Study on the Characteristics of Straw Fermentation by 
_Bacillus megaterium_ MYB3

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**Abstract**: Corn stover and rice straw fermentation were carried out using _Bacillus megaterium_ MYB3. The decomposition rates of cellulose and hemicellulose were analyzed at d3, d5, d10, d20. Organic acid concentration was determined by HPLC. The decomposition rates of cellulose and hemicellulose in corn stover were 54.29% and 48.21% in 20 days respectively. The decomposition rates of cellulose and hemicellulose in rice straw were 44.87% and 29.32%. Concentration of lactic acid was increased in the fermentation of corn stover and rice straw. And acetic acid was increased and then decreased. The concentration of propionate and butyric acid were measured after 10 days in the corn stover. Butyric acid was decreased from original concentration in 10 days at rice straw.

1 Introduction
China's total once-a-year output of straw had more than 700 million tons, ranking first in the world. It was that with the increase in corn production, a large amount of straw had been produced. Some of these straws were applied to biogas fermentation, culturing mushrooms [1], the vast majority of excess corn stalks were openly burnt. This has caused serious pollution to the air, soil and environment. This was the waste of valuable biological resources. In addition, straw addition, straw resource was one of the main components of straw in China, of which the straw resources accounted for 21.43%.

Crop Straw was mainly composed of cellulose, hemicellulose and lignin three components. In addition, it was a certain amount of pectin, nitrides and a small amount of inorganic salt nutrients [2]. And in the straw, cellulose and hemicellulose cross-linked with each other, showing intermittent, complex layered structure, this complex structure was the straw could not be easily decomposed an important reason.

Microbes play an important role. That could not replace in maintaining material transformation and ecological balance [3]. Microorganism had many types, reproduction fast, short life cycle characteristics, so the use of decomposition of cellulose and hemicellulose decomposing bacteria. And straw fermentation could not only improve the utilization rate, but also decreased pollutions. It is maintaining the effect of ecological balance, and suitable for large-scale production of straw.

In this study, the properties of straw fermentation were studied by using cellulose and hemicellulose degrading bacteria. This was of great significance to solve the economics, resources and environmental problems in Northeast China.
2 Materials and methods

2.1 Straw fermentation
Weighed 10 kg of corn stalks and 4 kg of straw into the sealed bag, its water content were adjusted to 75%, 3.5% seeding with Bacillus megaterium MYB3(10⁹ cfu/ml), incubating at 30℃. The strain MYB3 was isolated by the laboratory for degrading cellulose and hemicellulose bacteria.

2.2 Determination of cellulose and hemicellulose content
In this experiment, the content of cellulose in straw was determined by sulphuric acid and potassium dichromate oxidation method[4]. Weighed the dry straw(at 80℃, 4 h) to homogenized it, accurately weighed 0.10 g, and then added sulphuric acid and potassium dichromate solution to its oxidation. Then added potassium iodide solution, finally titrated with 0.2 mol/L sodium thiosulfate solution and calculated it. Hemicellulose content was used hydrochloric acid hydrolysis method[4]. And then, using DNS method at the OD₅₄₀nm detected reducing sugar content.

2.3 Determination of organic acids
Analysis organic acids using Chinese national standard GB/T 23877-2009[5], and the concentration were determined by HPLC.

2.3.1 Sample preparation for analysis
Weighed 5 g of the fermented straw, placed in 100 ml volumetric flask, added 60 ml of ultra-pure water, ultrasonic extracted 20 min at 50℃ watch bath, and then cooled to room temperature adjust volume, shaking and filtering with 0.45 μm filter.

2.3.2 Analysis conditions of HPLC. Column: ZOROBAX SB-C18, 4.6 mm × 15 cm × 5 μm
Column temperature: 35℃
Mobile phase: acetonitrile 25 ml + 0.1% phosphoric acid solution 975 ml (w / v)
Flow rate: 0.8 ml / min
Detection wavelength: 210 nm
Injection volume: 10 μl

