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Screening and breeding of a strain with high deodorization and lignocellulosic degradation

Meixiao Wu[1], Yuehua Wang[1], Yijing Wang[1], Xuefei Wang[2], Ming Yu[2], Guixia Liu[1*], Hui Tang[1*]

[1] *School of Life Sciences, Hebei University, Key Laboratory of Microbial Diverstiy Research and Application of Hebei Province, Engineering Laboratory of Microbial Breeding and Preservation of Hebei Province, Institute of Life Sciences and Green Development, Baoding 071002, People's Republic of China*
[2] *Hebei Research Center for Geoanalysis, Baoding 071002, People's Republic of China*
Corresponding author: Guixia Liu and Hui Tang

Abstract
The microbial fermentation bed for breeding cow can solve the problem of dairy manure pollution from the source. The cow's dung urine is degraded directly on the packing material, easy to clean, no odor, no pollution, zero discharge. The functional microorganism is the key of microbial fermentation bed. We obtained a strain Y3 that can efficiently degrade NH_3 and H_2S, and has the function of degrading cellulose and lignin. Y3 had been identified to be Hydrogenophaga bisanensis. And after mutation breeding by ARTP, we obtained the mutant strain Y3A. After Y3A was inoculated in the solid medium of bedding, NH3 and H_2S were reduced by 70.02% and 60.37% respectively after 7 days. And cellulose degradation rate of bedding reached 33.07% and lignin degradation rate was 32.55% after 14 days. This study provides a good strain for fermentation bed of raising cow.

Key words*: Microbial fermentation bed; Lignocellulose degrading bacteria; Deodorant bacteria; Microbial breeding*

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I. Introduction

With the improvement of people's quality of life, people's demand for dairy products is also increasing in recent years. Despite the impact of the COVID-19, Chinese people's demand for dairy products is still growing rapidly. In 2021, the dairy intake of the general public in my country will be 260 ml one day, an increase of 23 ml compared with 237 ml one day in 2019 (Liu 2022). Due to the growth of dairy products, the rapid development of dairy breeding industry in China, the standardization and scale of dairy farms have become the main development direction of dairy farming. In 2020, the proportion of large-scale breeding with more than 100 heads in China reached 67.2%, up 3.2% year-on-year and 18.9% higher than in 2015 (Zhang N and Zhang XG 2020). However, this scale of breeding model also has a lot of challenges. This scale of farm has a large and relatively concentrated number of cows, then cows produce a lot of feces that can affect the health of cows and cause serious pollution to the environment of farm.

Dairy farms may bring soil pollution, water pollution and air pollution. Data show that each cow produces 60~95Kg one day of fecal pollution, including 30~50 Kg one day of dung, 15~25 Kg one day of urine, 15~20L one day of sewage (Liu 2020). Farms produce a variety of noxious gases. The composition and properties of malodorous substances are difficult to distinguish, and the harm to humans and animals is positively related to their concentration and action time (Park et al. 2020). Deodorization methods can be divided into chemical method, physical method and biological deodorization method (Wysocka et al. 2019; Zhang 2015). Among them, biological deodorization method uses microbial metabolism to degrade odorous substances and achieve the purpose of air purification, which has certain advantages compared with physical and chemical methods (Zhang 2015)

The discharge of excrement, urine, sewage and odor in large-scale dairy farms has become an important factor restricting the development of dairy farming. Microbial fermentation bed is the use of microbial fermentation technology, rapid fermentation and decomposition of breeding waste, so that breeding pollution is greatly reduced, truly achieve green ecological breeding. Microbial fermentation bed also called deep-litter-system、in situ decomposition of mature. Microbial fermentation bed is a kind of livestock and poultry breeding mode, originated from Japan and South Korea, and then widely promoted in China. Microorganisms are the core of the microbial fermentation bed, microbial agents can produce cellulase, protease

and other components in the fermentation process of cushion materials, which can rapidly decompose feces and urine and convert them into nutrients such as heat biomass. The microbial fermentation bed contains aerobic bacteria and anaerobic bacteria, and aerobic bacteria include bacteria, yeast, actinomycetes, etc. In some studies, scholars believe that compound microbial can improve the activity of microorganisms, adjust the microbial community structure in the bedding, make the bedding rise to high temperature in a short period, which can inhibit the generation of pathogenic bacteria, also can accelerate the decomposition rate of feces in the bedding, reduce NH_3 and H_2S and other related harmful gases.

