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Effects of bar-transgenic rice on the intestinal microflora of the mice (*Mus musculus*)

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ABSTRACT

Microbial molecular ecology approaches were used to the effects of Bar-transgenic rice on Intestinal Microflora of the Mice (*Mus musculus*). Kunming mice (*Mus musculus*) of 100 SPF-grade (20 ± 2 g), half of which were male and the other half female, were randomly divided into five groups with four replications per group and five mice per replication to assess the safety of Bar-transgenic rice. Five diets meeting or exceeding the minimum nutrient requirement was fed for 180 days. After 90 days, parental generation (P) was bred to produce the first filial generation (F_1). Each generation was fed for 180 days. On the 180th day, five mice from each group were randomly sampled, and their intestinal contents were collected for DNA isolation. The V3 region of the 16S rDNA was amplified by polymerase chain reaction (PCR) and analyzed via denaturing gradient gel electrophoresis (DGGE). The resulting PCR-DGGE band number (bacterial species) was counted, and the banding patterns were analyzed by calculating the Sorenson's pairwise similarity coefficients (Cs), an index used to measure bacterial species found among all samples. The sequence analysis of bands was performed to identify the intestinal predominant microflora of the mice. The intergroup Cs values of the samples across all groups did not differ ($P > 0.05$) from each other. The effect of Bar-transgenic rice on the intestinal microflora of the mice was considered insignificant.

Keywords: Bar-Transgenic Rice; *Mus musculus*; Intestinal; Microflora; Denaturing Gradient Gel Electrophoresis

1. INTRODUCTION

Genetically modified organisms (GMO) are a group of organisms whose genomes have been altered using genetic modification. The cultivated area of transgenic herbicide-resistant crop is the largest among transgenic plants. Genetic modification has brought enormous economic and social values. However, since the emergence of genetic modification, there have been several controversies on the effect of transgenic crops and derived products on human health. These controversies continue to be a worldwide concern. With increased cultivation area and transgenic rice output, assessing the safety of transgenic rice is very necessary [1-3], especially toxicological assessments [4-13].

Intestinal microflora has important effects on immunity, disease prevention and treatment, digestion, absorption, and metabolism [14]. Diet composition can affect the number and type of bacteria in intestinal microflora because diets are the source of final metabolic substrates for intestinal microflora [15,16]. Therefore, intestinal microbial diversity reflects the quality of diets. The effects of different diets on intestinal microbes have been extensively studied. For example, the bacterial communities in the colon and feces of pigs fed with whole crop rice, the development of a bacterial community in the feces of weaning piglets, and the bacterial community and diversity in the gastrointestinal tract layer have been studied by Wang *et al.* [17], Zhu *et al.* [18], and Ni *et al.* [19] using denaturing gradient gel electrophoresis (DGGE). Molecular biotechnology has several advantages over traditional methods used to analyze intestinal microflora. For instance, polymerase chain reaction (PCR) coupled with DGGE can completely and accurately analyze microbial community structure and diversity. Thus, PCR-DGGE has been widely used over the past few years. However, there have only been a few studies on the effects of Bar-transgenic rice on intestinal bacterial microflora that used DGGE. The effects of Bar-transgenic rice on intestinal microflora were investigated in the present study using DGGE, which provided scientific and objective bases in assessing the safety of transgenic rice.

2. MATERIALS AND METHODS

2.1. Materials and Treatments

A total of 100 6-week-old SPF-grade *Mus musculus* [Num-

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ber of Animal License: SCXK (Xiang) 2009-0004] weighing 18 g to 22 g were purchased from Hunan Slca Jingda Experimental Animal Center Company, Limited. The mice were randomly divided into five groups. Five diets (**Table 1**) that met or exceeded the minimum nutrient requirements were fed to the mice. The ingredient and composition of the five experimental diets were almost identical, except for rice content. The experimental design is given in **Table 2**.

