



CENTRAL LUZON STATE UNIVERSITY



**MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION
OF LACTIC ACID BACTERIA FROM NAPIER, CALVES
CONCENTRATES, AND PROBIOTIC SUPPLEMENT**

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An Undergraduate Thesis Submitted to the Faculty of the Department of
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BACHELOR OF SCIENCE IN BIOLOGY

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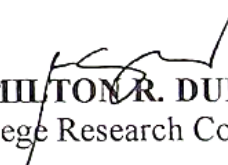

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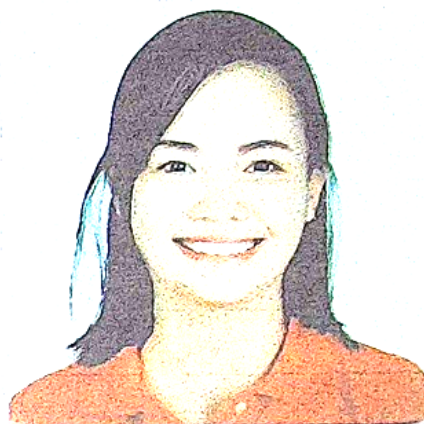
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ABSTRACT

JO-ELIZ MARTIN GONZALES, Bachelor of Science in Biology, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, July 2017. **MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION OF LACTIC ACID BACTERIA FROM NAPIER, CALVES CONCENTRATE, AND PROBIOTIC SUPPLEMENT.**

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This study aimed to isolate and characterize morphologically and physiologically the lactic acid bacteria present on the calves' diet and probiotic supplement. There were four isolates and marked as LAB1, LAB2, LAB3 and LAB4, isolates were then studied extensively for morphology and physiological growth responses to different pH representing digestive tract pH, and temperature of the environment. All isolates shared common staining - gram positive, nonsporing, and negative in catalase test and microscopic features – rounded end rod to cocci with single, pair, and chain arrangement with different morphology in MRS agar plate and slant and MRS broth. These isolates were categorized into two main genus *Lactobacillus* and *Streptococcus*. All isolates were found to be tolerant to extreme pH and temperature except for LAB3. All isolates were capable of lactic acid production in pasteurized milk.



LITERATURE CITED

- BUDDINGH G., AND BERGEYS MANUAL OF DETERMINATIVE BACTERIOLOGY 8TH EDITION. (1975).** The Williams and Wilkins Company, Baltimore Maryland. *The American Journal of Tropical Medicine and Hygiene*, 24:550-550
- CHOUDHURY, P. K., SALEM, A.Z.M., JENA, R., KUMAR, S., SINGH, R. AND A.K. PUNIYA. (2015).** Rumen microbiology: An overview. In *Rumen Microbiology: From Evolution to Revolution* (pp. 3-16). Springer India.
- CRAY, W. C., CASEY T. A., BOSWORTH B. T., AND M. A. RASMUSSEN. (1998).** Effect of dietary stress on fecal shedding of *Escherichia coli* O157:H7 in calves. *Applied Environmental Microbiology*. 64:1975–1979.
- DE KEERSMAECKER S.C., VERHOEVEN T.L., DESAIR J., VANDERLEYDEN J., AND I. NAGY. (2006).** Strong antimicrobial activity of *Lactobacillus rhamnosus* GG against *Salmonella typhimurium* is due to accumulation of lactic acid. *FEMS Microbiology Letters*; 259: 89-96.
- DEHORTY, B.A. AND C.G. ORPIN. (1997).** Development of, and natural fluctuations in, rumen microbial populations. In *The rumen microbial ecosystem* (pp. 196-245). Springer Netherlands.
- DE MAN J., ROGOSA M., AND M. SHARPE. (1960).** A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology* 3: 130-135
- EIZAGUIRRE I., URKIA N.G., ASENSIO A.B., ZUBILLAGA I., ZUBILLAGA P., VIDALES C. et al. (2002).** Probiotic supplementation reduces the risk of bacterial translocation in experimental short bowel syndrome. *Journal of Pediatric Surgery*; 37: 699-702.
- EWASCHUK J.B., NAYLOR J.M., CHIRINO-TREJO M., AND G.A. ZELLO. (2004).** *Lactobacillus rhamnosus* strain GG is a potential probiotic for calves, *The Canadian Journal of Veterinary Research*, 68:249–253
- FULLER R. (1989).** Probiotics in man and animals, *Journal of Applied Bacteriology* 1989, 66, 365-378
- GUPTA A., AND N. SHARMA. (2017).** Characterization of potential probiotic lactic acid bacteria- *Pediococcus acidilactici* ch-2 isolated from chuli- a traditional



apricot product of Himalayan region for the production of novel bioactive compounds with special therapeutic properties. *Journal of Food Microbiology Safety and Hygiene* 2: 119

GUARNER F., AND J.R. MALAGELADA. (1998). Gut flora in health and disease. *Lancet*; 361: 512-519.

HEMAISWARYA S, RAJA R., RAVIKUMAR R., AND I.S. CARVALHO. (2013). Mechanism of Action of Probiotics, IBB/CGB - Faculty of Sciences and Technology; Food Science Laboratory; University of Algarve; Campus de Gambelas, Education. 8, 8005-139

HOLZAPFEL W.H., HABERER P., GEISEN R., BJÖRKROTH J., AND U. SCHILLINGER. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. *American Journal of Clinical Nutrition*; 73 (suppl):365S-73S.

