Universe Scientific Publishing

ORIGINAL ARTICLE

Metagenomics Study on the Effect of β-lactam Antibiotic on the Structure of Rumen Bacteria in Calves

Lu Wang, Yongli Qu*, Wei Li

College of Animal Science and Technology, Heilongjiang Bayi Agricultural University, Daqing 25 163319

Abstract: (Objective) This paper aims to study the effect of β-lactam antibiotic on the structure of rumen bacteria in calves by metagenomics. (Method) In this experiment, 54 newborn calf were selected and randomly divided into 3 treatments according to the principle of similar birthday age. The test was divided into: 1) control group (CK), feeding no antibiotic milk group; 2) antibiotic milk group (AM); 3) pasteurization antibiotic milk group (APM). The trial period was 180 days. The rumen bacteria genomic library of calf was constructed from 60 g (T1), 90 d (T2) and 180 d (T3) calf rumen fluids in each group. (Results): The results indicate that (1) The high-quality reads of the nine samples accounted for more than 96% of the percentage of reads and high-quality sequence bases. (2) The reads bases obtained were in the 10%-90% range and the Q value was in the 35-40 range. (3) The bacteria with the highest abundance of 9 samples were Bacteroidetes (average abundance=69.8%), followed by Firmicutes (average abundance=9.8%). (Conclusion) Through screening, it was determined that the two bacteria of the rumen of the calf rumen played an important role in the digestion of nutrients in the r umen. Bacteroides for pasteurized milk containing β-lactam antibiotics More sensitive. The antibiotic milk with β-lactam antibiotic may cause food safety related drug resistance problems by affecting spirulina bacteria.

Keywords: Calf; Rumen; Antibiotic Milk; Flora; Metagenomics

Introduction

(Important Significance of Research) In pasture, anti-milk refers to the use of antibiotics in sick cows, which may cause cows to milk has antibiotic residues[1-2]. Long-term intake of anti-milk by human beings will have serious effects on health, such as allergic reactions, destruction of normal flora in intestinal tract, and increase of drug resistance[3]. In order to ensure human health, milk with antibiotic residues exceeding the standard will not be accepted by the manufacturers[4]. In order to reduce the economic loss of the pasture, the milk with anti-dairy and fresh milk will usually be pasteurized and fed to calves[5-6]. The phenomenon of feeding calves with anti-dairy in dairy farms in China is widespread. (Previous Research Progress) Metagenomics was originally used to obtain micro-biological genetic materials from microbial samples in specific environments. With the progress of sequencing technology, research and development can be carried out.

The researcher avoids the detour of culturing uncultured microorganisms, studies the diversity of microbial communities in a specific environment and evaluates their metabolic functions by constructing a metagenomic library and screening high-efficiency enzyme genes from the library. At present, this technology has been applied to study the diversity of various environmental microorganisms, such as deep-sea aquatic microorganisms and digestive tract ecosystem[7] of human beings and animals. In recent years, a large amount of cellulase (gene) related information is obtained by modern molecular biology techniques such as metagenomics. (Entry Point of this Study) There are many studies abroad on the effects of feeding anti-milk or waste milk on the growth and body weight of calves, but for calves.

doi: 10.18686/pas.v2i1.1313

There are few studies on the influence of rumen microflora changes of 55. (Key Issues to be Solved) The purpose of this experiment is to study the effect of β -lactams on rumen bacterial flora structure of calves by metagenomics. The changes of rumen flora can be comprehensively understood, which provides certain theoretical basis for utilization of anti-milk and improvement of calf health.

1. Materials and methods

1.1 Test material

In this experiment, 54 calves were randomly divided into 3 groups. The experiment was divided into: 1) control group (CK) and non-anti-fresh milk group. 2) β-lactams have anti-milk group. 3) pasteurized beta-lactams have anti-milk groups (APM). The test period is 180 d. Six calves were selected from each group 60 d, 90 d and 180 d after birth, rumen fluid 50 mL was taken by puncture, and the filtered rumen fluid was cryopreserved in liquid nitrogen for constructing rumen bacteria metagenomic library of calves.

