

A COMPARATIVE STUDY OF BACTERIA PRESENCE BETWEEN TWO TYPES OF COW POWDERED MILK

Yanti Yaacob*, Norsakina Zurina Zulkifli, Nunshaimah Salleh, Adibatul Husna Fadzil, Rosliza Ali, Nordiana
Suhada Mohamad Tahirudin
Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch Tapah Campus, 35400 Tapah Road,
Perak, Malaysia.

*yanti229@uitm.edu.my

Abstract: Given their crucial roles in the ecosystem which is present everywhere, bacteria are among the most significant species. The objectives of this preliminary study is to identify the presence of bacteria in different samples of powdered milk and to identify the types of bacteria in different samples of powdered milk which are Suffy Annis and Everyday powdered milk. The study was carried out because the microorganisms in the powdered milk samples included both hazardous and beneficial bacteria, each of which serves a specific purpose in supplying the human body with the necessary vitamins. In this experiment, bacteria were isolated from samples of each powdered milk using the techniques of agar streaking and gram staining with the goal of classifying the types of bacteria that had been noticed. According to the project's findings, the bulk of the bacteria generated were in the rod-shaped *Bacillus* sp., which is known to be gram positive. It states that cow's milk powdered under the Everyday brand contains the majority of probiotic bacteria, living microorganisms intended to offer health advantages when taken by the body compared to Suffy Annis milk.

Keywords: *Powdered milk, bacteria, gram staining, gram positive, gram negative, rod shape, microscopic, macroscopic*

INTRODUCTION

There are many types of powdered milk that are sold at the market. Milk which has a high nutritional value and usually contains calcium and phosphorus and is the most significant source of calcium found in our food, is an excellent source of most of the nutrients that are needed by humans. Although, the composition of milk varies between animal species, it is nevertheless very important for human nutrition and acting in providing for people's dietary needs is crucial. Cow milk is higher in folate, selenium, and riboflavin as well as significantly higher in vitamin B12 as stated by Kumar et al., (2016). Cow milk is composed mostly of long chain fatty acids while goat milk has much more medium and even short chain fatty acids. The advantages of cow milk are improved immunity by increasing antioxidant activity and decreasing inflammation, improved liver function by promoting export of lipid in the form of lipoprotein from liver and improved bone function. The individuals who usually consume cow milk are infants, kids, and adults. This objectives of this project is to observe the presence of bacteria in two types of cows powdered milk which from this study and to identify the types of bacteria in different samples of powdered milk. The significance of this study is the safety and quality of the milk can be controlled. The factors that determine the bacteria colonies formed in dairy products such as growth, location and distribution of bacteria are important in ripening and flavour development of other food such as cheeses, yogurt, and sour creams. Microscopy techniques are used to observe the bacterial colonies and environments that surround them. Lactic acid bacteria are the most important starter cultures that are used in fermented dairy products that originate from dairy products such as milk. For food safety concerns, dairy products that are commercially produced are manufactured from pasteurized milk. The process of pasteurization helps inactivate the pathogenic bacteria, but it also acts as a reduction or inactivation of other occurring microflora populations. The benefits of the production of milk within society is that it consists of never-ending nutrients and benefits that will play an important role for the building of calcium, minerals, and other required substances inside the human body. According to research by Professor Peter Elwood in the year 2010, he stated that drinking milk will lessen the chances of dying from illness for example coronary heart disease and stroke by up to 20 percent. It is considered as such because the calcium and lactose in milk enhances the calcium absorption, which will reduce the cholesterol levels (Berman, 2021). Other health benefits of society that consumes milk includes increased bone strength, smoother skin, a stronger immune system, prevention of illnesses such as dental decay, hypertension, respiratory problems and even some forms of cancer. It can be said that the beneficial health nutrients obtained from milk are solely essential towards the growth of the human body and help prevent chronic ailments. As opposed to the consumption of milk within society can lead to severe anemia, osteoporosis, and other related illnesses. Hence, it is important to consume milk within its required measurement, to maintain good health and generate the energy and strength to proceed with our daily activities (Batta, 2016). Despite the consumption of milk within the society, milk production is one of the most crucial sectors of the world's agricultural industry and it also plays a key role in the economy of milkproducing countries. The managing role of dairy in local food systems is known to be a very complex challenge to be carried out all around the world. India is one of the countries that has the largest milk producing nation in the world (Brown et al., 2022). In the food industries, the processes of milk will produce an array of products that revolve