Each group contained 10 male and 10 female mice. Five groups of mice were fed with routine feed [20] [Production License: SCXK (Xiang) 2009-0009] for 3 days to 5 days before the start of the experiment. They were fed with five experimental diets, namely, two doses of genetically modified (GM) Bar68-1 rice, two doses of D68 (non-GM) rice, and routine feed for 180 days. The mice were kept under standard environmental conditions (temperature between 22˚C and 25˚C, humidity between 50% to 60%, and luminosity of 15 lx to 20 lx) with free access to food and water. After 90 days, the parental generation (P) produced the first filial generation (F_1). Each generation was fed for 180 days. On the 180th day, five mice from each group were randomly sampled, and their intestinal contents were collected for DNA isolation. All animal experiments were performed in accordance with the Guidelines for Use of Experimental Animals established by the College of Life Science, Hunan Normal University.

Bar-transgenic rice Bar 68 - 1 (Production License: Agriculture Basic Security Examination [2006] No.060) and the corresponding non-transgenic rice D68 were obtained from the Research Institute of Subtropical Agricultural Ecology of Chinese Academy of Science. All ingredients were mixed in certain proportions and then rolled into a root-shaped formula feed (diet) at the Animal Feed Manufacturer of Central South University.

2.1.1. Intestinal Samples

Five mice from each group were randomly sampled and sacrificed. After sterilizing the body surface with 70% alcohol, the mice were dissected under sterile conditions. All ileal and cecal luminal contents of the sacrificed mice were collected into sterile plastic tubes and thoroughly mixed as previously described [19,21]. The intestinal contents were snap-frozen in liquid nitrogen and stored at –70˚C until analysis.

2.1.2. DGGE Preparation

The reagent was placed into 15-mL clean EP tubes in succession. Low and high concentrations of the denaturing gradient gel solution were then prepared (**Table 3**).

2.2. Methods

2.2.1. Sample Pretreatment

After the samples were thawed at room temperature, 2 g

Table 1. Ingredients and composition of the experimental diets (%, dry weight).

Item	Treatment				
	Z_1	Z_2	C_1	C_2	R
Ingredient					
Rice	40.0 (Bar68-1)	60.0 (Bar68-1)	40.0 (D68)	60.0 (D68)	0
Wheat	30.0	10.0	30.0	10.0	70.0
Soybean meal	19.58	19.58	19.58	19.58	19.58
Fish meal	4.52	4.52	4.52	4.52	4.52
Peanut oil	3.39	3.39	3.39	3.39	3.39
$CaHPO_4$	1.51	1.51	1.51	1.51	1.51
Premix*	1.00	1.00	1.00	1.00	1.00
Total	100.0	100.0	100.0	100.0	100.0
Analyzed composition					
Crude protein	19.21	19.18	19.21	19.18	19.19
Coarse fiber	3.24	3.26	3.24	3.26	3.25
Ca	1.23	1.25	1.23	1.25	1.24
P	0.49	0.50	0.49	0.50	0.48
NaCl	0.35	0.35	0.35	0.35	0.35
Lys	1.06	1.04	1.06	1.04	1.05
Met	0.48	0.47	0.48	0.47	0.47

*Content per kg of premix: VA 11,000 IU, VD 2800 IU, VE 45 IU, VK 1.60 mg, VB_1 2.4 mg, VB_2 6.5 mg, VB_6 3.6 mg, VB_{12} 38 μg, nicotinic acid 92 mg, folacin 0.85 mg, pantothenic acid 31 mg, biotin 115 μg, choline 285 mg, Fe 170 mg, Cu 240 mg, Mn 50 mg, Zn 160 mg, I 1.4 mg, and Se 0.4 mg.

Table 2. Experimental design.

Generation	Group 1	Group 2	Group 3	Group 4	Group 5
P	Z_1	Z_2	C_1	C_2	R
F_1	Z_1	Z_2	C_1	C_2	R

of the intestinal contents were suspended in 1.5 mL of 0.2 mol/L sterile PBS (pH 7.4), followed by 5 min vortexing in a 2-mL tube. The suspension was centrifuged at 500 r/min for 10 min, and the supernatant was collected in new sterile EP tubes. About 1 mL sterile PBS was added to the pellets and vortexed for 5 min. The suspension was centrifuged at 12,000 r/min for 5 min, and the supernatant was also collected in a new tube. The two sets of supernatant were mixed and the mixture was centrifuged at 500 r/min for 6 min to remove coarse particles. The cells in the supernatant were collected and washed twice with PBS by centrifuging at 12,000 r/min for 5 min. The supernatant was stored in 1 mL PBS at –70˚C.