1.2 Test and determination method

Each sample was weighed 10 ml and 150 ml of phosphoric acid buffer was added to it and incubated 30 min in a 37℃ constant temperature shaker. Centrifuge the sample (800 r/min, 10 min). The supernatant was centrifuged again in a centrifuge tube (10,000 r/min, 5 min), for precipitation. Weigh 0.5 g of precipitate into centrifuge tube, add 1 ml of extraction buffer, then add 10% SDS 200 μl, C water bath for 2 h, then quick freeze with liquid nitrogen for 5 min, finally 80 C water bath for 5 min, freeze and thaw repeatedly for 3 times. Centrifuge the sample obtained in the above steps (8000 r/min, 5 min), take the supernatant into a centrifuge tube, add equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, volume ratio) and centrifuge (12,000 r/min, 10 min), repeat this step. Then 0.7 times the volume of isopropanol is added to precipitate 3h at -20℃, centrifuged (12,000 r/min, 20 min) and rinsed with 1 ml ethanol (70% is evaporated and dried at room temperature after precipitation. The precipitate dissolved in 200μ l ddh 2 o. The PCR product was 1% stained with EB Agarose gel electrophoresis detection.

Bacterial genes in rumen of calves were extracted and corresponding libraries were established. Bioinformatics analysis was carried out after sequencing with illumina MiSeq.

2. Results

2.1 Rumen fluid sample test results

Using TBS380 combined with picogreen to detect the concentration and purity. According to the concentration detection results, 0.8% agarose gel electrophoresis (20 min) with a voltage of 120V was used to detect the integrity of the sample. The results of agarose gel electrophoresis are shown in Figure 1.

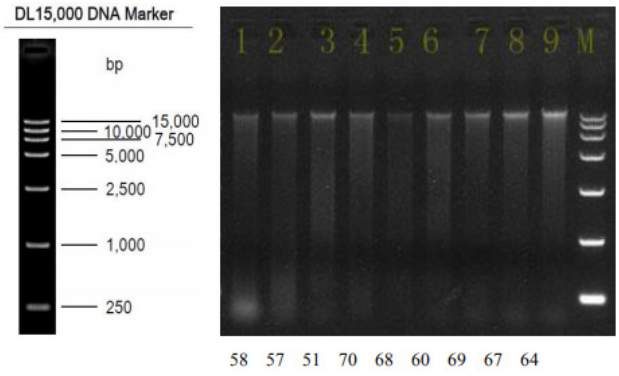

Figure 1. The test results of agarose gel electrophoresis

2.2 Filtering data quality assessment

FAST QC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) is used to control the quality of data. The specific results are shown in Figure 2. Generally, the base quality of the middle part of reads is relatively high, while the base quality of the 5 and 3 ends is relatively low. As shown in Figure 2, the q value of the reads bases in the 10%-90%

range is distributed in the 35-40 range. Therefore, the average quality of data obtained after sequencing and filtering in this test is very high.

