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# Colony Growth Rate and Morphological Characterization of Bovine Rumen Bacteria in Fermented Liquid

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**Abstract** Bovine rumen bacteria consist of diverse microorganisms essential for processing manure, compost, and liquid organic fertilizer, enhancing soil fertility and nutrient availability while suppressing pests and pathogens. These bacteria include cellulose-digesting species such as *Bacteroides succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus*, which produce cellulase enzymes for organic matter decomposition. Morphologically, cellulolytic bacteria are categorized into coccus forms (*Ruminococcus flavefaciens* and *Ruminococcus albus*) and rod forms (*Bacteroides succinogenes* and *Butyrivibrio fibrisolvens*), with Gram-positive and Gram-negative cell walls. This study examined the growth and morphology of bovine rumen bacteria in fermented liquid over three weeks. Colony counts increased from $4.75 \times 10^6$ CFU/mL in week 1 to $2.92 \times 10^7$ CFU/mL in week 2 and $6.2 \times 10^8$ CFU/mL in week 3. Colony morphologies included colors (pink, yellow, blue center), sizes (small, medium, large), shapes (circular, irregular), elevations (raised, convex, unbonated), surfaces (smooth, mucoid), and edges (regular, irregular). Predominant bacterial cell shapes were bacillus, with Gram-positive staining. These findings underscore the potential of bovine rumen bacteria in organic waste fermentation and nutrient cycling.

**Keywords:** Colony growth rate, Morphological characterization, Bovine rumen bacteria, Fermented liquid

## INTRODUCTION

Ruminants such as bovine have four stomach compartments consisting of the rumen, reticulum, omasum, and abomasum. The rumen is the largest compartment and has a diverse microbial community consisting of bacteria, archaea, protozoa, and fungi.

Bovine rumen bacteria consist of a collection of severalmicroorganisms that are very useful in the processing of manure, compost, liquid organic fertilizer, and at the same time are able to improve soil fertility and provide life in the soil. Microorganisms contained in the bovine rumen bacteria can increase the fermentation of waste and organic waste, increase the availability of nutrients for plants, and suppress the activity of insects, pests, and pathogenic microorganisms.

Bovine rumen bacteria consist of 3 parts, namely in the rumen fluid 30%, food particles 70%, and bacteria attached to the epithelium of the rumen wall generally amounting to 1-10 billion/ml of rumen fluid. This has the potential as a decomposer of organic matter (Purbowati et al., 2014, Zubaidah et al., 2019). A group of cellulose-digesting bovine rumen bacteria include *Bacteriodes succinogenes*, *Ruminicoccus flavefaciens*, *Ruminicoccus albus*, *Cillobacterium cellulosolvens* which can produce cellulose enzymes in hydrolyzing glucoside bonds B 1,4, cellulose and cellobiose dimers (Veiganata, 2020).

Morphologically, cellulolytic bacteria in the rumen are divided into coccus forms (*Ruminococcus flavefaciens* and *Ruminococcus albus*) and rod forms (*Bacteroides succinogenes*, *Butyrivibrio fibrisolvens* and *Clostridium lochheadii*), while the cell wall types of each of these morphological groups are Gram- positive and Gram-negative (Hungate, 1966; Ogimoto & Imai, 1981). Aside from being a cellulose digester, *R. flavefaciens*, *R. albus* and *B. fibrisolens* are also anemicellulose digester (Hungate, 1966). *B. fibrisolens* is even better known as a hemicellulose digester (Stewart, 1988).

Cellulolytic bacteria can hydrolyze cellulose complexes into smaller oligosaccharides and finally into glucose using cellulase enzymes. Cellulase enzymes work by breaking down cellulose chains which were originally polysaccharide chains that were difficult to digest into disaccharides or monosaccharides in the form of glucose which can be digested easily (Ibrahim & Dewany, 2007).

Cellulase enzymes are decomposing agents that have specific properties to hydrolyze β-1.4-glycosidic bonds of cellulose chains and their derivatives. Deng & Tabatabai (1994) stated that the cellulase enzyme complex generally consists of three main enzyme units, namely endo-β-1.4-glucanase (Cx) which plays a role mainly in the amorphous part of the cellulose chain, exo-β-1.4-glucanase (C1) or cellobiohydrolase which plays a role in breaking down the crystalline part of the cellulose chain and β-glucosidase is an important enzyme unit in producing glucose from breaking down cellobiose. According to Da Silva et al. (2005) that the cellulase enzyme or enzyme known as the systematic name β-1.4 glucan-4-glucanohydrolase is an enzyme that can hydrolyze cellulose by breaking the β-1.4 glycosidic bonds in cellulose, cellodectrin, cellobiose, and other cellulose

derivatives into simple sugars or glucose. The system for breaking down cellulose into glucose consists of three types of cellulase enzymes, namely endo-β-1.4-glucanase, exo-β-1.4-glucanase, and β-glucosidase. Endo-β-1.4-glucanase attacks the middle of the chain randomly, exo-β-1.4-glucanase (selobiohydrolase) breaks down disaccharide units (cellobiose) from the end of the chain, and β-glucosidase breaks down cellobiose into glucose.

