两株枯草芽孢杆菌协同降解黄曲霉毒素 B_1 的研究

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引言

黄曲霉毒素（Aflatoxin AFT）是由黄曲霉等在适宜条件下分泌的代谢物质，有强毒性和高致癌性，是食源性的二级真菌毒素[1]。黄曲霉毒素分为 B、G 和 M 三类，自然条件下被霉菌毒素污染的饲料中黄曲霉毒素 B_1（AFB_1）性质最稳定、毒性最强。因此，如何快速有效地抑制 AFB_1 对农作物、人畜及环境的污染是急需解决的问题，是国内外学者研究的热点。传统降解 AFT 的方法有物理和化学方法，但存在处理效率低、时间长、产生污染物等缺点，生物法降解 AFT 具有效率高且安全无污染成为近年研究的热点。张盼筛选出 9 株芽孢杆菌发酵全菌液对 AFB_1 降解率达 80%以上[2]。本研究拟利用前期筛选出的降解 AFB_1 的两株菌 K-3、K-TM，以降解 AFB_1 为指标，对发酵条件进行优化。

材料与方法

将两株枯草芽孢杆菌 K-3、K-TM 进行生理生化特性鉴定和 16s rRNA 序列测定确定分类地位后，以 AFB_1 降解率为依据，分别进行优化培养并观察协同降解作用。AFB_1 降解率的测定，发酵结束后分别取 900μL 上述不同处理组的发酵液加入 100μL AFB_1 标准品（400ng/mL）于 1.5mL 无菌离心管中，同时以无菌发酵培养基加 AFB_1 作空白对照，利用 ELISA 试剂盒检测含量。

结果与讨论

结果表明，两株芽孢杆菌鉴定为枯草芽孢杆菌；菌株 K-3 优化条件为装液量 50mL/250mL、温度 37℃、4%接种量、初始 pH 7.0、发酵周期 48 h，150 r/min 振荡培养，对 AFB_1 降解率为 88.07%（$P<0.05$）；菌株 K-TM 装液量为 50mL/250mL、温度 37℃、6%接种量、初始 pH 7.0、发酵周期 60h，150 r/min 振荡培养，对 AFB_1 降解率为 84.81%（$P<0.05$）；K-3、K-TM 按体积 1:2 组合，对 AFB_1 协同降解率达到 88.43%。

本研究对两株高效降解 AFB_1 的菌株 K-3、K-TM 进行优化培养以便产生更好降解作用，当培养基固定时，不同发酵条件对菌体和代谢物质的产量影响巨大。通过优化发酵条件可以提高菌株繁殖能力和分泌更多降解 AFB_1 的产物。温度对微生物的生长至关重要，对菌体代谢及代谢产物的合成有影响，也会影响蛋白质和酶的活性以及微生物膜的液体结构，进而影响微生物生理活动[3]。发酵时间也是影响菌株对 AFB_1 降解作用的重要因素。将不同菌株按一定比例组合协同降解 AFB_1 是未来发展的一个方向，本研究表明 K-3 和 K-TM 协同降解 AFB_1 降解率比单一菌株效果提升，这可能与两株菌的生长及代谢有关，不同菌株活性物质的协同、互补促进降解效果。本试验采用单因素筛选法对枯草芽孢杆菌 K-3、K-TM 培养条件进行优化，还需要进行多因素正交分析及降解 AFB1 的机理需进一步研究。

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表达结核分枝杆菌 ESAT-6-Ag85A 基因的侵入型乳酸菌免疫特性研究

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引言

结核病(Tuberculosis，TB)是一种由结核分枝杆菌(*Mycobacteria tuberculosis, MTB*)引起的人兽共患病。目前用于预防 TB 的唯一疫苗是卡介苗（BCG），但只是在婴幼儿上发挥作用[1]，在保护青少年和成年人的肺病中却受限 [2]；BCG 是全菌疫苗，可引起淋巴结肿大和化脓等异常反应，所以改造卡介苗和开发安全有效的新疫苗势在必行。ESAT-6 是一个大小为 6kDa 的早期分泌靶抗原，是最具有免疫能力的特定靶向抗原，具有增强细胞免疫反应的能力。结核分枝杆菌中主要的保护性抗原是抗原 85（Ag85） [3]。本文目的是在侵入型乳酸菌的基础上，转入表达 ES85A 抗原的真核质粒，构建双质粒表达的侵入型重组乳酸菌，研究表达 ESAT-6-Ag85A(ES85A)融合基因的侵入型乳酸菌对小鼠体内免疫特性的影响。