2.2.2. Genomic DNA Extraction

Genomic DNA was isolated from the samples as previously described [22] with some modifications according to the specification in the DNA extraction kit, and was then stored at –20˚C.

2.2.3. Amplification of Genomic DNA by PCR

The V3 region of 16S rDNA was amplified by PCR using primers specific for the bacteria. In the present study, a pair of primers was designed for PCR by Sangon Biotech (Shanghai) Company, Limited. The oligonucleotides used were as follows: the upstream primer was HAD-1-GC-F(5'-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG-3'); and the downstream primer was HAD-2-R(5'-GTATTACCGCGGCTGCTGGCA-3').

PCR was performed using a PCR kit (MBI Fermentas) in a 30-μL flask containing 3.5 μL of the template DNA (50 ng/μL), 20.0 μL ddH_2O, 1.0 μL dNTP (10 mmol/L), 3.0 μL of 10× buffer, 1.0 μL of each primer, and 0.5 μL Taq DNA polymerase (2.5 U/μL, Mg^{2+} plus).

The reaction was denatured at 95˚C for 5 min, followed by 35 cycles of 30 s at 95˚C, 30 s at 56˚C, 40 s at 72˚C, and an extension of 10 min at 72˚C. The PCR products were separated using 1.5% agarose gel.

2.2.4. DGGE

After visual confirmation of the PCR products using agarose gel electrophoresis, DGGE was performed using the BioRad Dcode system as previously described [23]. Up to 35% to 65% linear DNA-denaturing gradients were formed in 8% polyacrylamide gels using a Bio-Rad Gradient Former to separate the PCR fragments. Bacterial V3 16S PCR products were loaded in each lane, and electrophoresis was performed in 1× TAE Buffer at 60˚C at 100 V for 16 h to 18 h. The denaturing gradient was parallel to the direction of electrophoresis. After electrophoresis, the gels were silver-stained and scanned using a ChemiDoc XRS system (Image lab software version 3.0) (BioRad). Each individual amplicon was then visualized as a distinct band representing at least one bacterial species on the gel.

2.2.5. Identification of Dominant Microflora in Mouse Intestines

The DGGE objective gels were cut using a disposable operation blade and collected into 1.5-mL sterile EP tubes (enzyme free). After the gels were pounded, 20 μL ddH_2O was added and the mixture was kept overnight at 4˚C. PCR was performed on the template objective gels using HAD-1-GC-F and HAD-2-R primers under the same reaction conditions stated in Section 2.2.3. After the PCR products were separated by electrophoresis on 1.5% agarose gel, the objective fragments were purified with Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced by BGI (Beijing). The sequence data were analyzed, and a basic local alignment search tool (http://www.ncbi.nlm.nih.gov/BLAST/) search was performed to identify the sequences.

Table 3. Composition and content of the denaturing gradient gel solution.

Composition	35% Denaturant (LOW)	65% Denaturant (HIGH)
50× TAE Buffer	300 μL	300 μL
Acrylamide/Bisacrylamide	3.75 mL	3.75 mL
Deionized Formamide	2.1 mL	3.9 mL
Urea	2.205 g	4.095 g
DCode Dye	0 μL	150 μL
10% (w/v) Ammonium Peroxydisulfate	150 μL	150 μL
TEMED	15 μL	15 μL
ddH_2O	To 15 mL	To 15 mL

The 100% denaturant is equivalent to 7 mol/L urea and 40% (v/v) deionized formamide.

2.2.6. Data Analysis

Quantity One (Version 4.4) (BioRad) was used to analyze PCR-DGGE banding patterns by measuring the migration distance and band intensity within each gel lane. This information was then used to analyze the banding patterns by measuring the community diversity, including band number and Sorenson's pairwise similarity (Cs).

The data were expressed as mean ± SD. The statistical significance ($P < 0.05$) of difference between the means was determined using ANOVA with SPSS (Version 17.0).