At present, microbial fermentation bed culture is facing some problems, which need to be solved urgently. For example, some fermentation beds have been used for less than half a year, and the phenomenon of dead beds has not been solved in time (Li 2012). Some raw materials are scarce and difficult to obtain, and when they are overpriced. Microbial fermentation beds are easy to harden, and maintenance is time-consuming and laborious. The fermentation strains on the market are complex and there are many bacterial communities. However, due to the different geographical environment of different regions, the local microbial community is also very different. Therefore, the selection of appropriate strains is the primary task to solve the problem of microbial fermentation bed. There is a lot of lignocellulose in the cushion material, and its degradation is conducive to the subsequent compost decomposing, and the secondary utilization can truly realize the significance of the new culture technology of microbial fermentation bed.

II. Materials and Methods

2.1 Collection of Samples

Vinasse was from a winery in Baoding city of China. Wheat straw and sawdust were taken from Baoding city of China. Three kinds of microbial farm deodorants, 4 kinds of fermentation bed microorganisms.

2.2 Enrichment, isolation and purification of functional microorganism in microbial fermentation bed

The fermentation bed microorganisms, microbial farm deodorants were added to large tubes containing mixed bedding and cow manure, and set up a blank control group. It was treated with nine kinds of processing. And then ferment in 37℃ incubators for 30 days. Strains with high efficiency in removing NH_3 and H_2S and degrading lignin and cellulose were isolated from bedding after fermentation. The fermentation cushion leachate was enriched in various liquid enrichment media at at 37 ℃ and shaken at 180 r/min for 5 days, and enriched for 3 times consecutively. The enriched microbial was diluted to an appropriate gradient and spread on heterotrophic nitrification plate, sulfur oxidation plate, CMC-Na plate and alkaline lignin plate and then cultured at 37℃. The single colony was selected for plate scribing and the strain was purified.

2.3 The first screen of the microbial fermentation bed function microorganism

The purified strains were inoculated into 250mL triangle bottles containing 100mL liquid heterotrophic nitrification medium and sulfur oxidation medium at 1% inoculation volume, respectively. And 12g feces of cow was added into the medium. The deodorization effect of microorganisms was determined by sensory methods.

The purified colonies were inoculated on cellulose congo red plate, lignin aniline blue plate and lignin bright blue plate, respectively, and cultured at 37℃ for 4 days. The colony diameter (d/mm) and hydrolytic ring diameter (D/mm) were measured, and the strain with larger D/d were selected for the next experiment.

2.4 Multiple screening of the microbial fermentation bed function microorganism

We added 200ml fermentation bed mixed padding, 12g cow manure in the 250ml triangle bottle. The amount of inoculation was 10 percent of the quality of the gasket, and the bacteria were developed to 10^8cfu/ml. the number of spore of actinomycetes and fungi were 10^6 cfu/ml. Adjust the moisture content to 60%, mix evenly, and press a little bit. The fermentation bottle and absorption liquid were connected by the device in the figure1, which was placed at 37℃for 7 days. Use boric acid absorption and hydrochloric acid titration to measure NH_3 content. And for specific determination method. The amount of H_2S was determined by zinc acetate absorption and iodometry.

The device is developed seven days later to seal the 250ml triangle bottle and continue to cultivate 7 days. And the content of cellulose and lignin was determined, and the determination method was based on Wang Wanyu's method (Wang WY 1987).

Fig. 1 Odor detection device

2.5 Identification of strains

Extraction and amplification of DNA. Dip an appropriate amount of bacteria into 10μL lysis solution and lysis at 99℃ for 30 min. The DNA is amplified by 1μL template DNA. Bacterial amplification system: 1μL template DNA, primer 27F 0.5μL, 1525R 0.5μL, Mix 10uL, ddH2O 8μL. Amplification procedure: 94℃ 10min→30 cycles (94℃ 30s→55℃ 30s→72℃ 1min) →72℃ 5min→4℃.

Detection of amplification products. The purity and fragment size of PCR products were detected by agarose gel electrophoresis. Prepare 1.0% agarose gel blocks in advance. The electrophoresis solution was 1TAE, the voltage was set to 100V, the current was adjusted to 120mA, and the time was 30min. Stored PCR products were placed in ice boxes for sequencing. Sequence homology analysis by BLAST (http://www.ncbi.nlm.nm.gov/BLAST).