材料与方法

将真核表达载体 pValac 与 ES85A 连接构建真核表达质粒，并将其分别电转到重组乳酸菌 NC8-pSIP-409 和 NC8- pSIP-409-FnBPA 感受态中，构建双质粒的乳酸菌 pValac-ES85A/409 和 pValac-ES85A/FnBPA。将其免疫 BALB/c 小鼠，检测表达 ES85A 的侵入型乳酸菌对小鼠树突状细胞（dendritic cells, DCs）亚群 CD80 和 CD83 的影响以及血清中细胞因子的表达水平。

结果与讨论

Western blot 和免疫荧光证明 ES85A 蛋白成功表达；与空载体组相比，在小鼠免疫表达 ES85A 的侵入型乳酸菌后，小鼠脾细胞中 $CD11^+CD80^+$ （$P< 0.001$）和 $CD11^+CD83^+$ （$P< 0.01$）以及血清中细胞因子 IL-4（$P< 0.05$）的表达都有所增加。结果表明表达 ES85A 的侵入型乳酸菌能促进小鼠体内 DCs 的分化和成熟以及提高血清中细胞因子 IL-4 的表达。

乳酸菌属于益生菌，可以作为 DNA 疫苗的递送载体，但其递送效率不高[4]，为了提高递送效率，我们构建了表达 FnBPA 的侵入型植物乳杆菌 NC8[5]。这种新型的乳酸菌递送载体可以提高表达外源抗原的递送效率，构建了表达 ESAT-6-Ag85A 抗原的侵入型乳酸菌。将构建好的表达 ES85A 的侵入型乳酸菌免疫 BALB/c 小鼠，检测 DCs 亚群 CD80 和 CD83 的表达，结果表明 ES85A 蛋白的表达能促进 DCs 的分化和成熟，尤其是以侵入型乳酸菌为递送载体的效果更好。这或许是因为表达 FnBPA 的侵入型乳酸菌由于具有侵入细胞的能力，进而增加了与 DCs 的结合效率，为后期研制抗结核病的 DNA 的疫苗奠定了良好的基础。

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Isolation and identification of *Enterococcus faecium* from swine and biological properties

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Introduction

Probiotics are such micro-organisms when delivered live,through the diet,enhance the growth of the host.Theprobiotic micro-organisms is an alternative to take the place of use the antibiotics for prevent diseases in between animals and humans,thus emerging as a novel field which iscurrently under investigation for its applications in farmingand aquaculture,as well in the development of prophylactictreatments for animals and humans. In this study, the strain LY001 belonged to *Lactobacillus* and was isolated from healthy pig feces, the biological characteristics of the grow rate, acid resistance, bile salt tolerance, antibacterial activity and safety performance showed that LY001 has a potential probioticalproperties and could serve as new probiotics for fodder industry with highresistance to gastro-intestinal stresses.

Materials and Methods

The experiments included bacterial identification, resistance acid and salt assay, resistance high temperature assay, biofilm formation assay, antibacterial activity assay, mice virulence assay》

Results

The LY001 genomic DNA was successfully isolated, phylogenetic analysis suggested that the 16s RNA of LY001 remain highly homologycompared with the *E.faecium* (GQ337884.1).The growth rate is very fast in growth stage at 1-6 h post inoculation. The produce acid resulted that when incubation time was prolonged the produce acid more and more increasingly at 1-6 h post inoculation, and reach stable growth phase at 8 h post inoculation.LY001 strain could survive in high salt medium with 6% NaCl and strong acidity at pH=3 for 8 hours. The LY001 strain was able to grow at 55 ℃ for 4 h after inoculation. The strain was tested for biofilm associated biological activity. The OD570nm value of LY001was significantly higher thancontrol. The LY001 strains can be antibacterial activityto *E.coli, Salmonella*and*Staphylococcus*, Especially to inhibit the growth *staphylococcus*. The mouse virulence test showed that the mice in the sterile PBS control group and1.0×10^{10}, 1.0×10^{9}and1.0×10^{8}CFU three groups did not die. In high dose group, mice appeared in the spirit of malaise, but soon returned to normal.

Discussion

In order to exploit a probiotic strain that can be used in feed supplement for swine. A strain of *Enterococcus faecium*(*E.faecium*) was determined by microscopic examination, biochemical test and 16s RNA sequencing from healthy piglets, named LY001. Further phylogenetic tree analysis showed that the isolate was closest to the Chinese isolates of *E.faecalis* KLDS4.0341 (GenBank GQ337884.1). Growth characteristics and acid-producing ability of the isolates were observed. The results showed that the strain was transferred to fresh MRS medium at a ratio of 1: 100, and reached a stationary phase after 6 h, indicating that the isolates grew faster and the growth period and the acidity was stable at 8 h after culture. The characteristics of acid production and the trend of the growth rate were basically the same.