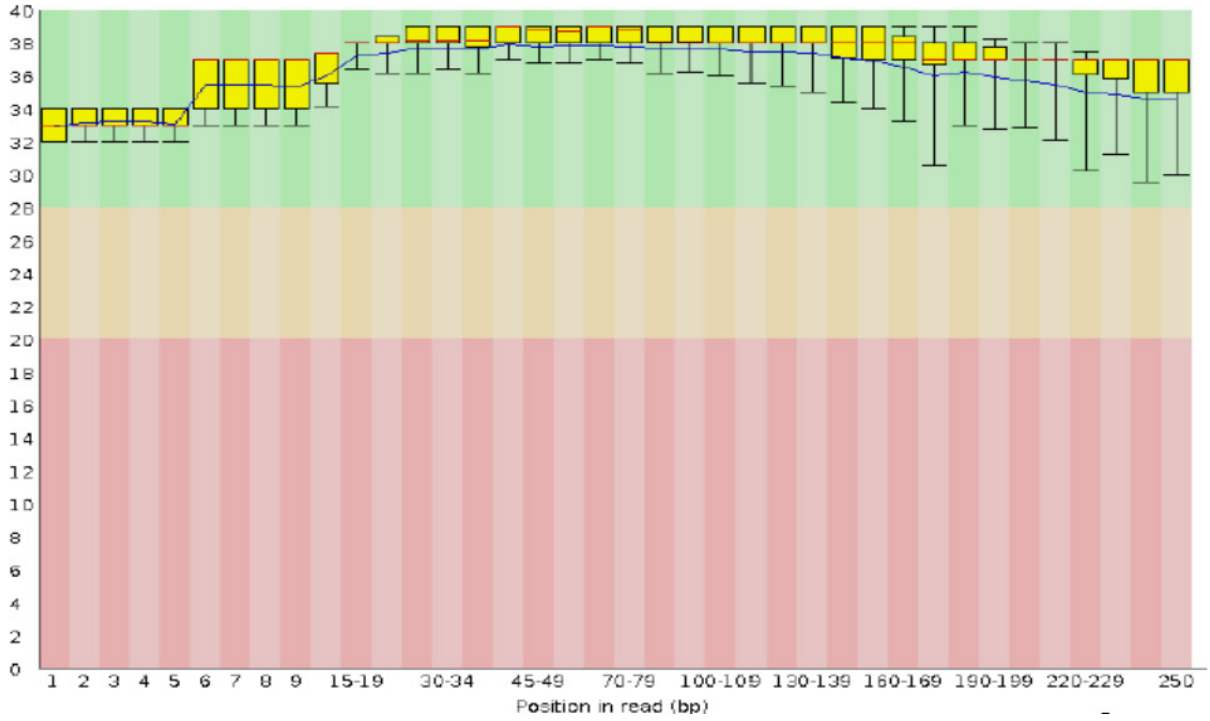

Figure 2. Distribution of single-base quality.

2.3 Analysis of species abundance

Because each sequence may have multiple alignment results and at the same time obtain multiple different classification levels, in order to ensure its biological significance, LCA algorithm (applied to the systematic classification of MEGAN software) is adopted. This classification takes the classification level before the first branch appears as the species annotation information of the sequence.

Figures 3 and show species abundance at the gate level. Among the 9 samples, the 5 species with relatively high abundance are Bacteroidetes, Firmicutes, Proteobacteria, fibrobacterias. Actinobacteria From the comparison of the samples fed pasteurization, β -lactam with milk resistance, B1T1, B1T2, and B1T33, it can be seen that the bacteria of Firmicutes and Bacteroidetes show a gradual downward trend with the growth of calf's month age. However, actinomycetes, Fibrobacteres and Actinobacteria have opposite trends. From the comparison of feeding, β-lactam with milk resistance, B2T1, B2T2, and B2T33 samples, we can know that the microflora Verrucomicrobia and Proteobacteria are gradually increasing with the growth of calf's month age, while the abundance of other phyla has no obvious change rule. Proteobacteria can be seen from the comparison of B3T1, B3T2, B3T3 and 3 samples fed with fresh milk (Proteobacteria). Bacteria are on the rise. In general, 2, feeding in month-old calves, β-lactams have anti-milk group. (B2T1) and fresh milk feeding group (B3T1) have relatively high species abundance at the gate level. The two groups contain 11,754,882 and 11,753,187 bacteria respectively. Therefore, it can be seen that 2 month-old calves are richer in rumen microflora than 3 month-old calves and 6 month-old calves, and milk obtained by pasteurization method has a greater impact on rumen microflora structure of calves, which may be caused by fewer bacteria in pasteurized milk.

