



GLOBAL JOURNAL OF SCIENCE FRONTIER RESEARCH: C
BIOLOGICAL SCIENCE

Volume 14 Issue 5 Version 1.0 Year 2014

Type : Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Isolation and Phenotypic Characterization of Lactobacillus Sakei and Pediococcus spp. Antagonists from Algerian Meat

By M. Naimi & M. B. Khaled

Djillali Liabes University of Sidi Bel Abbes, Algeria

Abstract- The aim of the present work was to isolate antagonist cultures in order to use them in biopreservation. LAB were isolated from Algerian meat and characterized at the genus level based on phenotypic characteristics. That following these spot agar test was achieved to assess their potential antagonistic towards pathogens: *Bacillus cereus*, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC8739, *Salmonella Typhimurium* ATCC14028, *Staphylococcus aureus* ATCC6538, and *Pseudomonas aeruginosa*. Biochemical tests had ended this study to characterize the potent isolates at the species level. As a results, thirty LAB had been differentiated to: 53% belong to *Lactobacillus* or *Lactobacillus*-like; 23% to *Pediococcus*; 20% to *Lactococcus* or *Vagococcus*; and 4% to *Streptococcus*. The antagonist test had observed activity of five isolates against only *St. aureus* with inhibition zone ranging from 0.58 to 5.16 mm.

Keywords: *exploratory test, lab, crude bacteriocins, spoilage, pathogens.*

GJSFR-C Classification : *FOR Code: 069999*



Strictly as per the compliance and regulations of :



Isolation and Phenotypic Characterization of *Lactobacillus Sakei* and *Pediococcus* spp. Antagonists from Algerian Meat

M. Naimi^α & M. B. Khaled^σ

Abstract- The aim of the present work was to isolate antagonist cultures in order to use them in biopreservation. LAB were isolated from Algerian meat and characterized at the genus level based on phenotypic characteristics. That following these spot agar test was achieved to assess their potential antagonistic towards pathogens: *Bacillus cereus*, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC8739, *Salmonella Typhimurium* ATCC14028, *Staphylococcus aureus* ATCC6538, and *Pseudomonas aeruginosa*. Biochemical tests had ended this study to characterize the potent isolates at the spice level. As a results, thirty LAB had been differentiated to: 53% belong to *Lactobacillus* or *Lactobacillus*-like; 23% to *Pediococcus*; 20% to *Lactococcus* or *Vagococcus*; and 4% to *Streptococcus*. The antagonist test had observed activity of five isolates against only *St. aureus* with inhibition zone ranging from 0.58 to 5.16 mm. The five potent isolates vary mainly by the fermentation of: raffinose, sorbitol, dulcitol, l'esculine and D-mannitol, thus one had been identified as *Lactobacillus sakei* and four as *Pediococcus* spp.. This work showed our isolates as potential inhibitors to the growth of pathogens, suggesting the possibility to improve the hygienic quality of meat.

Keywords: exploratory test, lab, crude bacteriocins, spoilage, pathogens.

I. INTRODUCTION

Meat is rich in nutrients, so provided a desired environment for growth for different groups of micro-organisms (Guiraud et al. 1980; Stiles 1994; Bibek et al. 2008). Lactic acid bacteria are part of the initial microbiota, typically mesophilic which can grow easily at 5-45°C, under aerobic, anaerobic or microaerobic terms. These bacteria form a group of diverse genera with *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* that form the core of the group. However, from a practical food-technology point of view, the following genera are considered the principal: *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. Lactic acid bacteria; may be characterized as Gram-positive sphere or rod shaped, non-spore-forming, oxidase and catalase negative, do