Several types of bacteria/microbes present in the contents of the rumen are (a) lipolytic bacteria/microbials, (b) acid-forming bacteria/microbials, (c) amylolytic bacteria/microbials, (d) cellulotic bacteria/microbials, (e) bacteria/microbes proteolytic. The number of microbes in the bovine's rumen varied, including: proteolytic microbes 2.5 x $10^9$cells/g rumen contents, cellulolytic microbes 8.1 x $10^4$cells/gram rumen contents, amylolytic 4.9 x $10^9$cells/g contents, acid-forming microbes 5, 6 x $10^9$cells/g contents, lipolytic microbes 2.1 x $10^{10}$cells/g contents and lipolytic fungi 1.7 x $10^3$cells/g contents. These microorganisms can digest starch, sugar, fat, protein, and non-protein nitrogen to form microbes and B vitamins (Sutrisno et al., 1994).

The research results of Jannah et al., (2017) showed that the bacterial colonies filled with free-range chicken cecum were white, were inside and on the surface of the agar, had flat edges, colony diameters of 2-3 mm and clear zone diameters of 18 mm and 29 mm. Bacillus-shaped bacteria react negatively to gram staining, while cocci-shaped bacteria react positively and negatively to gram staining. The total number of bacteria in the contents of the free-range caecum was 5.3 x 105 cfu/g.

## METHOD

### *Bovine Rumen Bacteria*

Put the shrimp paste into the boiling water and add the molasses and stir. Put the potatoes in the water in the pot. Separate the potatoes with the potato extract solution. Put the corn bran into the bucket then close tightly and add the shrimp paste and molasses solution and extra potato solution. Stir until evenly distributed and closed. PDA medium for inoculation of bacteria present in the epithelium of the bovine rumen wall. Insert the chopped bovine rumen epithelium into the PDA medium. Put the pieces of pineapple into the blender and add water, the results of the crushed pineapple blend, then put it into the PDA solution. Stir the medium until evenly distributed and closed tightly. Put the pieces of pineapple into the blender and add water, the results of the crushed pineapple blend, then put it into the PDA solution. Stir the medium until evenly distributed and closed tightly.

### *Total of Bacterial Colonies*

Sample dilution was carried out in multiples of 1:10. Each dilution suspension was grown using the pour plate method. Bacteria will reproduce on agar medium and form colonies after incubation for 18-24 hours. Counted using a colony

counter equipped with an electronic recorder. The bacteria that are counted are bacteria that are still alive, by diluting the bacterial medium, for example, up to 3 times in a test tube. Then the bacteria were planted and incubated, after which the growing colonies were counted.

*Morphological Characterization of Bacterial Colonies*

Macroscopic observations were made by observing the shape and color of the bacterial liquid colonies. It is divided into three, namely observation from above for the overall shape of the colony, observation from above for the shape of the edge of the colony and observation from the side for the height of the colony.

*Gram Staining Technique*

Aquades was taken and dripped on a glass object, added with 1 ose of sample culture, then fixed over a fire. Drop violet liquid coloring and leave for 1 minute, wash with running water, then drop Lugol leave for one minute and wash again with running water. Next, drip 96% liquid, leave it for 10-20 seconds, wash it with running water and add safranin, leave it for 20-30 seconds, then wash it again with running water. The next step is to dry it using absorbent paper and add immersion oil and observe it under a microscope. If the results of the staining are red, then the bacteria are gram-negative, whereas if purple-colored bacteria are obtained, the bacteria are gram-positive.

## FINDINGS AND DISCUSSION

The results of this study are as follows.

In Figure 1. Shows the growth of the number of colonies in the rumen of bovine in the fermented liquid. The number of bovine rumen colonies was 4.75 X $10^6$CFU/ml in week 1, increased by 2.92 X $10^7$CFU/ml in week 2 and increased again by 6.20 X $10^8$CFU/ml in week 3. This shows that bacteria can still grow It is suspected that the nutrients and media still support the growth of bacteria.