Sorenson's pairwise similarity (Cs) was estimated using the formula below:

$$\mathrm{Cs}(\%) = (2j)/(a+b) \times 100\%$$

where a is the number of total bands in the PCR-DGGE pattern for one sample, b is the number of total bands in the PCR-DGGE pattern for another sample, and j is the number of common bands shared by both samples.

3. RESULTS AND ANALYSIS

3.1. Genomic DNA and PCR Products

The DNA was first checked for integrity by electrophoresis analysis on 1.5% agarose gel, and then quantified using a spectrophotometer (Thermo). DNA bands amplified by the primers appeared clear and bright. The purity and quality of the DNA obtained by this method were satisfactory. The ratio of OD_{260} to OD_{280} was between 1.75 and 1.83.

After the V3 region of the 16S rDNA was amplified by PCR, the evaluated PCR products by electrophoresis were as expected. The size of the amplified fragments of the 16S rDNA ranged from 200 bp to 500 bp (**Figure 1**).

3.2. Effect of Bar-Transgenic Rice on Band Numbers of 16S rDNA Using PCR-DGGE

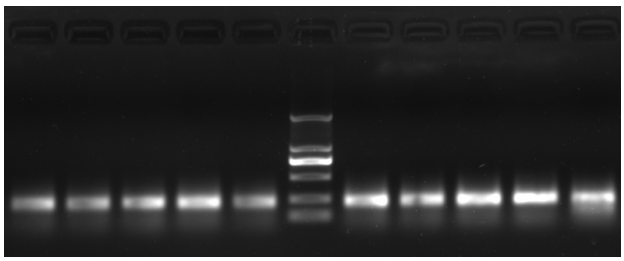
As shown in **Figure 2**, many bands were obtained after DGGE. These bands had different magnitudes and mobilities. The resulting DGGE band numbers were counted (**Figure 2**). The effects of Bar-transgenic rice on band numbers in each sample using PCR-DGGE were compared with each other (**Table 4**). In P and F_1, the band numbers did not differ ($P > 0.05$) among Groups 1, 3 and 5, and did not differ ($P > 0.05$) among Groups 2, 4 and 5.

3.3. Effect of Bar-Transgenic Rice on Sorenson's Pairwise Similarity Coefficient (Cs)

The effect of Bar-transgenic rice on 16S rDNA PCR-DGGE banding patterns was further assessed by comparing the Sorenson's pairwise similarity coefficient (Cs) of each group, as presented in **Tables 5** and **6**. As shown in **Table 5**, the Cs values ranged from 87% to 91%. The higher the Cs values, the higher the homogeneity. In P and F_1, intergroup Cs values did not differ ($P > 0.05$) among Groups 1, 3 and 5, and did not differ ($P > 0.05$) among Groups 2, 4 and 5 (**Table 6**). This result indicated a higher homogeneity among groups.

3.4. Dominant Microflora in Mouse Intestines

The 11 dominant microflora were identified as follows:



Lanes 1-5: Groups 1-5 of P; Lanes 1'-5': Groups 1-5 of F_1; M: DNA marker (from below to above) ranging from 200, 300, 500, 750, 1000 to 2000 bp.

Figure 1. PCR products separated on 1.5% agarose gel.

Table 4. Effect of Bar-transgenic rice on band numbers using PCR-DGGE.