not reduce nitrates to nitrite and sulfate to sulphide, able to produce lactic acid either by Homofermenter or Heterofermenter way; and are associated not only with meat but also with beverages, vegetables and dairy as well normal microbiota of mouth, intestinal and vaginal microbiota of mammals (Carr et al. 2002; Axelsson 2004; Doyle et al. 2006). Since do not pose any health risk to human there are designated as GRAS « Generally Recognized As Safe » organisms (Klaenhammer et al. 2005; Castellano et al. 2008; Dortu et al. 2009; Jeevaratnam et al. 2005). At this time, lactic acid bacteria are exploited as one of three cultures: probiotic, protective or starter (Carr et al. 2002; Castellano et al. 2008; Lücke 2000; Holzapfel 1995; Gálvez et al. 2007). Also using their antimicrobial end products such bacteriocins as dual anti- and probiotics is well-known. Lactic acid bacteria have been isolated and characterized from meat and meat products (Schillinger, and Lücke 1987; Morishita, and Shiromizu 1986; Samelis et al. 1994; Najjari et al. 2008; Bromberg et al. 2004; Jones et al. 2008; De-Martinis, and Freitas 2003; Al-Allaf et al. 2009; Chaiyana 2007; Castellano et al. 2004). As no universal selective medium exists for the cultivation of all genera, elective media appear for more than one genus and selective media assigned for well-defined genera while changing pH, addition of inhibitory agents, or used with other temperature-time terms (Reuter 1985), as well as some color indicators (Najjari et al. 2008; Dallbello et al.). Moreover, the choice of the medium is related to the biotope, and for example, for meat and meat products MRS medium are often used. Therefore, this medium has been recommended for the isolation of "LLPW" group (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Weissella*) other secondary genera as *Lactococcus* and *Streptococcus* can grow over (Reuter 1985; Schillinger and Holzapfel 2003; Carr et al. 2002). Self-evidently, characterization is largely based on morphology, mode of glucose fermentation, lactic acid produce, ability to grow at different temperatures, at high salt concentrations, and acid or alkaline tolerance. These characteristics are a basic and still very important to identify lactic acid bacteria (Axelsson 2004; Doyle et al. 2006). The objective of this study was to isolate and characterize, through phenotypic characteristics, *Lactobacillus* and *Pediococcus* from Algerian meat, in

Author ^α ^σ: Department of life and natural sciences, Djillali Liabes University, B.P N° 89 Sidi Bel Abbès, Algeria.
e-mail: mostecoc@yahoo.fr.

order to initiate an isolates collection and to probable use as bioprotective agents for meat products.

II. MATERIAL AND METHODS

a) Sample Meat Collection

Six samples, each one in three units, every unit about one hundred gram measuring five centimeters cub of fresh lamb meat, liver, and small intestine were cut out according to destructive technique using sterile instruments (scalpel, clamp) under aseptic condition (Larparent 1997), from retail stores and butchers in Saida region, (Algeria). Samples were introduced in label sterile bags, immediately transferred in isotherm box at 4°C to the laboratory, being analyzed on arrival.

b) LABMeatIsolation

Samples have been prepared for analysis according to ISO 6887-2, for each sample 25 g of meat cutting into small cubes was aseptically transferred to a sterile stomacher bag homogenized with 225 ml of saline-peptone water (NaCl 8.5 g/l; bactopectone 1g/l) for 1 min using stomacher (LAB BLINDER © 400) to obtain a 1:10 dilution. Serial dilutions 10^{-1} - 10^{-6} were then made directly or after enrichment for 1 day at room temperature, and 100 μ l aliquots were spread onto duplicate plates of MRS-BG agar (bromocresol green: 0.0025% (w/v)). Plates were incubated microaerobically at 30°C for 2 days (Najjari et al. 2008; Dallbello et al.) Bacterial count was performed according to ISO 4833. Colonies were selected from plates on the basis of their colors and size. Such colonies were sub cultured differentially on MRS-BG agar and pure isolates were maintained on MRS-BG as slants agar at temperature of 4°C for short-term use. Stock cultures were maintained frozen at -18°C on 20% glycerol (De valdez 2001). Each isolate was propagated twice on MRS broth before use. Overnight culture was employed in the tests. All isolates were initially subjected to macroscopic exams and orientation tests; Gram stain (Chaskes 2009), catalase (Hart and Shears 1997) and endospore (Guiraud et al. 1980).