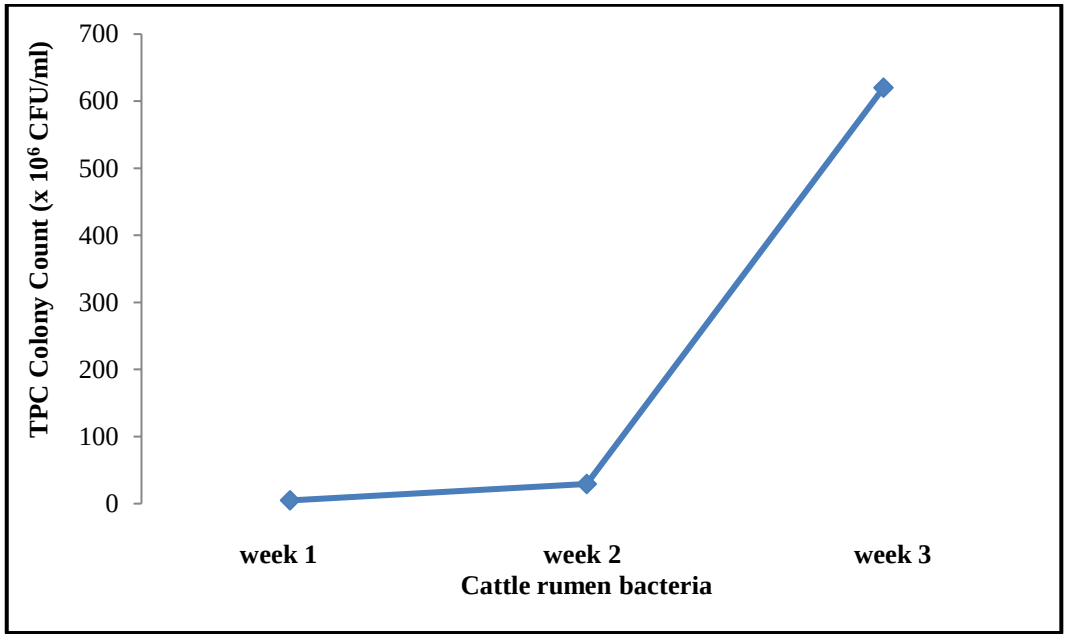


**Figure 1.** Development of the number of colonies of bovine rumen bacteria in fermented liquid.

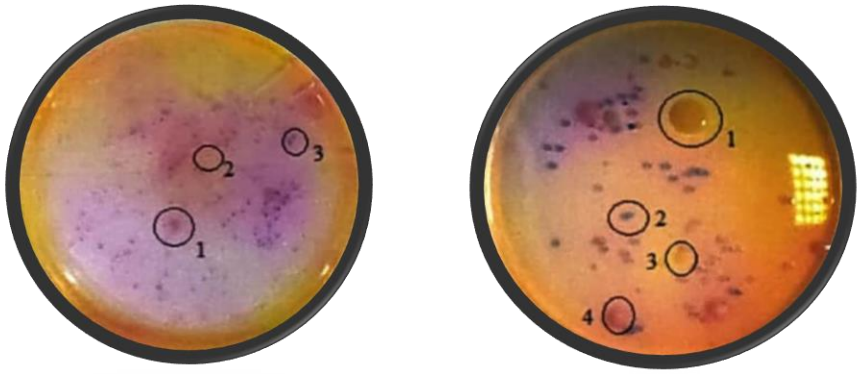


**Figure 2.** Development of the number of bovine rumen bacterial colonies (A and B).

**Table 1.** Morphology of bovine rumen colony week 1.

| ISOLATE CODE | COLONY MACROSCOPY | | | | | |
|---|---|---|---|---|---|---|
| | **Colony Color** | **Size** | **Colony Form** | **Elevation** | **Surface** | **Edge** |
| **1.** | Pink | Currently | Irregular | Raised | Smooth | Irregular |
| **2.** | Yellow | Currently | Irregular | Raised | Smooth | Irregular |
| **3.** | Center blue | Small | Irregular | Raised | Smooth | Irregular |

**Table 2.** Morphology of bovine rumen bacterial cells week 1.

| ISOLATE CODE | CELL MORPHOLOGY | |
|---|---|---|
| | Cell Shape | Grams |
| **1.** | Basil | Positive |
| **2.** | Basil | Positive |
| **3.** | Basil | Positive |