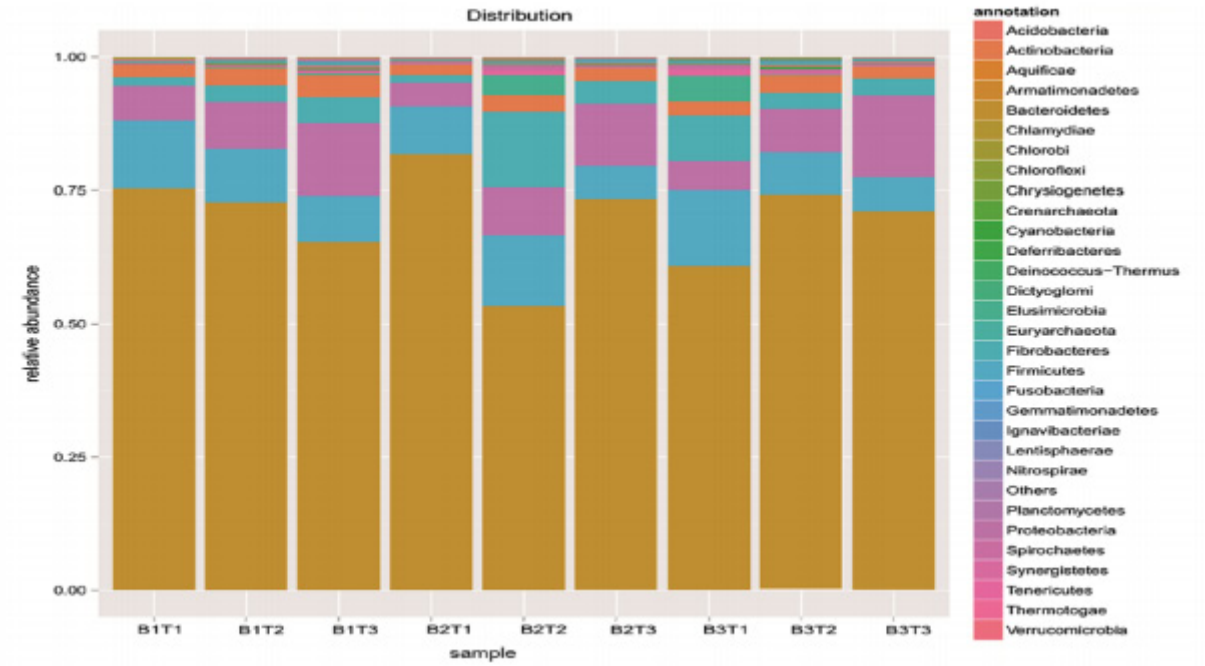

Figure 3. Relative species abundances of the samples in the level of phylum. x axis represents sample, and y axis indicates relative abundance.

The species abundance at genus level is shown in Figure 4. Among the 9 samples, the 5 bacteria with relatively high abundance are Platforms (Prevotella) > Bacteroides) > fibrobacilli > barnesilla > aliestipes. From the comparison of samples fed with Bavarian disinfection, β -lactam milk-resistant calves, B1T1, B1T2, and B1T33, it can be concluded that the abundance of fibrobacilli increases with the increase of age, while the abundance of Barnesiella, Bacteroides, Prevotella decreases. From the comparison of the samples of calves fed with β -lactam with milk resistance, B2T1, B2T2, and B2T33, it can be concluded that with the increase of the age of the month, the fibrinous bacteria shows an upward trend, and from the comparison of the samples fed with fresh milk, B3T1, B3T2, and B3T3, this, 3, it shows a downward trend.

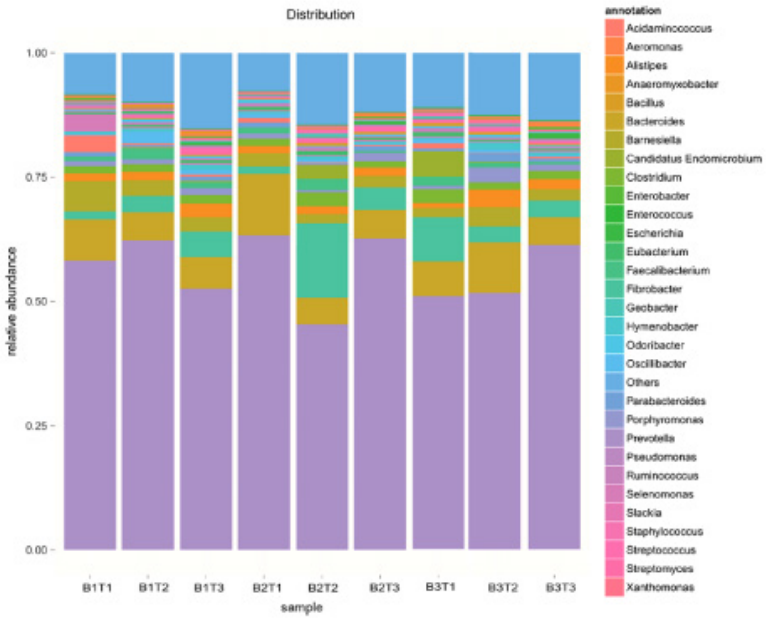

Figure 4. Relative species abundances of the samples in the level of genus. x axis represents sample, and y axis indicates relative abundance.

3. Discussion

High-quality reads accounts for more than 96% of the quality filtered raw data. After the sequence is assembled, a

total of 262,753 scaffolds and 3,498,534 contigs are obtained. In the sample, the abundance of the phylum Fibrobacter and the genus fibrobacter are relatively high compared with other species. We have learned that the genus Bacillus and two species (F and succinogenes and fibrin intestinalis) in the Bacillus can degrade plant cellulose in the intestines of herbivores, which indicates that a remarkable characteristic of the Bacillus is cellulose degradation[8-9]. As an effective cellulolytic bacterium, fibrobacter plays an executive role in rumen and can be used as a source of biological new energy, with potential for development and utilization[10]. according to the previous studies of 16s rRNA ,it was found that many components in fibrobacilli play an important role in the cellulose decomposing ecosystem[11] and the physiological and ecological studies of F. succinogenes showed that it is an important cellulose hydrolyzing microorganism and plays an important role in the degradation process of rumen cellulose[12-13]. Among them, xylanase, esterase, glucanases and arabinofuranosidase act on cellulose and hemicellulose through complex processes to degrade plant cell walls[14] Bacteroides includes four classes (Bacteroides, Sphingobacteria, Flavobacteria and Cytophagia), with about 7,000 species. Among them, Flavobacterium is the largest class, which contains more than four times as many species as the remaining three classes. Its members are the main microorganisms in gastrointestinal tract, and it is considered to be an important bacterium specialized in degrading high molecular organic substances (such as carbohydrates and proteins)[15-16].