c) Characterization and Differentiation of LAB Meat Isolates to the Genus Level

A preliminary identification in order to differentiate isolates at the genus level (Axelsson 2004; Doyle et al. 2006) was carried out using the following tests: CO₂ from glucose, growth at different temperatures, salt tolerance at 6.5-10 % (w/v) and pH tolerance at pH 3.9, 4.4, 9.6. Incubations were made at: 30°C for 3 days, 7-10°C for 7 to 10 days, 15-45°C for 3 to 5 days, 30°C for 2 to 3 days, in the same order (Schillinger and Lücke 1987). Lactic acid production was determined according to NF V.04.206.

d) Antagonism Test

To select antagonists among lactic acid bacteria isolates an antagonism test was achieved

based on the agar spot test, according to (Schillinger and Lücke 1989) originally described by Fleming et al. (1975), on TSA-YE medium (Tryptic Soy Agar supplemented with 0.6% Yeast Extract) towards the following pathogens: *B. cereus*, *B. subtilis* ATCC 6633, *E. coli* ATCC 8739, *S. typhimurium* ATCC 14028, *St. aureus* ATCC 6538, and *P. aeruginosa*. Incubation was carried out at 30°C for 2 days under anaerobic conditions means to reduce lactic acid and hydrogen peroxide effect. Isolates were selected on the basis of positive results showed the presence of clear zone around spots.

e) Characterization of the Selected LAB Meat Isolates to Species Level

A secondly identification at the species level was carried out by both assimilation and production tests: Arginine (Schillinger and Lücke 1987), Nitrate, Urea, and Hydrogen sulfide (Guiraud et al. 1980; Larparent and Gourgaud 1990; Forouhandeh et al. 2010). Incubations were made at: 30°C for 2 days, 30°C for 3 days, and 30°C for 2 weeks, in the same order. Carbohydrate fermentation profile was determined on MRS-BCP (bromocresol purple: 0.017% (w/v)). Sterile solutions of the sugars at 10 % (w/v) were added at final sugar concentration of 2 % (w/v). All strains were tested for fermentation of the following sugars: L-Arabinose, D (+) Glucose, Starch, D (+) Maltose, D (+) Galactose, Saccharose, D Mannitol, L Rhamnose, D (+) Lactose, Esculine, Arabinose, D Fructose, Raffinose, D Xylose, Sorbitol, D Cellulose, Ducitol. 100 μ l aliquots of sterile liquid paraffin were added to ensure anaerobic conditions. Incubations were made at 37°C for 2 days.

III. RESULTS

Thirty-three isolates were pricked from dilutions 10^{-4} and 10^{-5} . In the case without enrichment only eight isolates were pricked from dilutions 10^{-1} . So a total of forty-one bacteria were isolated from different parts of fresh lamb meat, including liver and small intestine. Loads of 2.10^2 , 2.10^6 and 3.10^6 UFC / g for directly, after enrichment, liver and small intestine, were taken in. Colonies macroscopic exams show five colors (green, light green with green center, white, white with green center and grey), two forms (punctiform and circular), opaque with smooth surface, of sizes from 1 to 3 mm. The colonies were picked from plates with 100 to 150 total colonies. Thirty isolates were non-spore-forming, catalase negative and Gram-positive (bacilli/cocci-bacillior cocci, some of them form tetrad). These lactic acid bacteria isolates produce various ratios of lactic acid of 0.74 to 1.26% and were farther characterised at the genus level, most (66.66%) seems to be true psychrotrophic growing at 7°C, as: fifteen mesophilic-mo fermentative bacilli (*atypical Streptobacteria*), eight among them were able to grow at 10-15°C but not at