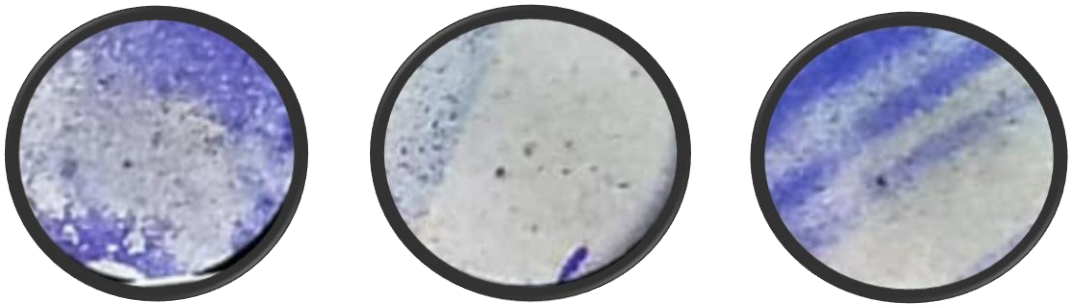

**Figure 3.** Results of gram staining for week 1 bovine rumen bacteria.

Based on the results of observations of the bovine rumen bacterial colony isolates in week 1, 3 (three) colony colors were obtained. In pink colony, medium size, irregular shape (irregular/not edged), flat elevation on the entire surface (raised), smooth texture (smooth), irregular edges. In yellow colony, medium size, irregular shape (irregular/not edged), even elevation on the entire surface (raised), smooth texture, smooth surface, irregular edge. In center blue colony, small size, irregular shape (irregular/not edged), even elevation over the entire surface (raised), smooth surface, irregular edges, bacillus, and gram-positive cell shape.

**Table 3.** Morphology of week 2 bovine rumen bacterial colonies.

| ISOLATE CODE | COLONY MACROSCOPY | | | | | |
|---|---|---|---|---|---|---|
| | Colony Color | Size | Colony Form | Elevation | Surface | Edge |
| **1.** | Yellow | large | Circular | Convex | Mucoid | Regular |
| **2.** | Center blue | small | Irregular | Unbonated | Smooth | Irregular |
| **3.** | Yellow | Currently | Irregular | Raised | Smooth | Irregular |
| **4.** | Pink | Currently | Circular | Convex | Smooth | Regular |

**Table 4.** Morphology of sugarcane bovine rumen bacterial cells week 2.

| ISOLATE CODE | CELL MORPHOLOGY | |
|---|---|---|
| | Cell Shape | Grams |
| **1.** | Basil | Positive |
| **2.** | Basil | Positive |
| **3.** | Basil | Positive |
| **4.** | Basil | Positive |

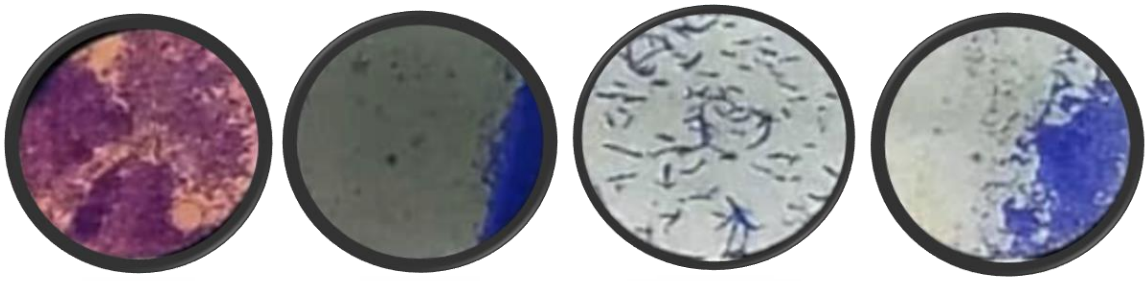

**Figure 4.** Results of gram staining of bovine rumen week 2.

Based on the results of observations on the morphological isolates of the 2nd week of bovine rumen bacterial colonies, 4 (four) colony colors were obtained. In yellow colony, large size, circular in shape, convex in elevation, smooth surface, regular edges. In center blue colony, small size, irregular shape (irregular/not edged), unbonated elevation, mucoid surface, regular margin. In yellow colony, medium size, shapeirregular (irregular/not edged), elevationevenly over the entire surface (raised), mucoid surface, irregular edges. In pink colony, medium size, circular shape (circular/round edged), convex elevation, mucoid surface, regular edge, bacillus, and gram-positive cell forms.

## CONCLUSION

The growth of the number of bovine rumen bacterial colonies was 4.75 X $10^6$CFU/ml in week 1, increased by 2.92 X $10^7$CFU/ml in week 2 and increased again by 6.20 X $10^8$CFU/ml. at week 3 in fermented liquid. The morphology of the bovine rumen bacterial colonies from week 1 to week 2 obtained colony colors: yellow, pink, blue, milky white, blue center, colony size: pith/point, medium, large, colony shape: circular, irregular, elevation: convex, flat, surface: smooth, mucoid, edge: regular, irregular. Bacterial cell shapes: bacillus and gram positive.

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