2.6 Mutation breeding by ARTP

Measure the OD value at 600nm (0.6 ~ 0.8), add 10μL of diluted bacteria solution to the special metal plate, and use the atmospheric pressure room temperature plasma breeding machine (ARTP) produced by Siqingyuan Biotechnology to mutate the strain. Helium gas was used as the working gas in this study. The operating conditions of ARTP are as follows: input power: 120W, irradiation interval: 2mm, gas flow rate: 10L/min, treatment time: 10 s, 30 s, 50 s, 70 s, 90s. The ARTP-treated bacterial suspension was fully oscillated and diluted to an appropriate dilution, and coated on Congo red plate, lignin aniline blue plate and lignin bright blue plate, and cultured at 37℃. The growth status of bacteria was observed in time, and those with large D/d were screened for further screening. And then the determination of the ability of NH_3, H_2S, cellulose and lignin degradation were gaged by the method of 2.4.

III. Results

3.1 Preliminary results

The strains with good deodorization effect were screened by sensory methods. The degradation rates of cellulose and lignin were then measured. And the Table 1 shows the D/d ratio of hydrolytic circles generated by these strains on cellulose Congo red plate, lignin aniline blue plate and lignin bright blue plate.

Tab.1 The D/ d ratio of strains CMC-Na Congo red agar plate, lignin aniline blue agar plate and lignin bright blue agar plate.

Serial number	CMC-Na Congo red D/d	Lignin aniline blue D/d	Lignin bright blue D/d
Y1	2.1	2.0	2.2
Y2	4.0	3.5	3.0
Y3	7.5	10.7	5.7
Y4	3.5	2.7	3
Y5	2.5	3	2.3
Y6	2.4	3.3	2.7

(A) (B) (C)

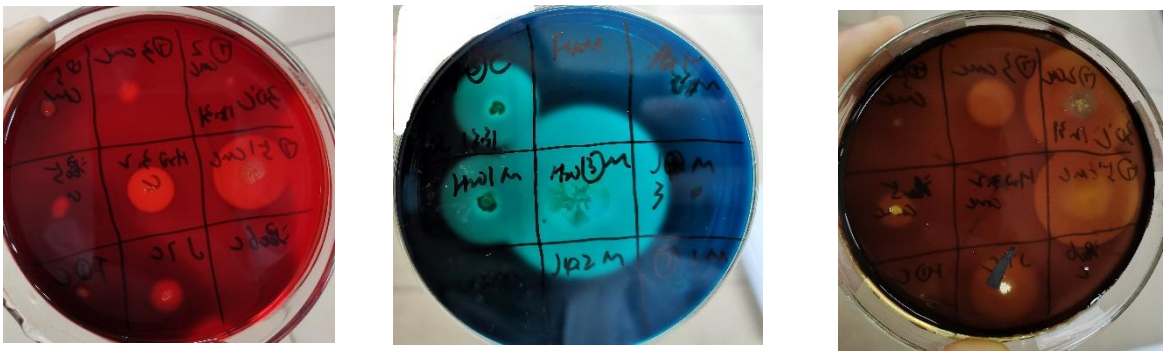
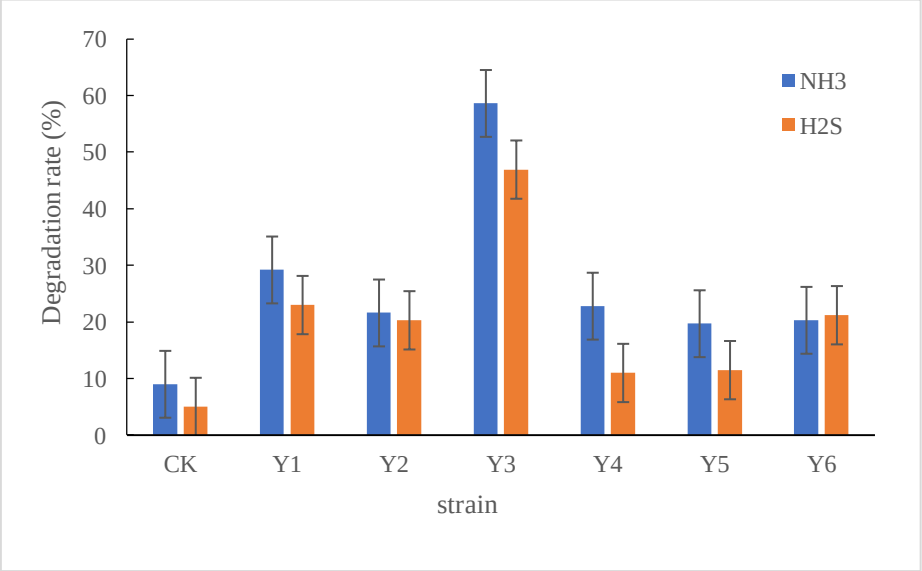


Fig. 2 (A)were hydrolytic circles generated by some strains on CMC-NA Congo red plate, (B) were hydrolytic circles generated by some strains on lignin bright blue plate, and (C) were hydrolytic circles generated by some strains on lignin aniline blue plate.