45°C, failed to stand in the presence of 10-6.5% NaCl, as well to different pH except 9.6, thus, they appears belong to the genera: *Lactobacillus* and or *Lactobacilluslike*. While the others were able to grow at 10-15°C but not at 45°C, also in the presence of 10% but not at 6.5% of NaCl, unable to bear different pH except 9.6 other than one isolate, they appears belong to the genus *Lactobacillus*; one thermophilichomofermentative bacilli (*Thermobacteria*) the only able to grow at 45°C but not at 15°C, stand in the presence of 6.5% NaCl and to pH 9.6, such description be like the genus *Lactobacillus*; seven mésophilichomofermentativecocci (*Streptococcus*), six of whom were able to grow at 10 15 °C but not at 45°C, do not with 10-6.5% NaCl and on different pH except 9.6, that to say *Lactococcus* or *Vagococcus*, only one isolate are unable to grow at 10°C appears to be *Streptococcus*; and seven mésophilichomofermentativecocci (*Tetracoccus*) able to grow at 10°C except for two, grow all at 15°C but not at 45°C, can't do it at 10-6.5% NaCl, as well to different pH except 9.6, characteristics of genus *Pediococcus*. The antagonist test point out five isolates potency bacteriocinogenic, were antagonistic to Gram-positive target strains: *B. cereus*, *B. subtilis* ATCC 6633 and *St. aureus* ATCC 6538 with inhibition diameters ranging from 0.5 to 5.16 mm. On the basis of biochemical tests carried towards their characterization at the spice level; one isolate assumed *Lactobacillus* or *Lactobacilluslike* are arginine positive, urea negative, are neither nitrite nor H₂S producer, ferment weakly esculine, mannitol, D-sorbitol, are negative reaction for L-rhamnose, L-arabinose, raffinose and dulcitol, take these specific characters with *Lactobacillus sakei*. Another are arginine and urea negative, are neither nitrite nor H₂S producer, ferment all sugars except for L-rhamnose, L-arabinose and sorbitol, weakly reaction for dulcitol; the three other isolates are Nitrate, H₂S, arginine and urea negative, ferment all sugars except for L-rhamnose, L-arabinose, raffinose, sorbitol and dulcitol. These four isolates were *Pediococcus* spp.

IV. DISCUSSION

Isolation been began with an enrichment so as to increase the initial biomass and to give a better chance to detect lactic acid bacteria. Whereon, total counts are 2.10² on fresh lamb meat, at attempt without enrichment, those counts may reflect the exact population of the products at the time of sampling. While, after enrichment counts are ranged from 2.10⁶ to 3.10⁶ UFC/g on fresh meat, liver and small intestine, respectively. Similar densities from fresh sheep-meat around 10⁶ CFU/g were found by Najjari et al. (2008). Liver and small intestine showed the highest bacterial population, these are in agreement with results obtained by Olaoye and Onilude (2009). Five different types of colony were observed on plates, where upon colonies

were picked on the basis of theirs colors and size that have the same colony morphology noted by Najjari et al. (2008) and Dallbello et al. (). Such colonies are: green; light green with green center; white; white with green center and grey. As result, forty-one bacteria were isolated; among them thirty isolates were non-spore-forming, catalase negative and Gram-positive. These results are consistent with the group of genera of lactic acid bacteria (Carr et al. 2002; Schillinger and Holzapfel 2003; Axelsson 2004; Doyle et al. 2006). These isolates displayed various forms: bacilli, coccobacillior cocci, some of them with tetrad formation. As to lactic acid, isomer L/D, and CO₂ production from glucose, such parameters were useful for the characterization (Hayward 1957; Axelsson 2004). Thereby, all our isolates converted glucose quantitatively to lactic acid suggesting that belonging to the homofermentative following genera *atypical Streptobacteria*, *Thermobacteria*, *Streptococcus* and *Tetracoccus* (Stiles et al. 1997; Axelsson 2004; Doyle et al. 2006). So with a wide prevalence of homofermentative. Similar to that observed by Niemand and Holzapfel (1984) having isolated 67 strains, only two were heterofermentative. In addition, homofermentative lactic acid bacteria are potency to be used for biopreservation of meat (Vermeiren et al. 2004). Titratable acidity shows deferent capacity to produce lactic acid from 0.74 to 1.26%, this may have an effect antagonist, on typical spoilage microbiota mainly Gram-negative bacilli, while decreasing pH (Niemand and Holzapfel 1984). Moreover, (Stiles 1994) noted suitable for use lactobacilli with are aciduric, producing low pH in meats. In fact Inhibitory activity of lactic acid lies in the reduction of pH, and in the action of undissociated acid molecules. Further, (L+) lactic acid is inhibitorier than (D-), since the (D-) isomer is not hydrolyzed by human lactate dehydrogenase and may cause health problems, only strains producing mainly (L+) lactic acid should be selected (Ammor and Mayo 2007). All our isolates except one were able to grow at 15°C most among them were psychrotrophic growing at 7°C. An advantage, since the psychrophilic character is noted as a key in the selection of protective cultures (Vermeiren et al. 2004). Mesophilichomofermentative were *Lactobacillus*, *Lactobacilluslike* genus *Carnobacterium* called "atypical meat lactics". This genus resemble lactobacilli but they do not grow on acetate media (Stiles et al. 1997, are unable to grow at 0°C and are arginine positive (Carr et al. 2002). Our *Lactobacillus* and *Lactobacilluslike* were: bacilli/coccobacilli; able to grow at pH 9.6 but do not at pH 3.9. Are therefore in agreement with description of *atypical Streptobacteria* found associated with red meat (Samelis et al. 1994; Carr et al. 2002). Growing at pH 9.6, this also was found in previous study (Chaiyana 2007). Such *atypical Streptobacteria* have been found inhibit the growth of *St. aureus* and others undesirable bacteria with bacteriocins (Carr et al. 2002). Bacteriocins can be