2.4 Evaluation standard for fermentation grade. Fermentation grade were according to silage quality assessment standard[6] as evaluation.
Table 1 standard for straw fermentation score and organic acid content

| Accounting for the total acid ratio (%) | Score | Accounting for the total acid ratio (%) | Score |
|----------------------------------------|-------|----------------------------------------|-------|
|                                        | Lactic acid | Acetic acid | Butyric acid | Lactic acid | Acetic acid | Butyric acid |
| 0.0-0.1                                | 25     | 50         | 28.1-30.0    | 5          | 20         | 10          |
| 0.2-0.5                                | 25     | 48         | 30.1-32.0    | 6          | 19         | 9           |
| 0.6-1.0                                | 25     | 45         | 32.1-34.0    | 7          | 18         | 8           |
| 1.1-1.6                                | 25     | 43         | 34.1-36.0    | 8          | 17         | 7           |
| 1.7-2.0                                | 25     | 40         | 36.1-38.0    | 9          | 16         | 6           |
| 2.1-3.0                                | 25     | 38         | 38.1-40.0    | 10         | 15         | 5           |
| 3.1-4.0                                | 25     | 37         | 40.1-42.0    | 11         | 14         | 4           |
| 4.1-5.0                                | 25     | 35         | 42.1-44.0    | 12         | 13         | 3           |
| 5.1-6.0                                | 25     | 34         | 44.1-46.0    | 13         | 12         | 2           |
| 6.1-7.0                                | 25     | 33         | 46.1-48.0    | 14         | 11         | 1           |
| 7.1-8.0                                | 25     | 32         | 48.1-50.0    | 15         | 10         | 0           |
| 8.1-9.0                                | 25     | 31         | 50.1-52.0    | 16         | 9          | -1          |
| 9.1-10.0                               | 25     | 30         | 52.1-54.0    | 17         | 8          | -2          |
| 10.1-12.0                              | 25     | 28         | 54.1-56.0    | 18         | 7          | -3          |
| 12.1-14.0                              | 25     | 26         | 56.1-58.0    | 19         | 6          | -4          |
| 14.1-16.0                              | 25     | 24         | 58.1-60.0    | 20         | 5          | -5          |
| 16.1-18.0                              | 25     | 22         | 60.1-62.0    | 21         | 0          | -10         |
| 18.1-20.0                              | 25     | 20         | 62.1-64.0    | 22         | 0          | -10         |
| 20.0-22.0                              | 24     | 18         | 64.1-66.0    | 23         | 0          | -10         |
| 22.1-24.0                              | 23     | 16         | 66.1-68.0    | 24         | 0          | -10         |
| 24.1-26.0                              | 22     | 14         | 68.1-70.0    | 25         | 0          | -10         |
| 26.1-28.0                              | 21     | 12         | > 70         | 25         | 0          | -10         |

Total Score 0-20 20-40 40-60 60-80 80-100

| grade | very bad | bad | general | good | excellent |

3 Results and Discussions

3.1 Changes of pH in corn stover and rice straw fermentation

The pH of corn stover on 3d, 5d, 10d and 20d were pH 6.43, pH 7.03, pH 5.73 and pH 5.69. The pH of rice straw on 3d, 5d, 10d and 20d were pH 6.62, pH 6.86, pH 5.63 and pH 5.61. The changes of pH of corn stover and rice straw were the highest at the 5th day. This was caused by strain MYB3 reproduced, which was growing with pH increasing. However, during the fermentation continued, organic acid gradually increased, so that pH changing, as shown in Fig.1.
3.2 Changes in cellulose and hemicellulose content in corn Stover and rice straw

The cellulose of corn stover contents were 24.58%, 17.91%, 14.5% and 13.33% at 3d, 5d, 10d and 20d respectively. The decomposition rates were 15.72%, 38.59%, 50.28% and 54.29% respectively (Fig. 2-a). Control decreased from 28.95% to 28.37% during fermentation. Hemicellulose contents were 25.68%, 18.83%, 15.74% and 14.93% at 3d, 5d, 10d and 20d respectively. Its decomposition rates were 10.93%, 34.68% 45.4% and 48.21% respectively. Control decreased from 28.55% to 28.26%, as shown in Fig.2-b.

Fig. 2 Content and decomposition rate of cellulose (a) and hemicellulose (b) in the corn stover