HUNGATE R.E. (1966). The rumen and its microbes. Department of Bacteriology and Agricultural Experiment Station. University of California. Davis, Academic Press New York, 1-533,

IBOURAHEMA C., DAUPHIN R.D., JACQUELINE D., AND P. THONART. (2008). Characterization of lactic acid bacteria isolated from poultry farms in Senegal. *African Journal of Biotechnology* Vol. 7 (12), pp. 2006-2012

IRIANTO A. (2002). Austin B. Probiotics in aquaculture. *Journal of Fish Diseases*; 25: 633-642.

KAILA M., ISOLAURI E, VIRTANEN E., AND H. ARVILOMMI. (1992B). Preponderance of IgM from blood lymphocytes in response to infantile rotavirus gastroenteritis. *Gut*; 33: 639-642.

KAILA M., ISOLAURI E., SOPPI E., VIRTANEN E., LAINE S., AND ARVILOMMI H. (1992A). Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatric Research*; 32: 141-4.

KATHARINA E., SCHOLZ-A., PETER A., BERIT M., PETRA W., WOLFRAM T., ET AL. (2007). Prebiotics, Probiotics and Symbiotic Affect Mineral Absorption, Bone Mineral Content, and Bone Structure. *Journal of Nutrition*; 137: 838S- 846S.



- LEEDLE, J. A. Z., BRAYANT, M.P. AND R.B. HESPEL. (1982).** Diurnal Variations in Bacterial Numbers and Fluid Parameters in Ruminant Contents of Animals Fed Low- or High-Forage Diets, 44, 2.
- LONGENBACH, J. I. AND A. J. HEINRICHS. (1998).** A review of the importance and physiological role of curd formation in the abomasum of young calves. *Animal Feed Science and Technology* 73:85-97.
- MANGELL P., NEJDFORS P., WANG M., AHRNE S., WESTROM B., THORLACIUS H., et al. (2002).** *Lactobacillus plantarum* 299v inhibits *Escherichia coli* induced intestinal permeability. *Digestive Disorder and Sciences*; 47: 511-6.
- MD. KHALIL I., AND MD N. ANWAR. (2016).** isolation, identification, and characterization of lactic acid bacteria from milk and yoghurts. e-ISSN: 2321 6204, p-ISSN: 2347-2359.
- MOHKBER-DEZFOULI M.R., OLOURCHI P.B., AND H. MAHMOUDZADE. (2007).** effect of probiotic supplement in daily milk intake of newborn calves on body weight gain, body height, diarrhea occurrence and health condition, department of animal health and nutrition, faculty of veterinary medicine, journal of biological sciences, 10 (18): 3136-3140.
- NORIN E., MIDTVEDT T., AND B. BJO'RKSTE'N. (2004).** Development of faecal short-chain fatty acid pattern during the first year of life in Estonian and Swedish infants. *Microbial Ecology in Health and Diseases Journal*; 16: 8-12.
- RAJA R., HEMAI SWARYA S., ASHOK KUMAR N., SRIDHAR S., AND R. RENGASAMY. (2008).** A perspective on the biotechnological potential of microalgae. *Critical Reviews in Microbiology*; 34: 77-88
- RAJA R., HEMAI SWARYA S., AND R. RENGASAMY (2007A).** Exploitation of *Dunaliella* for β -carotene production. *Applied Microbiology and Biotechnology*; 74: 517-523.
- REID G. (2002).** Safety of *Lactobacillus* strains as probiotic agents. *Clinical Infectious Diseases*; 35: 349-350.
- SALMINEN S., BOULEY C., BOUTRON RAULT M.C., CUMMINGS J.H., FRANCK A., GIBSON GFR., et al. (1998).** Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition*; 80: S147-S171.



- SANDERS M. E., AND KLAENHAMMER. (2001).** The scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *Journal of Dairy Sciences*. 84: 319–331.
- SUSKOVIC J., KOS B., GORETA J., AND S. MATOSIC. (2001).** Role of lactic acid bacteria and bifidobacteria in symbiotic effect. *Food Technology and Biotechnology*; 39: 227–235.
- TAHERI H. R., MORAVEJ H., TABANDEH F., ZAGHARI M., AND M. SHIVAZAD. (2009).** Screening of lactic acid bacteria toward their selection as a source of chicken probiotic, (88):1586–1593.
- TAMBEKAR, D. H. AND S. A. BHUTADA. (2010)** Studies on antimicrobial activity and characteristics of bacteriocins produced by *Lactobacillus* strains isolated from milk of domestic animals. *The Internet Journal of Microbiology*; 8:1-6.
- TIMMERMAN H. M., MULDER L., EVERTS, D. C. VAN ESPEN, E. VAN DER WAL, G. KLAASSEN, S. M. G. ROUWERS, R. HARTEMINK, F. M. ROMBOUTS, AND A. C. BEYNEN. (2005).** Health and Growth of Veal Calves Fed Milk Replacers With or Without Probiotics, *American Dairy Science Association*, 88:2154–2165
- VENKAT H.K., SAHU N.P., AND K. KAMAL. (2004).** Jain Effect of feeding *Lactobacillus* based probiotics on the gut microflora, growth and survival of post larvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture Research*; 35: 501-507.
- WASSIE M. and T. WASSIE. (2016).** Isolation and Identification of Lactic Acid Bacteria from Raw Cow Milk. *International Journal of Advanced Research in Biological Sciences*. 3(8): 44-49