used with or without released cultures as food-grade (Hugas 1998; Lücke 2000; Vermeiren et al. 2004; Savadogo et al. 2006; Castellano et al. 2008; Carr et al. 2002). Mésophilichomofermentative cocci were: *Lactococcus* or *Vagococcus*, except one unable to grow at 10°C was *Streptococcus*; those with tetrad formation were *Pediococcus* (Schillinger and Lücke 1987). Antagonism test carried out *in vitro* to assess isolates potential inhibitor has ended to select five isolates potency bacteriocinogenic against Gram-positive: *B. cereus*, *B. subtilis* ATCC 6633 and *St. aureus* ATCC 6538, with inhibition diameters ranging from 0.5 to 5.16 mm.. This result isn't wonder; as known the inactivity of bacteriocins against the Gram-negative due to the protective barrier provided by the lipopolysaccharides (Abee et al. 1995; De-Martinis and Freitas 2003; Bromberg et al. 2004) and bacteriocins are only active against Gram-positive (Dortu et al. 2009). Biochemical characteristics allowed to identify one isolate as *L. sakei* (Schillinger and Lücke 1987; Korkeala and Mäkelä 1989; Carr et al. 2002) this isolate shows properties of the subgroup S6/b include this spice described by Morishita and Shiromizu (1986), therewith weak reaction; fermenting D-xylose, mannitol, sorbitol, esculine; and little growing in 7.5% NaCl. The same identity description of group 4 represented only by *L. sakei* reported by Korkeala and Mäkelä (1989) therewith weak reaction; fermenting D-xylose, sorbitol and little growing in 8% NaCl. Also his pattern agrees with *L. sakei* identified by Samelis et al. (1994) there with fermenting D-xylose and growing in 8 % NaCl. Furthermore, this isolate is saccharose positive, another clean character to *L. sakei* (Carr et al. 2002). So it is of great importance to note that various researchers have used different criteria to describe a typical *Streptobacteria* which sometimes makes it difficult to make a point comparison and to take meaning conclusions (Carr et al. 2002). Others four isolates, were distinctive for tetrad formation, identified at genus as *Pediococcus*, three isolates have common fermenting all sugars except L-rhamnose, L-arabinose, raffinose, dulcitol, while the fourth has ability to ferment all sugars except L-rhamnose, L-arabinose, sorbitol, dulcitol. Due too far differences patterns from those described in the literature, themselves ever changing, sometimes even contradictory, this was mainly attributable to high variability observed in the same species of the genus. It seems at that time not possible to make clear statements about *Pediococcus* sp.