By comparing the samples of B1T1, B1T2 and B1T33, it is found that the abundance of bacteroides decreases with the increase of the age, while that of fibrobacilli increases. Through the comparison of B2T1, B2T2 and B2T3, it can be found that with the increase of the age of the month, the fiber.

The bacilli showed an upward trend, while the 3 samples of B3T1, B3T2 and B3T3 showed a downward trend. This is basically consistent with the changes in the water level. The above results show that in rumen digestion, the phylum fibrobacilli and bacilli play a crucial role, and the phylum bacteroides is more sensitive to pasteurized milk.

4. Conclusion

Through screening, it is determined that the two bacteria of the phylum fibrobacilli and the genus Bacillus play an important role in the rumen digestion of calves. Bacteroides is more sensitive to pasteurized milk containing β -lactam antibiotics. β-lactam antibiotics are resistant to milk, which may lead to drug resistance problems related to food safety by affecting spirochete bacteria.

References

1. Briyne N D, Atkinson J, Borriello S P, *et al.* Antibiotics used most commonly to treat animals in Europe.Veterinary Record,2014,175(13):325.
2. Pereira R V, Siler J D, Bicalho R C, *et al.* Multiresiduescreening of milk withheld for sale at dairy farms in central New York State. Journal of Dairy Science,2014,97(3):1513-1519.
3. Hershkovich J, Broides A L, Smith H, *et al.* Beta lactamallergy and resensitization in children with suspected beta lactam allergy. Clinical & Experimental Allergy,2009,39(5):726.
4. Bai Guotao, Chu Xiaogang, Pan Guoqing, *et al.* Research progress on detection technology of β -lactam antibiotic residues. Food Science, 2008(7):485-489.
5. Duse A, Waller K P, Emanuelson U, *et al.* Farming practices in Sweden related to feeding milk and colostrum from cows treated with antimicrobials to dairy calves.Acta Veterinaria Scandinavica,2013,55(1):1-9.
6. Brunton L A, Duncan D, Coldham N G, *et al.* A survey of antimicrobial usage on dairy farms and waste milk feeding practices in England and Wales. Veterinary Record, 2012,171(12):296.
7. Ransom-Jones E, Jones DL, McCarthy AJ, *et al.*The Fibrobacteres: an important phylum of cellulose-degrading bacteria. Microb Ecol2012, 63:267-281.
8. Zhao Jingwen. Screening and diversity analysis of rumen microbial protease and dipeptidyl peptidase IV genes in dairy cows. Beijing: China Agriculture Department College, 2013.
9. Rosenberg E.The phylum Fibrobacteres. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds). The prokaryotes. Springer, Berlin, 2014: 641-642.
10. Jewell KA, Scott JJ, Adams SM, *et al.* A phylogenetic analysis of the phylum Fibrobacteres[J].Syst Appl Microbiol

1.,2013,36:376-382.
11. Ransom-Jones E, Jones DL, Edwards A, *et al.* Distribution and diversity of members of the bacterial phylum Fibrobacteres in environments where cellulose degradation occurs. Syst Appl Microbiol,2014,37:502-509.
12. Kobayashi Y, Shinkai T, Koike S.Ecological and physiological characterization shows that Fibrobacter succinogenes is important in rumen fiber digestion: review.Folia Microbiol,2008, 53:195-200.
13. Shinkai T, Ueki T, Kobayashi Y. Detection and identification of rumen bacteria constituting a fibrolytic consortium dominated by Fibrobacter succinogenes. Anim Sci J,2010, 81:72-79.
14. Jun H, Qi M, Ha J, Forsberg C.Fibrobacter succinogenes, a dominant fibrolytic ruminal bacterium: transition to the post genomic era.Asian Australas J Anim Sci ,2007,20:802.
15. Thomas F, Hehemann JH, Rebuffet E, *et al.* Environmental and gut Bacteroidetes: the food connection. Front Microbiol 2011,2:93.
16. Fernández-Gómez B. Ecology of marine Bacteroidetes: a comparative genomics approach.ISME J 2013,7:1026-1037.