The results showed that LY001 isolates could survive for a long time in adverse environment. The probiotics had some time in the gastric juice and bile salt after oral administration. Probiotics were concentrated in the bacteria solution or granulation and transport storage process, are inevitably high temperature and even heat treatment; therefore, tolerance to high temperature selection of probiotics as one of the indicators, the results show that the isolate can reach the intestinal tract through the stomach in order to play a prebiotic role prerequisites. Antibiotic susceptibility test showed that LY001 isolates were sensitive to three antibiotics, such as norfloxacin, cefradine and cefotaxime, and were sensitive to seven antibiotics such as penicillin, ciprofloxacin and amoxicillin.

Biofilm test results show that the isolate has a strong ability to form biofilm, Abdelwaheb et al reported that the formation of biofilm bacteria can resist the adverse environmental resistance, it may be its acid and high salt and high temperature resistance characteristics,it is a certain relationship. Probiotics play a major role in animal intestinal, so the main pathogens in the intestinal tract of *E.coli*, *Salmonella* and *Staphylococcus aureus* antibacterial test, the results confirmed that LY001 isolates of these three intestinal common pathogenic bacteria have a certain inhibitory effect, in particular, the role of *Staphylococcus*aureus the most obvious, and Munoz-Quezadaet separation results are basically the same, but the antibacterial effect is better; animal safety test results show that the 10^{10}CFU of the isolates were not killed by intraperitoneal injection, which indicated that the isolates had better safety.

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Changes in the Composition of Fecal Microbiota of Domestic Cats Associated with Feline Panleukopenia Virus Infection

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Background

Gut microbiota is a dynamically complex microflora and plays a vital role in maintaining the host's gastrointestinal health. Recent studies have investigated gut microbiome alterations in cats with various gastrointestinal diseases, including inflammatory bowel disease, acute and chronic diarrhea and helminth infections; however, limited information is available on the relationship between the gut microbiome and enterovirus infections. The aim of this study was to evaluate the fecal microbiota alterations in cats in response to naturally-acquired feline panleukopenia virus (FPV) infection.

Materials and Methods

Fresh fecal samples were collected from 14 cats of which 8 with naturally-acquired FPV infection and 6 FPV-uninfected controls. The fecal microbiota was characterized through high-throughput sequencing of the V3-V4 regions in the 16S rRNA. The differences in fecal microbiota between FPV-infected cats and uninfected controls were evaluated using alpha diversity index, nonmetric multidimensional scaling (NMDS) analysis and principal component (PCA) analysis.

Results and Discussion

A total of 1,264,380 paired-end (PE) reads from the 16S rRNA V3-V4 region were generated from 14 fecal samples. These sequences were assigned to 130 OTUs and classified into different taxa including 5 phyla, 14 classes, 18 orders, 34 families, and 68 genera. For uninfected controls, Firmicutes (mean, 68.53%) was predominant, followed by Bacteroidetes (15.58%), Proteobacteria (10.12%), Actinobacteria (5.56%) and Fusobacteria (0.11%), while the percentages of these phyla in the FPV-infected cats were 44.81%, 13.39%, 26.56%, 5.34% and 9.70%, respectively. The abundance of dominant flora changed markedly in FPV-infected cats. After FPV infection, the percentage of Firmicutes was markedly decreased, while the populations of Proteobacteria and Fusobacteria were remarkably increased, similar to previous observations in human, dogs and piglets with GI disease. Alpha diversity index analysis showed that no significant differences were seen in feline fecal flora diversity between the two groups, but the floral richness of the FPV-infected cats was greater than that of the uninfected controls. NMDS analysis results showed that fecal samples from FPV-infected cats and uninfected controls formed separated clusters suggesting that the feline fecal microbiota compositions were destroyed by the FPV infection. The PCA displayed consistent results with the NMDS analysis in classifying the fecal samples. In addition, the PCA results showed that the fecal microbiota compositions of FPV-infected individuals varied more than those of the uninfected controls. We further analyzed the specific bacterial taxa associated with FPV infection at the genus level. Among these markedly different

genera, the relative abundances of Streptococcus, Peptostreptococcus, Escherichia-Shigella and Fusobacterium were significantly higher in FPV-infected cats compared with healthy controls. Conversely, the relative abundances of Enterococcus, Lactobacillus, Megasphaera, Dialister and Prevotella were significantly lower in the FPV-infected group compared with the contros group. These results suggest that FPV infection alters the ecal bacterial community composition and induces abundances changes in the FPV-associated bacterial taxa.

Conclusions

The compositions of fecal microbiota were different between FPV-infected cats and uninfected controls. FPV infection may destabilize the fecal microbiota composition in cats, thereby causing bacterial dysbiosis.

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