V. ACKNOWLEDGMENT

We are; obliged to the Algerian Centre of Quality Control and Packaging, Saida, Algeria; thankful to messes Hadj Ahmed Belaoui and Mohammed EL Amine Bendaha, for invaluable help.

REFERENCES RÉFÉRENCES REFERENCIAS

1. Abee T, Krockel L, Hill C. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology*. 1995; 28: 169-185.
2. Al-Allaf MAH, Al-Rawi AMM, Al-Mola AT. Antimicrobial activity of lactic acid bacteria isolated from minced beef meat against some pathogenic bacteria. *Iraqi Journal of Veterinary Sciences*. 2009; 23: 115-117.
3. Aly S, Ouattara cat, Bassole IHN, Traore SA. Bacteriocins and lactic acid bacteria. *African Journal of Biotechnology*. 2006; 5 : 678-683.
4. Ammor MS, Mayo B. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production. *Meat Science*. 2007; 76: 138-146.
5. Axelsson L. Lactic acid bacteria, Classification and physiology. In: Salminen S, Von-Wright A, Ouwehand A. Lactic acid bacteria, third edition. Marcel Dekker: New York; 2004. 20-86.
6. Bibek R, Arun B. Fundamental food Microbiology. fourth edition. CRC Press: 2008. 492p.
7. Bromberg R, Moreno I, Zaganini CL, Delboni RR, De Oliveira J. Isolation of bacteriocin-producing lactic acid bacteria from meat and meat products and its spectrum of inhibitory activity. *Brazilian Journal of Microbiology*. 2004; 35:137-144.
8. Carr FJ, Chill D, Nino M. The lactic acid bacteria: A literature survey, *Critical Reviews in Microbiology*. 2002; 28: 281-370.
9. Castellano P, Belfiore C, Fadda S, Vignolo G. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. *Meat Science*. 2008; 79: 483-499.
10. Castellano PH, Holzapfel WH, Vignolo GM. The control of *Listeria innocua* and *Lactobacillus sakei* in broth and meat slurry with the bacteriocinogenic strain *Lactobacillus casei* CRL705. *Food Microbiology*. 2004; 21: 291-298.
11. Chaiyana J, Boonrang S, Sinsuwongwat S. Isolation and screening of bacteriocin producing bacteria from fermented meat products. *Biotechnology for gross national happiness*. 2007; 108: 245-258.
12. Chaskes S. Stains for Light Microscopy. In: Goldman E, Green LH. Practical Handbook of Microbiology. Second Edition, CRC Press: 2009; 37-51.
13. De-Martinis ECP, Freitas FZ. Screening of lactic acid bacteria from Brazilian meats for bacteriocin formation. *Food Control*. 2003; 14: 197-200.
14. De-valdez GF. Maintenance of lactic acid bacteria. In: Spencer JFT, Spencer ALR. Food Microbiology-protocols, Humana Press: 2001; 163-172.
15. Dortu C, Thonart P. Les bactériocines des bactéries lactiques: caractéristiques et intérêts pour la