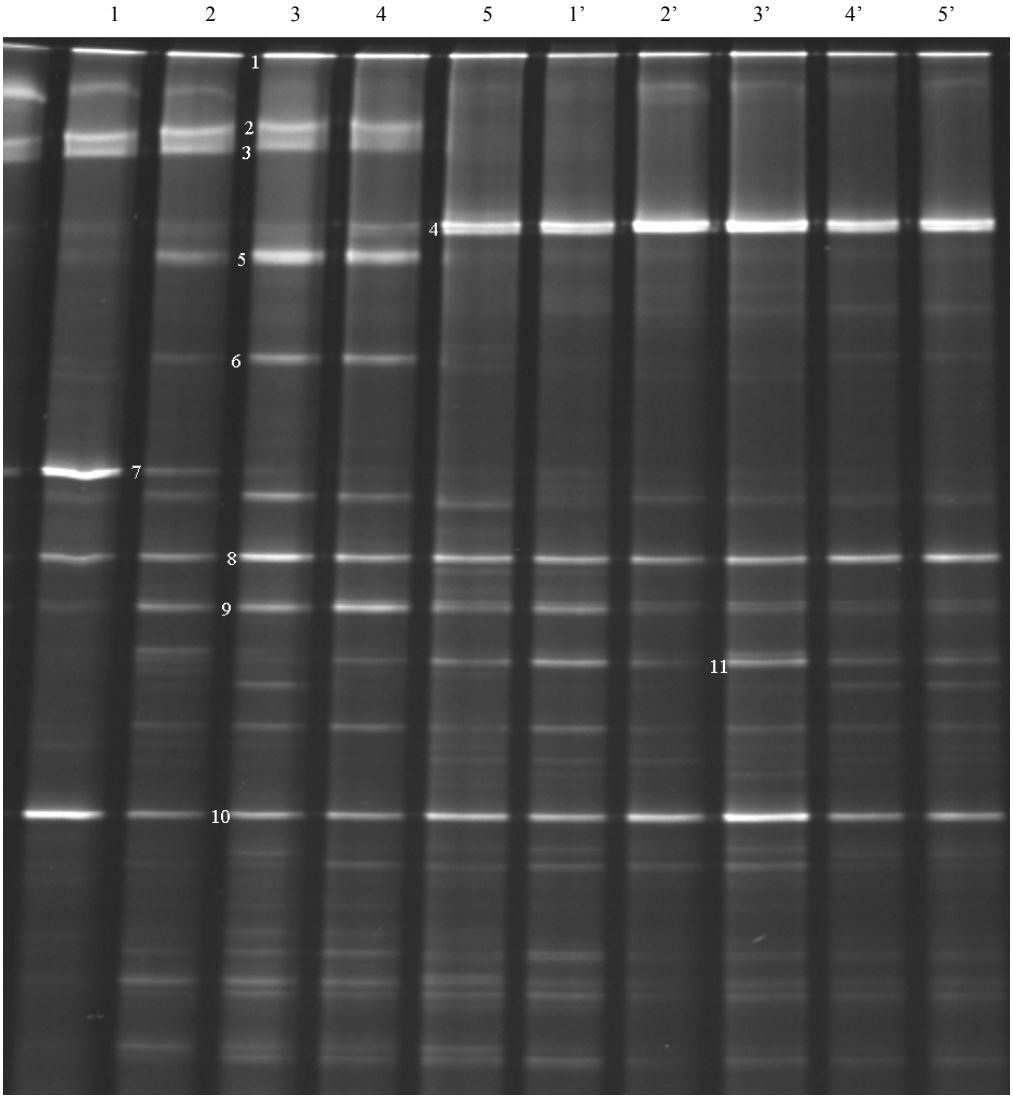
Group	*P*	F_1	Group	*P*	F_1
1	36.7 ± 1.5^a	35.8 ± 1.3^b	2	36.4 ± 1.6^c	35.8 ± 1.3^d
3	37.0 ± 1.7^a	35.6 ± 1.5^b	4	36.8 ± 1.4^c	35.7 ± 1.5^d
5	35.8 ± 1.3^a	36.1 ± 1.8^b	5	35.9 ± 1.2^c	36.1 ± 1.7^d

Values are expressed as Mean ± SD. $^{a, b, c, d}$: Values sharing a common letter in a column are not significantly different from each other ($P > 0.05$).

Table 5. Sorenson's pairwise similarity coefficients (Cs) of intestinal microflora in mice/%.

Group	*P*				F_1			
	1	2	3	4	1	2	3	4
2	89.3 ± 1.3	-	-	-	88.6 ± 1.2	-	-	-
3	88.7 ± 1.2	90.2 ± 1.2	-	-	87.9 ± 1.1	89.3 ± 1.3	-	-
4	87.8 ± 1.1	89.1 ± 1.3	91.3 ± 1.3	-	88.2 ± 1.3	88.7 ± 1.2	90.7 ± 1.3	-
5	88.4 ± 1.1	88.5 ± 1.2	89.6 ± 1.2	87.7 ± 1.3	89.5 ± 1.2	88.9 ± 1.4	88.9 ± 1.2	87.5 ± 1.1

Values are expressed as Mean ± SD.