around milk such as cheese, butter, cream, yogurt, ghee etc. The quality of these products truly depends on the quality of milk that is received as it is also important to both the farmers and dairies that process the milk into other products. Each industry that exports the production milk is required to oversee the storage time of the milk as good quality milk takes longer to deteriorate. Another factor that needs to be acknowledged is the range of products that can be produced. As an example, high quality milk can be used to process high value products such as UHT milk, ice-cream, yogurt and others. This is important as good quality products will bring benefit to the food industry, as the outcome of processed food centres around a specific product such as milk.

METHODOLOGY

Milk Sample Preparation

The powdered milk sample was prepared by firstly preparing a solution of nutrient broth using peptone water. Peptone water is a product of enzyme digestion of proteins where it provides small peptides that microorganisms can use. Traces of elements and vitamins are present in sufficient quantities in this example of complex media to support the growth of many organisms. Hence, it is why peptone water is used to grow the bacteria that resides in powdered milk of both samples. The procedure of both milk samples was prepared. Firstly, 2.22g the powdered milk of each sample was weighed. Later, the powdered milk was placed into a universal bottle and 20mL sterile peptone water was added and incubated 48 hours at 37°C. The peptone water turned turbid once the bacteria growth. The bacteria from the peptone water is transferred onto the nutrient agar plate by streaking techniques. Then the nutrient agar was placed into the incubator for 48 hours at 37°C. Later, the agar media could be observed for the presence of bacteria culture inside the nutrient broth after 48 hours. The data was recorded.




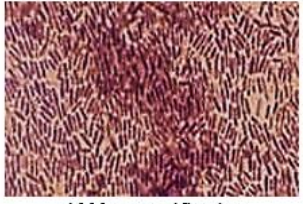
Gram Staining

The purpose of the gram staining method functions is to identify the type of bacteria in a sample. The bacteria can be distinguished and classified into 2 groups which are gram-positive and gram negative. Gram-positive bacteria will leave a purple stain on the bacteria sample. It is because they have a thick cell wall without an outer membrane. The gram- negative bacteria will leave a red stain with gram stain. It is because they have a thin cell wall and outer membrane. The procedure of gram staining was conducted, first preparing a smear of bacteria. 1 drop of distilled water was placed at the centre of a clean slide. The wire loop was flamed using a Bunsen burner and it was let to cool. The wire loop was touched lightly on the selected bacterial colony. The lid was opened slightly for safety reasons. The sample was mixed with the water on the slide. The cells were smeared out on the slide to create a thin layer. The smear was heated fixed 3 times to allow the bacteria to stick to the slide. The loop flamed again. Second was to prepare a Bacteria Staining. The slide was dropped with crystal violet solution and allowed to act for 60 seconds. The slide was rinsed immediately by using distilled water. The slide was dropped with iodine solution and allowed iodine to act for 60 seconds. Excess iodine was rinsed off immediately by using distilled water. The acetone was dropped to the slide and left for 5 seconds. The slide was rinsed immediately with distilled water. Those organisms that are gram-negative are no longer visible after the acetone is decolourized. Safranin was applied counterstain for 30 seconds. The slide was rinsed with distilled water. The smear was heat fixed 3 times to ensure the bacteria stuck to the slide and it was allowed to dry. The bacteria were viewed under a light microscope. A drop of the immersion oil was added when using 1000X magnification.