*Bacillus megaterium* MYB3 was inoculated in the rice straw for fermentation. The cellulose contents were 24.77%, 19.44%, 15% and 14.7% at 3d, 5d, 10d and 20d respectively. Cellulose decomposition rates were reached 7.11%, 27.1%, 43.75% and 44.87%. Control did not change significantly (26.51% ~ 26%), as shown in Fig.3-a. Hemicellulose contents were 19.62%, 18.52%, 17.95% and 17.65% at 3d, 5d, 10d and 20d respectively. The decomposition rate was 22.48%, 26.3%, 28.28% and 29.32%. Control group did not change significantly (25.9% ~ 25.53%), as shown in Fig.3-b.
According to the results, we could be use *Bacillus megaterium* MYB3 for corn stover fermentation. Cellulose decomposition rate was up to 50.28%, hemicellulose decomposition rate was up to 48.21% at 20 days. To rice straw, cellulose decomposition rate was up to 44.87%, and hemicellulose decomposition rate was up to 29.32%. It indicated that decomposition effect on the corn stover was higher than rice straw by *Bacillus megaterium* MYB3. Several researchers were reported for degrading cellulose and hemicellulose. Li Jing et al. [7], used the isolated of the highly efficient cellulose degrading bacteria, and using complex bacteria was obtained the highest decomposition rate of 31.8%. Li Yi et al. [8], through the different pretreatment experiment that the straw cellulose and hemicellulose decomposition rate were 51.46% and 32.98% each other. In this study, we obtained decomposition rate of cellulose, and hemicellulose were 50.28% and 48.21%. Cui ZJ et al. [9], the bacteria isolated from compost decomposed rice straw to 81%, which greatly limits the applied biodegrading.

### 3.3 Changes in organic acid content

Lactic acid concentration was kept highest level during the corn stover fermentation. And it was gradually to go on rising. It concentration rose 3070.15 mg/kg at 20 days. Acetic acid was increasing in the early stage. The concentration could be reached 1352.69 mg/kg at 5 days, and then it decreased after fermentation until the 10th day. Acetic acid was keeping on the increasing after 10 days, its concentration was 1824.67 mg/kg at 20 days. Propionic acid and butyric acid were begun to be detected at 10 days. These organic acids were detected to 33.97 mg/kg and 184.10 mg/kg at 20 days respectively (Fig. 4-a).

At rice straw of fermentation, lactic acid and acetic acid were produced similar to corn stover at early state. Lactic acid was increased continuously all the time. And detected 1972.86 mg/kg at 20 days. Acetic acid was reached the highest level of 2879.48 mg/kg at 5th day. And then it was decreasing quickly after fermentation. And finally, it was detected to 399.49 mg/kg at 20 days. The propionic acid and butyric acid were decreased in the early fermentation. These organic acids were not detected at 10th day. After this period, these organic acids concentration were gradually increasing to 533.37 mg/kg and 440.74 mg/kg at 20 days, as shown in Fig. 4-b.
Fig. 4 Changes of organic acids of corn stover(a) and rice straw(b) fermentation

In this experiment, the changes of lactic acid displayed similarly the trend for corn stover and rice straw. The concentration was lowest the level at 3rd day. And then the concentration of lactic acid was increased gradually with fermentation. Lactic acid level could have important role preservation of silage fermentation. This was consistent with the research of Liu GY and Allis I et al. The acetic acid produced in the silage was mainly derived from acetic acid bacteria, heterofermentative lactic bacteria and homofermentative lactic bacteria. The amount of acetic acids produced were negatively correlated to the quality of silage. Acetic acid concentration was continuously high level in the early stage of fermentation. After 5th day, its level was decreased value. This was due to rapid into the anaerobic fermentation at early. It was for create favorable conditions for acetic acid production. And then higher lactic acid content inhibited the formation of acetic acid. This was consistent than the results from the study of Liang Yu et al.

Propionic acid was mainly produced with propionic acid bacteria. It effected good inhibition of the aerobic rotting of silage, but at Kung et al., it was shown that propionic acid did not affect the quality of straw fermentation. Butyric acid was produced from decomposition of proteins, glucose and lactic acid by the spoilage bacteria and tyrosine bacteria. Butyric acid was decreased from original concentration in 10 days at rice straw. It was showing that inhibition growth of spoilage bacteria and tyrosine bacteria to improve silage quality. Later stage, butyric acid was gradually produced at two groups.

This fermentation was evaluated the quality by the standard. Fermentation of corn stover got 84, 82, 97 and 75 score at 3, 5, 10 and 20 days respectively, and the fermentation grade was in most of the excellent. The fermentation of rice straw got 48, 43, 90 and 73 score at 3, 5, 10 and 20 days respectively, and the fermentation grade was excellent at 10 days.

4 Conclusions
Corn stover and rice straw were fermentation using Bacillus megaterium MYB3. After 20 days of fermentation, the decomposition rate of cellulose and hemicellulose in the corn stover could get 50.28% and 48.21%. In the rice straw, it could get 44.87% and 29.32%. Fermentation grade was obtained "good" with the organic acid level. This Bacillus megaterium MYB3 could be used a candidate for straw fermentation of silage, to improve the reusing and utilization of effect for straw.

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