Lettuce	*L. salivarius*; *L.plantarum* 1	3.3; 3.63 respectively

Table 2: Antibacterial Activity* of LAB strains on Standard Bacteria

Standard strains	*L. cellobiosus*	*P. pentosaceus* 2	*L. salivarius*	*L. plantarum* 1
***P. aeruginosa* ATCC 27853**	7	13	13	11
***E. faecalis* ATCC 29212**	10	14	12	R
***E. coli* ATCC 12900**	8	11	10	R
***P. penneri* ATCC 13315**	R	11	9	8

**** (Inhibition zones in millimeter)***

Pediococcus pentosaceus 2 and *L. salivarius* showed inhibitory effects on all the standard strains tested while *L. plantarum* 1 showed no inhibitory activities against *E. faecalis* and *E. coli* but inhibited *P. aeruginosa* and *P. penneri. L. cellobiosus* showed inhibition against *E. faecalis, P. aeruginosa* and *E. coli* (Table 2). All the controls showed no zones of inhibition on the culture plates while there were ranges of inhibition on the plates incubated with LAB supernatant. The pH of control before and after incubation were 6.49 and 5.64 respectively while the pH range of all MRS broth cultures were within 3.30 and 3.92 (Table 1).

DISCUSSION

Lettuce, cucumber and cabbage are often served raw as supplements to some cooked food as salads. There are not many reports on the occurrence of LAB in these vegetables (Patil *et al.,* 2010). In this study, the results of the API identification system confirmed that three out of the four strains isolated from these vegetables were members of the genus *Lactobacillus*. All the isolates have morphological characteristics typical of lactic acid bacteria.

Although, other LAB species including *Lactobacillus plantarum* "group" have been recognised as predominating organisms in fermented cabbage (Tamang *et al*., 2005), in this present study, *Lactobacillus cellobiosus* was the main LAB isolated from cabbage. The major LAB species from fermented Chinese cabbage using Ca^{2+} medium plates were *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus minor and Lactobacillus fermentum* (Xiao *et al*., 2006). Differences between these studies and the present investigation could be ascribed to the isolation procedures. In order to optimise the isolation of LAB from these sources, it may therefore be necessary to investigate various isolation techniques in subsequent research. Nevertheless, strain isolated from cucumber was identified as *Pediococcus pentosaceus* 2. A similar study documented the isolation of *Pediococcus pentosaceus* from curd and cucumber (Patil *et al*., 2010).

Zwielehner and others concluded that in view of the importance of salad in many diets, lettuce may contribute to a constant supply of LAB (Zwielehner *et al.*, 2008). This study confirmed lettuces as reasonable source of LAB as *Lactobacillus salivarius and* Lactobacillus *plantarum* 1 were isolated from the vegetable. Previously, studies have also shown that different strains of LAB possess inhibitory activity against other bacterial pathogens (Olotu, 2007; Savino *et al*., 2011; Kivanc *et al*., 2011). The *in vitro* antibacterial assays employed in this study revealed that all the LAB strains had antibacterial activity against the standard strains, though at varied inhibitory spectrum. Contrary to our results, a study performed by Gomez *et al*. (2002), showed that none of the lactic acid bacteria strains from lettuce juice displayed inhibitory activity against *E. cloacae*, *A. hydrophila*, and *E. coli.* However, Kivanc *et al*. (2011) demonstrated the inhibitory performances of some lactobacilli from Turkish boza against a wide range of pathogenic organisms including *Pseudomonas aeruginosa.*

In this study, the pH range of the LAB culture supernatant was quite low, probably due to production of lactic acid and/ or other metabolites like hydrogen peroxide and diacetyl by these bacteria. This, however, needs to be confirmed by further study. Nonetheless, lactic acid bacteria produce metabolites which are widely reported to be responsible for their antagonistic properties (Gomez *et al*., 2002; Padmaja *et al*., 2011; Kivanc *et al*., 2011). For instance, *L. plantarum* has been shown to produce plantaricin (Bruno *et al.,* 1998) and Barrett *et al*. (2007) demonstrated that *L. salivarius* possessed some anti-listerial properties. In this study, both species were isolated from lettuce. Previous studies have shown that most of the selected strains of lactic acid bacteria had good antagonistic activity against pathogens including

Escherichia coli (Savino *et al*., 2011). The entire LAB identified in this study, except *P. pentosaceus* are known probiotics (Klein *et al.,* 1998). The association of pH, metabolites responsible for inhibitory activities and the probiotics potentials of the isolated LAB are being investigated in an on-going study. This will make it possible to recommend the bacteria as probiotics, especially in the management of certain diseases such as diarrhoea in our setting. Hypothetically, the anti-diarrhoeal activities of cabbage might be associated with the LAB contents since this vegetable is served raw.