FINDINGS

Table 1 showed the macroscopic and microscopic observation characteristics of the bacteria colony of Suffy Annis and Everyday powdered milk. For Suffy powdered milk macroscopic observation, the colonies formed on the surface of the nutrient agar was shaped irregularly and dry colonies. The colour of each colony produced are white in color. On each colony of the bacteria, it was carefully determined that the edges were shaped irregularly. Hence, the margin observed was undulated. For microscopic observation which used to identify the gram type of the bacteria through the gram staining method and to identify the shape of the bacteria that was formed in the sample Suffy powdered milk. Based on the microscopic observation, the gram type of the bacteria was gram positive, which indicates that the bacteria had retained the purple colour of the crystal dye due to the thickness of the peptidoglycan layer of its cell wall. The shape of the bacteria was identified to be a rod-shaped bacterium with the formation of its spore around its cell. Based on the Suffy powdered milk, can be summarised that the gram type of the bacteria is gram positive and most of the bacteria was rod shaped with formation of spores from its cell. From Suffy powdered milk, it was identified that the cell was undergoing sporulation of bacteria where according to Cho & Chung (2020), spores are generally formed when the organism is stressed by reducing the amount of nutrients and pathogens will allow it to survive the dormant state outside the aerobic or anaerobic environment of the intestinal tract until a new host is colonised.

Table 1. Macroscopic and microscopic observation of bacteria isolated from Suffy Annis and Everyday powdered milk.

SAMPLE	MACROSCOPIC OBSERVATION	COLONIES MORPHOLOGY CHARACTERISTICS	MICROSCOPIC OBSERVATION	GRAM TYPE	SHAPE
Suffy Annis		<ul style="list-style-type: none"> • Colony is shaped irregularly. • Dry surface. • White colony. • Undulated margin. 	 1000x magnification	<ul style="list-style-type: none"> • Gram +ve 	<ul style="list-style-type: none"> • Rod shape with spore
Everyday		<ul style="list-style-type: none"> • Colony is shaped irregularly. • Smooth surface. • Creamy colony. • Undulated margin. 	 1000x magnification	<ul style="list-style-type: none"> • Gram +ve 	<ul style="list-style-type: none"> • Rod shape

From the table above, also showed the result of Everyday powdered milk and it can be identified by microscopic observation that the characteristics of the bacterial colony formed on the surface of the agar plate that the whole colony was shaped irregularly with smooth and wet surface. The colour formed on the agar plate was cream in colour. The whole colony of the bacteria shown in the result was irregularly. The elevation of the colony formed was convex. The edge margin of the colony was undulating (wavy). Another microscopic observation was conducted to identify the gram type of the bacteria and the shape of the bacteria through the gram staining method in these sample. As shown in the result, the bacteria can be identified as gram positive based on the purple colour shown on the bacteria seen under the microscope. The shape of the bacteria was identified to be rod shaped. From everyday powdered milk sample, it was identified that the cell was a *Lactobacillus* sp. that according to Bratcher (2018), Lactobacilli are small, slender, nonmotile, gram-positive bacilli in chains. Immature colonies appear smooth, convex, and translucent. According to Iwasa et al., (2013), *Lactobacillus* was fermented milk that had many benefits based on its antioxidative and anti-inflammatory properties. Hence these bacteria inside the everyday was the beneficial bacteria.

CONCLUSIONS

In conclusion, from this comparative study between the two types of cow powdered milk, the differentiation of the bacteria presence and the type of bacteria formed within these two powdered milks has been determined. It can be summarised where the bacteria that was identified from each powdered milk consists of similar characteristics where it has the traits of rod-shaped bacteria which is known as the *Bacillus* sp. which are known as rod-shaped bacteria. This comparison was indicated by the method of agar streaking and gram staining that was performed throughout the project. The milk that mostly had a higher number of probiotic bacteria was Everyday powdered milk. As according to Taye et al., (2021), known probiotic and bacteriocinogenic properties such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus* and *Streptococcus* species form an integral part of the microbiota. One of the probiotic bacteria that was observed based on the results, would be *Lactobacillus* sp. and *Bacillus subtilis*. It is because these bacteria had the same characteristics where it is rod-shaped, clumped, and both of these bacteria were gram-positive. Most of the bacteria that was observed, were gram positive, by using the gram staining. Based on the Suffy powdered milk, can be summarised that the gram type of the bacteria is gram positive and most of the bacteria was rod shaped with formation of spores from its cell. From Suffy powdered milk, it was identified that the cell was undergoing sporulation of bacteria where according to Cho & Chung (2020), spores are generally formed when the organism is stressed by reducing the amount of nutrients and pathogens which are not safe to be consumed might be due to the cross contamination during sample preparation. However, the results of this study should be treated with caution due to the lack of details regarding the specificity type of bacteria that is present in each agar plate. Future research could further examine the specificity type of bacteria that is present between the different samples of cow powdered milk and the functionality of each bacteria that is studied upon. As this would contribute to a deeper understanding between the classification of each bacteria that is observed within the project.

