

**MICROBIOLOGICAL ASSESSMENT AND CHARACTERIZATION OF
BACTERIA ASSOCIATED WITH DAIRY CARABAO'S MILK-BASED
(*Bibingka*)**

JUNNIDA B. MIRANDA

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ABSTRACT

MIRANDA, JUNNIDA B., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines May 2018, **MICROBIOLOGICAL ASSESSMENT AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH DAIRY CARABAO'S MILK-BASED DELICACY**

Adviser: ARMAN M. PARAYAO, Ph. D.

Co-Adviser: MINA P. ABELLA, M. Sc.

Foodborne microorganism can lead to serious outbreaks in the community hence, food safety is vital for the consumer. This study was focused on microbiological assessment and characterization of bacteria associated with dairy carabao's milk-based *bibingka* sold in local dairy cooperatives. Physical assessment of the product was performed prior the bacteriological assessment. Conventional method and Petrifilm™ was used in enumerating microorganisms. Aerobic plate count, presumptive confirmatory and completed coliform and *E. Coli* enumeration were utilized for the conventional method while Petrifilm™ Aerobic Count Plate and Petrifilm™ *E. coli* / Coliform Count Plate was used in 3M Petrifilm™.

The physical assessment of the product showed no objectionable traits in terms of odor, color, appearance and taste. Results showed that within the two samplings, the dairy carabao's milk-based *bibingka* satisfied some of the given standards for its properties. Parallel results was obtained among conventional methods and Petrifilm™ for analyses of aerobics, total coliforms and *Escherichia coli*.

Three isolates were the food-prevalent bacteria that appeared in both samplings. Three Gram positive bacterial isolates were characterized based on its cultural and morphological characteristics.

LITERATURE CITED

- Ali, A.A., Irshad, N., Razaz, S.A., & Manahil, A.A. (2010). Microbiological safety of raw milk in Khartoum state, Sudan: 1-Khartoum and Omdurman cities. *Pakistan Journal of Nutrition*, 9(5), 426-429.
- Aganga, A.A., Amartiefio, J.O., & Nkile, N. (2002). Effect of stage of lactation on nutrient composition of tswana sheep and goat's milk. *Journal of Food Composition and Analysis*, 15(5), 533-543
- Bacteriological Analytical Manual (2001). Aerobic plate count. Retrieved on: fda.gov/Food-ScienceResearch/LaboratoryMethods.
- Bhat, Z. F. & Bhat, H. (2011). Milk and dairy products as functional foods: a review. *International Journal of Dairy Science*, (6), 1-12.
- Cappucino, G. & Welsh, C. (2018). Microbiology: A laboratory manual. Eleventh Edition. Edinburgh Gate, England: Pearson Education Limited.
- Corpuz, A.M. (2018). DNA barcoding and characterization of microflora associated with *bibingkas gatas*, a carabao-based product of dairy cooperatives of nueva ecija. *Unpublished thesis*. Central Luzon State University.
- Creamer, L. K., Pearce, L. E., Hill, J.P. & Boland, M. J. (2002). Milk and dairy products in the 21st century. *Journal of Agriculture and Food Chemistry*, 50(25), 7187-7193.
- Das, S., Hasan, G.A., & Parveen, S. (2015) Evaluation of microbial load and quality of milk & milk based dairy products, *Octa Journal of Biosciences*, 3(1), 1-4
- De Marchi, M., Bittante, G., Zotto, R.D., Dalvit, C., & Cassandro, M. (2008). Effect of holstein friesian and brown swiss breeds on quality of milk and cheese. *Journal of Dairy Science*, 91(10), 4092-4102
- Drake, M.A. Invited review: sensory analysis of dairy products. *Journal of Dairy Science*. 90(11), 4925-4937.
- Faye, B., Konuspayeva, G., Messad, S., & Loiseau, G. (2008). Discriminant milk components of Bactrian camel (*Camelus bactrianus*), dromedary (*Camelus dromedarius*) and hybrids, *Dairy Science & Technology*, 88(6), 607-617
- Feng, P., Weagant, S., Grant, M., & Burkhardt, W. (2002). Bacteriological analytical manual, 8th Edition, Revision A, 1998. Chapter 4.
- FAO. (2007). Food Labelling: Codex General Standard for the Labelling of Prepackaged Foods. Codex Alimentarius 5th Ed.