In conclusion, lettuce, cucumber and cabbage have been found in this study to be carriers of some LAB and present potential sources of the organisms. So far, to the best of our knowledge, there is no record in the literature on the isolation of LAB from vegetables grown in Nigeria. Nevertheless, more samples of these vegetables from different localities would be investigated to establish the isolated LAB as their normal flora. A major limitation of the present investigation, however, is that the identification of the reported LAB species was based only on phenotypic characteristics and LAB strains were not confirmed by molecular methods. Detailed studies on the isolation and characterisation of these isolates and their potential as probiotics are currently being investigated.

ACKNOWLEDGEMENT

The authors appreciate the technical/scientific input of Mr. Bamidele Moses and Dr Francisca Nwaokorie.

REFERENCES

Adeniyi BA, Ayeni FA, and Ogunbanwo ST (2006). Antagonistic Activities of Lactic Acid Bacteria Isolated from Nigerian Fermented Diary food against Organisms Implicated in Urinary Tract Infection. *Biotech.* **5**: 183- 188

Agerholm-Larsen L, Raben A, Haulrik N, Hansen, A.S., Manders, M. and Astrup, A. (2000). Effect of 8 Week Intake of Probiotic Milk Products on Risk Factors for Cardiovascular Diseases. *Eur J Clin Nutr*. **54**: 288–289

Barrett E, Hayes M, O'Connor P, Gardiner G, Fitzgerald GF, Stanton C, Ross RP and Hill C (2007). Salivaricin P, one of a Family of Two-component Antilisterial Bacteriocins Produced by Intestinal Isolates of *Lactobacillus salivarius*. *Appl Environ Microbiol.* **73**:3719–3723

Bernet-Camard MF, Lievin V, Brassart D, Neeser JR, Servin AL, and Hudault S (1997). The Human *Lactobacillus acidophilus* Strain LA1 Secretes a Non-bacteriocin Antibacterial Substance (s) Active *In vitro* and *In vivo*. *Appl Environ Microbiol.* **63**:2747–2753

Bruno BJM, Sineriz F, Gonzalez de llano D, Rodriguez A and Suarez EJ (1998). Chemostat Production of Plantaricin C by *Lactobacillus plantarum* LL441. *Appl Environ Microbiol.* **64**: 3512–3514

Chang BL, Sheik YH, Wang LH, Liao CK and Gill HS (2000). Enhancing Immunity by Dietary Consumption of a Probiotic Lactic Acid Bacterium (Bifidobacterium lactis HN019): Optimization and Definition of Cellular Immune Responses. *Eur J Clin Nutr*. **54**:849-855

Chen Y, Wu H and Yanagida F (2010). Isolation and Characteristics of Lactic Acid Bacteria Isolated from Ripe Mulberries in Taiwan. *Brazilian J Microbiol.* **41**: 916- 921

Coconnier MH, Bernet MF, Kerne´is S, Chauvie`re G, Fourniat J, and Serving AL (1993). Inhibition of Adhesion of Enteroinvasive Pathogens to Human Intestinal Caco-2 Cells by *Lactobacillus acidophilus* strain LB Decreases Bacterial Invasion. *FEMS Microbiol Lett.***110**: 299- 306

Fayol-Messaoudi D, Berger CN, Coconnier-Polter M, Liévin-Le Moal V and Servin AL (2005). pH-, Lactic Acid-, and Non-Lactic Acid-Dependent Activities of Probiotic Lactobacilli against *Salmonella enterica* Serovar Typhimurium. *Appl Environ Microbiol*. **71**: 6008-6013

Gomez R, Munoz M, de Ancos B and Cano MP (2002). New Procedure for the Detection of Lactic acid Bacteria in Vegetables Producing Antibacterial Substances. *Lebensmittel-Wissenschaft und-Technologie*. **35**: 284-288

Graver MA and Wade J (2011). The Role of Acidification in the Inhibition of *Neisseria gonorrhoeae* by Vaginal Lactobacilli During Anaerobic Growth. *Annals Clin Microbiol Antimicrob.* **10**:8-12

Khay E, Idaomar M, Castro LMP, Bernárdez PF, Senhaji1 SN and Abrini J (2011). Antimicrobial Activities of the Bacteriocin-like Substances Produced by Lactic Acid Bacteria Isolated From Moroccan Dromedary Milk. *Afr J Biotechnol*. **10**(51):10447-10455