REFERENCES

- Batta, A. Importance of Milk. *Int J Res Rev.* 2016; 3(2):96-102.
- Berman, R. (2021). Could Milk Reduce Coronary Heart Disease Risk?. *Medical News Today.*
- Bratcher D. F. (2018). 133 - Other Gram-Positive Bacilli. *Principles and Practice of Pediatric Infectious Diseases (Fifth Edition) 2018*, Pages 786-790.e4. <https://doi.org/10.1016/B978-0-323-40181-4.00133-X>
- Brown, K.A., Venkateshmurthy, N.S., Potubariki, G. et al. (2022). *The Role of Dairy In Healthy And Sustainable Food Systems: Community Voices From India.* BMC Public Health 22, 806. doi: 10.1186/s12889-022-13194-w.
- Cho, W. I., & Chung, M. S. (2020). Bacillus spores: a review of their properties and inactivation processing technologies. *Food science and biotechnology*, 29(11), 1447–1461.
- Elwood, P. C., Pickering, J. E., Givens, D. I., & Gallacher, J. E. (2010). The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. *Lipids*, 45(10), 925-939.
- Iwasa, Masayo; Aoi, Wataru; Mune, Keitaro; Yamauchi, Haruka; Furuta, Kaori; et al. *Nutrition Journal*; London Vol. 12, (2013). DOI:10.1186/1475-2891-12-83.
- Kumar, H., Yadav, D., Kumar, N., Seth, R., & Goyal, A. K. (2016). Nutritional and nutraceutical properties of goat milk-a review. *Indian J. Dairy Sci*, 69, 513-518.
- Taye, Y., Degu, T., Fesseha, H., & Mathewos, M. (2021). Isolation and Identification of Lactic Acid Bacteria from Cow Milk and Milk Products. *The Scientific World Journal*, 2021, 4697445. <https://doi.org/10.1155/2021/4697445>

Surat kami : 700-KPK (PRP.UP.1/20/1)

Tarikh : 20 Januari 2023

Prof. Madya Dr. Nur Hisham Ibrahim
Rektor
Universiti Teknologi MARA
Cawangan Perak



Tuan,

PERMOHONAN KELULUSAN MEMUAT NAIK PENERBITAN UiTM CAWANGAN PERAK MELALUI REPOSITORI INSTITUSI UiTM (IR)

Perkara di atas adalah dirujuk.

2. Adalah dimaklumkan bahawa pihak kami ingin memohon kelulusan tuan untuk mengimbas (*digitize*) dan memuat naik semua jenis penerbitan di bawah UiTM Cawangan Perak melalui Repositori Institusi UiTM, PTAR.

3. Tujuan permohonan ini adalah bagi membolehkan akses yang lebih meluas oleh pengguna perpustakaan terhadap semua maklumat yang terkandung di dalam penerbitan melalui laman Web PTAR UiTM Cawangan Perak.

Kelulusan daripada pihak tuan dalam perkara ini amat dihargai.

Sekian, terima kasih.

“BERKHIDMAT UNTUK NEGARA”

Saya yang menjalankan amanah,

SITI BASRIYAH SHAIK BAHARUDIN
Timbalan Ketua Pustakawan

nar

Setuju.

27.1.2023

PROF. MADYA DR. NUR HISHAM IBRAHIM
REKTOR
UNIVERSITI TEKNOLOGI MARA
CAWANGAN PERAK
KAMPUS SERI ISKANDAR