- bioconservation des produits alimentaires. *Biotechnol. Agron. Soc. Environ.* 2009; 13: 143-154.
16. Doyle MP and Meng J. Bacteria in food and beverage production. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E. The Prokaryotes. Third edition. Springer, 2006; 795-809.
 17. Dal-Bello F. Ecological studies of the *Lactobacillus* biota in the human digestive tract and adaptation of intestinal lactobacilli to the sourdough ecosystem. Thèse de doctorat des sciences, université de Hohenheim. Université de Hohenheim, 2005; 98p.
 18. Fleming HP, Etchells JL, Costilow RN. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. *Applied Microbiology*. 1975; 30: 1040-1042.
 19. Forouhandeh H, Vahed SZ, Hejazi MS, Nahaie MR, Akbari DM. Isolation and phenotypic characterization of lactobacillus species from various dairy products. *current research in bacteriology*. 2010; 3: 84-88.
 20. Gálvez A, Abriouel H, López RL, Ben Omar N. Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology*. 2007; 120: 51-70.
 21. Guiraud J, Galzy P. L'analyse Microbiologique dans les industries alimentaires. L'usine, 1980 ; 235p.
 22. Hart T, Shears P. 1997. Atlas de poche de Microbiologie. Flammarion, Paris. 1997; 314p.
 23. Hayward AC. Detection of gas production from glucose by heterofermentative lactic acid bacteria. *J. gen. Microbiol.* 1957; 16: 9-15.
 24. Holzapfel WH, Geisen R, Schillinger U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology*. 1995; 24: 343-36.
 25. Hugas M. Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products. *Meat Science*. 1998; 49: 139-150.
 26. Jeevaratnam K, Jamuna M, Bawa AS. Biological preservation of foods-bacteriocins of lactic acid bacteria. *Indian journal of biotechnology*. 2005; 4: 446-454.
 27. Jones RJ, Hussein HM, Zagorec M, Brightwell G, Tagg JR. Isolation of lactic acid bacteria with inhibitory activity against pathogens and spoilage organisms associated with fresh meat. *Food Microbiology*. 2008; 25: 228-234.
 28. Klaenhammer TR, Barrangou TR, Buck R, Azcarate-Peril BL, Altermann MA, Genomic features of lactic acid bacteria effecting bioprocessing and health. *FEMS Microbiol Rev.* 2005; 29.
 29. Korkeala H, Mäkelä P. Characterization of lactic acid bacteria isolated from vacuum-packed cooked ring sausages. *International Journal of Food Microbiology*. 1989; 9 : 33-43.
 30. Larpent JP, Microbiologie des viandes. In: Larpent. Microbiologie alimentaire, Technique de laboratoire. Lavoisier. Paris, 1997 ; 860-870.
 31. Larpent JP, Larpent- Gourgau M. Mémento technique de Microbiologie. Lavoisier:paris,1990; 417p.
 32. Lücke FK. Utilization of microbes to process and preserve meat, *Meat Science*. 2000; 56: 105-115.
 33. Morishita Y, Shiromizu K. Characterization of lactobacilli isolated from meats and meat products. *International Journal of Food Microbiology*. 1986; 3: 19-29.
 34. Najjari A, Ouzari H, Boudabous A, Zagorec M. Method for reliable isolation of *Lactobacillus sakei* strains originati from Tunisian seafood and meat products. *International Journal of Food Microbiology*. 2008; 121: 342-351.
 35. Niemand JG, Holzapfel WH. Characteristics of lactobacilli isolated from radurised meat. *International Journal of Food Microbiology*. 1984; 1: 99-110.
 36. Olaoye OA, Onilude AA. A study on isolation of presumptive technologically important microorganisms from Nigerian beef. *American-Eurasian Journal of Sustainable Agriculture*. 2009; 3: 75-83.
 37. Reuter G, Elective and selective media for lactic acid bacteria International, *Journal of Food Microbiology*. 1985; 2: 55-68.
 38. Samelis J, Maurogenakis F, Metaxopoulos J. Characterisation of lactic acid bacteria isolated from naturally fermented Greek dry salami. *International Journal of Food Microbiology*. 1994; 23: 179-196.
 39. Schillinger U, Holzapfel WH. Culture media for lactic acid bacteria Chapter 8. In: Corry JEL et al. Handbook of culture media for food Microbiology. 2003; 127-140.
 40. Schillinger U, Lücke FK. Antibacterial Activity of *Lactobacillus sakei* isolated from meat. *applied and environmental Microbiology*. 1989; 55: 1901-1906.
 41. Schillinger U, Lücke FK. Identification of lactobacilli from meat and meat products. *Food Microbiology*. 1987, 4: 199-208.
 42. Stiles ME, Wilhelm H, Holzapfel B. Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology*. 1997; 36: 1-29.
 43. Stiles ME. Potential for biological control of agents of foodborne disease. *Food Research International*. 1994; 27: 245-250.
 44. Vermeiren L, Devlieghere F, Debevere J. Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology*. 2004; 96: 149-164.

GLOBAL JOURNALS INC. (US) GUIDELINES HANDBOOK 2014

WWW.GLOBALJOURNALS.ORG