Kivanc M, Yilmaz M and Cakir E (2011). Isolation and Identification of Lactic Acid Bacteria from Boza, and Their Antimicrobial Activities Against Several Reporter Strains. *Turk J Biol*. **35**: 313-324

Klein G, Pack A, Bonaparte C and Reuter G (1998). Taxonomy and Physiology of Probiotic Lactic Acid Bacteria. *Int J Food Microbiol*. **41**:103-125

Lievin VI, Peiffer S, Hudault F, Rochat D, Brassart JR, Neeser, and Servin AL (2000). *Bifidobacterium* Strains from Resident Infant Human Gastrointestinal Microflora Exert Antimicrobial Activity. *Gut*. **47**:646–652

Ljungh A and Wadstrom T (2006). Lactic Acid Bacteria as Probiotics. *Curr. Issues Intest. Microbiol.* **7:**73–89

Marteau P, de Vrese, M., Cellier CJ and Schrezenmeir J (2001). Protection from Gastrointestinal Diseases with the Use of Probiotics. *Am J Clin Nutr*. **73**: 430S–436S

Olotu O (2007). Antagonistic Effect of *Lactobacillus* Isolates from Kunnu and Cowmilk on Selected Pathogenic Microorganisms. *Int J Food Safety*. **9**: 63-66

Padmaja GA, Ramachandra B, Manjunath H, Prabh R, Krishna R and Shankar PA (2011). Characterization of Lactic Acid Bacteria Isolated from Fruits and Vegetables for their Antibacterial Activity. *J Dairying Foods Home Sci*. **30**: 85-89

Parvez S, Malik KA, Ah Kang S and Kim HY (2006). Probiotics and their Fermented Food Products are Beneficial for Health. *J Appl Microbiol.* **100**: 1171–1185

Patil MM, Pal A, Anand T and Ramana KV (2010). Isolation and Characterisation of Lactic Acid Bacteria from Curd and Cucumber. *Indian J Biotech*.**9**:166-172

Piard JC and Desmazeaud M (1992). Inhibiting Factors Produced by Lactic Acid Bacteria: 2. Bacteriocins and other Antibacterial Substances. *Lait*. **72**(2): 113-142

Ravi V, Prabhu M and Subramanyam D (2011). Isolation of Bacteriocin Producing Bacteria from Mango Pulp and its Antimicrobial Activity. *J Microbiol Biotech Res.* **1**: 54-63

Saikali J, Picard V, Freitas M and Holt P (2004). Fermented Milks, Probiotic Cultures, and Colon Cancer. *Nutr Cancer*. **49**: 4–24

Sarkono, Faturrahman and Sofyan Y (2010). Isolation and Identification of Lactic Acid Bacteria from Abalone (*Haliotis asinina*) as a Potential Candidate of Probiotic. *Biosci Biotechnol Biochem*. **2**: 38-42

Savino F, Cordisco L, Tarasco V, Locatelli E, Di Gioia D, Roberto Oggero R and Matteuzzi D (2011). Antagonistic Effect of Lactobacillus Strains against Gas-producing Coliforms Isolated from Colicky Infants. *BMC Microbiol*. **11**:157 *http://www.biomedcentral.com/1471-2180/11/157*

Tamang JP, Tamang B, Schillinger U, Franz CMAP, Gores M and Holzapfel WH (2005). Identification of Predominant Lactic Acid Bacteria Isolated from Traditionally Fermented Vegetable Products of the Eastern Himalayas. *Int J Food Microbiol*. **105**: 347–356

Trias R, Bañeras L, Montesinos E and Badosa E (2008). Lactic Acid Bacteria from Fresh Fruit and Vegetables as Biocontrol Agents of Phytopathogenic Bacteria and Fungi. *Int Microbiol.* **11**: 231-236

Xiao J, Sun j, Wang X and Ren J (2006). Isolation and Identification of Lactic Acid Bacteria from Fermented Chinese Cabbage. *China Brewing*. **5**: *en.cnki.com.cn/Article_en/CJFDTOTAL-ZNGZ200605004.htm*

Zwielehner J, Handschur M, Michaelsen A, Irez S, Demel M, Denner EB and Haslberger AG (2008). DGGE and Real-time PCR Analysis of Lactic Acid Bacteria in Bacterial Communities of the Phyllosphere of Lettuce. *Mol Nutr Food Res*. **52**:614-623