- Fox, E. M., Fanning, S., Corsetti A. & Jordan, K. (2017). Microbial food safety along the dairy chain. *Frontiers in Microbiology*, 8(16), 1-12.
- Gaucheron, F. (2011). Milk and dairy products: a unique micronutrient combination. *Journal of the American College of Nutrition*, (30), 400S-409S
- Hashmi, S. & Saleem, Q. (2015). An investigation on microbiological and chemical quality of buffalo milk supplies. *Int.J.Curr.Microbiol.App.Sci*, 4(1), 78-83
- Heck, J.M.L., Van Valenberg, HJF., & Dijkstra, J., Van Hooijdonk, ACM. (2009). Seasonal variation in the Dutch bovine raw milk composition. *Journal of Dairy Science*, 92(10), 4745-4755
- Hill. D. R. & Newburg, D. S. (2015). Clinical applications of bioactive milk components. *Nutrition Reviews*, 73(7), 463-476
- Ledenbach, L. H. & Marshall, R. T. (2009). Microbiological spoilage of dairy products. *Compendium of the Microbiological Spoilage of Foods and Beverages*, 7(10), 41-67.
- Makerere University. (2016). Module on: Dairy products quality and safety. Retrieved on: onegreenplanet.org
- Marina, A. M. & NurulAzizah, S. (2014). Use of coconut versus dairy milk products in Malaysian dishes: comparison of nutritional composition and sensory evaluation. *Journal of Food and Nutrition Research*, 2(4), 204-208
- Maturin, L., & Peeler, J. (2001). Bacteriological Analytical Manual, Edition 8, Revision A, 1998. Chapter 3.
- Menrad, K. (2003). Market and marketing of functional food in Europe. *Journal of food engineering*, (56), 181-18
- Motarjemi, Y., Moy, G.G., Jooste, P.J., & Anelich, L.E. (2014). Milk and dairy products. *Food safety management*, 83-117
- Muehlhoff, E., Bennett, A. & McMahon, D. (2015). Milk and dairy products in human nutrition. *Food and Agriculture Organization of the United Nations*, 243-273.
- NSW Food Authority. (2009). Microbiological quality guide for ready-to-eat foods, a guide to interpreting microbiological results. NSW/FA/CP028/0906
- Oliver, S.P., Jayarao, B.M., & Almeida, R.A. (2005). Foodborne pathogens in milk and the dairy farm environment; food safety and public health implications. *Foodborne Pathogens & Disease*, 2(2), 115-129
- Philippines News Agency. (2016). Carabao-based dairy farming gains ground.

- Recommended International Code Of Practice-General Principles of Food Hygiene. (2003). CAC/RCP 1-1969, Rev. 4-2003 1
- Roberfroid, M.B., (2000). Concepts and strategy of functional food science: The European perspective. *The American journal of clinical nutrition*, (71),1660s-1664s.
- Robinson, R. K. (2002). Dairy microbiology handbook. Retrieved from [http://dlx.bok.org/genesis/997000/180eafceb6e13356a40cc649894a9885/_as/%5B%5D_Dairy_Microbiology_Handbook_The_Microbiology_o\(b-ok.org\).pdf](http://dlx.bok.org/genesis/997000/180eafceb6e13356a40cc649894a9885/_as/%5B%5D_Dairy_Microbiology_Handbook_The_Microbiology_o(b-ok.org).pdf)
- Sakar, S. (2015). Microbiological Considerations: Pasteurized Milk. *International Journal of Dairy Science*, 10(5), 206-218
- Salmorin, L. M. (2018). Microbial isolation and characterization of carabao-based product (Espasol de Leche) sold in dairy cooperatives of Nueva Ecija. Unpublished Article. Central Luzon State University
- Slots, T., Butler G., Leifert, C., Kristensen, T., Skibsted, L.H., & Nielsen, J.H. (2009). Potentials to differentiate milk composition by different feeding strategies. *Journal of Dairy Science*, 92(5), 2057-2066
- Soomro, A. H., Raunaq, S., Sheikh, S.A., Khaskheli, M., & Talpur, A. (2016). Assessment of microbial quality of farm buffalo milk. *Pak. J. Agri. Agril. Engg., Vet. Sci*, 32 (2), 268-276
- Tamime, A.Y. (2009). Milk Processing and Quality Management, Blackwell Publishing Limited. ISBN: 978-1-405-14530-5
- Walsh, S., Buckley, F., Pierce, K., Byrne, N., Patton, J., & Dillon, P. (2008). Effects of breed and feeding system on milk production, body weight, body condition score, reproductive performance, and postpartum ovarian function. *Journal of Dairy Science*, 91(11), 4401-4413
- Young, P. W.(2017). Handbook of milk of non- bovine mammals 2nd Ed. Wiley Blackwell Publishing 2017.
- Zhong Han, B., Meng, Y., Li, M., Yang, Y.X., Ren, F.Z., Zeng, Q.K., & Robert Nout, M.J. (2007). A survey on the microbiological and chemical composition of buffalo milk in China. *Food Control*, 18(6), 742-746