3.2 Multiple screening

The degradation rate of odor and lignocellulosic were determined for all the obtained strains. The determination results are shown in Figure 3. Y3 had the highest removal rates of NH_3 and H_2S, which were 58.62% and 46.93%, respectively. And its degradation rate of cellulose is 21.08%, and lignin is 20.68%. And Y3 is *Hydrogenophaga bisanensis.*

(A)



(B)

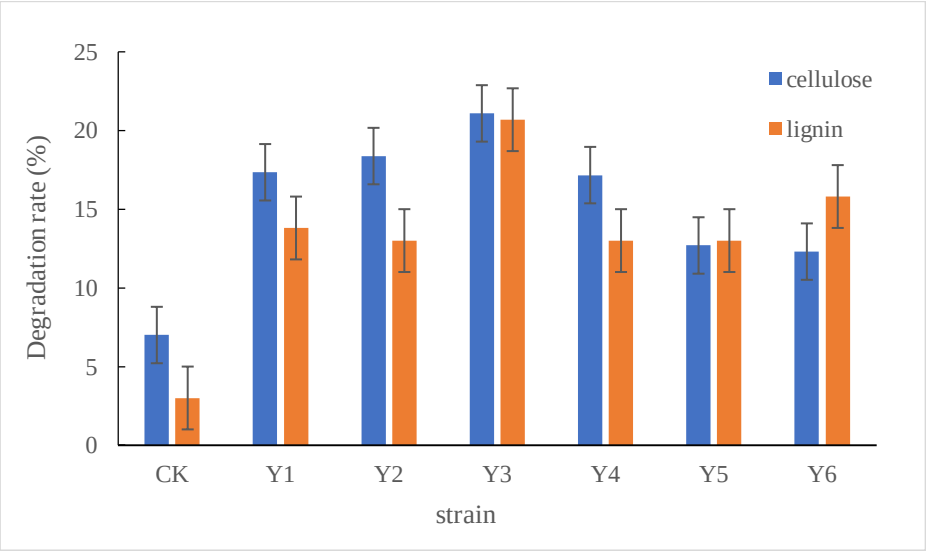


Fig. 3 (A) shows the degradation ability of NH_3 and H_2S, (B) shows the degradation ability of cellulose and lignin.

3.3 Mutation breeding

The D/d which was produced on three screening plates by Y3A, was significantly larger after mutation breeding. After Y3A was inoculated in solid fermentation medium in 2.4 for 7 days, the removal rates of NH_3 and H_2S were 72.02% and 60.37%, respectively. After 14 days, the degradation rates of cellulose and lignin were 33.07% and 32.55%, respectively.

IV. Discussion

Hydrogenophaga bisanensis was isolated from wastewater of a textile dye works in 2008, this is the first time *Hydrogenophaga bisanensis* was discovered and named (Yoon JH et al. 2008). It has been studied to remove ammonia nitrogen contained in wastewater (Feng XJ 2017). Zhang found that Hydrogenophaga existed in the new static aerobic composting process of cow manure, and it was the dominant genus in the initial stage of composting (Zhang WH 2018). *Paenibacillus Lautus* and *Bacillus Niacini*, which were screened by Yang, had over 40% scavenging capacity of NH_3 and H_2S respectively, and they also had high cellulose degradation capacity (Yang et al. 2018). There is no report that *Hydrogenophaga bisanensis* can degrade lignocellulose. And *Hydrogenophaga bisanensis* in this study has a high ability to degrade NH_3, H_2S and lignocellulose.

V. Conclusion

We obtained Y3 *Hydrogenophaga bisanensis*. It can efficiently degrade NH_3 and H_2S, and has the function of degrading cellulose and lignin. And after mutation breeding by ARTP, the mutant strain Y3A had the high removal rates of NH_3 and H_2S, which were 70.02% and 60.37%, respectively. And its degradation rate of cellulose is 33.07%, and lignin is 32.55%. This study provides a good strain for fermentation bed of raising cow.

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Author contributions

H.T. (Professor) planned and designed this study. M.W. (graduate student) analyzed data and wrote this article.

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Availability of data and materials

Data is available under request.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All coauthors have consent and approve the paper for publication.

Competing interests

The authors declare they have no conflict of interests.

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