Lanes 1-5: Groups 1-5 of P; Lanes 1'-5': Groups 1-5 of F_1.

Figure 2. PCR-DGGE bands in mouse intestinal samples.

Table 6. Comparison of intergroup sorenson's pairwise similarity coefficient (Cs).

Intergroup	*P*	F_1	Intergroup	*P*	F_1
1 vs. 3	88.7 ± 1.2^a	87.9 ± 1.1^b	2 vs. 4	89.1 ± 1.3^c	88.7 ± 1.2^d
1 vs. 5	88.4 ± 1.1^a	89.5 ± 1.2^b	2 vs. 5	88.5 ± 1.2^c	88.9 ± 1.4^d
3 vs. 5	89.6 ± 1.2^a	88.9 ± 1.2^b	4 vs. 5	89.6 ± 1.2^c	87.5 ± 1.1^d

Values are expressed as Mean ± SD. ^{a,b,c,d}: Values sharing a common letter in a column are not significantly different from each other ($P > 0.05$).

1-*Lactobacillus gasseri*; 2,3-*Uncultured bacterium*; 4-*Lactobacillus johnsonii*; 5-*Staphylococcus lentus*; 6-*Staphylococcus cohnii*; 7-*Lactobacillus intestinalis*; 8-*Lactobacillus murinus*; 9-*Uncultured bacterium*; and 10-*Staphylococcus schleiferi*.

4. DISCUSSION

Since the first time DGGE was applied in investigating the microbial community structure by Muyzer *et al*. in 1993, it has been widely used in every field in molecular microbial ecology. DGGE has now become one of the main methods in studying the microbial community structure.

PCR-DGGE banding profiles are different among individuals due to individual differences among hosts, and this finding has already been reported [18,21,24]. Genomic DNA was isolated from the mixed intestinal contents of five mice from each group in the present study to eliminate the sample differences, which was noted by Gong, *et al.* [21]. Thus, the mixed samples in the present study are more representative of the actual intestinal contents.

In a general way, the number of samples should be as large as possible. With the disadvantages limits of samples quantity, there were only five samples from each

group in this study. It was not enough and worth the further improvement.

The higher the number of DGGE bands, the more abundant the mouse intestinal microbial species. PCR-DGGE banding profiles of genomic DNA of intestinal microbial species obtained in the present study have complicated quantities and locations, which reflect the diversity of intestinal microbes in mice. Upon detailed analysis, the chief reason behind this complexity is that the main component of the diets is grain (rice and wheat) that contains more cellulose. The cellulose is decomposed by a large numbers of intestinal microorganisms. Thus, the intestine becomes the place of growth and reproduction of microorganisms. However, intestinal microbes and their metabolites affect nutrient digestion and absorption, energy balance, immunologic function, and other important physiological activities. Intestines and microbes keep and maintain a mutualistic relationship, which results from mutual selection and adaptation between the host and intestinal microbes in the course of long-term coevolution.

The dominant microfloras were identified, and the dominant genera were *Lactobacillus*, *Staphylococcus* and *Clostridium*, among others. These genera are microflora normally found in mammalian intestines, which is consistent with the results of Zhu [25].

The experimental results show that the diversity of intestinal microbes in mice fed with GM Bar68-1 rice is the same as that in mice fed with D68 (non-GM) rice. Moreover, the homogeneity of intestinal microbes in mice fed with GM Bar68-1 rice is higher compared with the mice fed with D68 (non-GM) rice. The effect of Bar-transgenic rice on the intestinal microflora is considered insignificant due to the following reasons:

1) The bialaphos resistance gene is derived from *Streptamyces hygroscopicus*, and its expression products are phosphinthricin acetyltransferase (PAT). PAT acetylizes the free amino of phosphinothricin (PPT), which is the main component of glufosinate in herbicides. However, PAT cannot restrain the activity of glutamine synthetase. Thus, PAT can induce transgenic crops to become resistant to herbicide, and the toxicity caused by glufosinate is eliminated [26]. *S. hygroscopicus*, which are a part of the biosphere, are widespread in nature. In *Streptomyces*, a few strains are related to the pathogens of human beings, animals, and plants.

2) PAT, which is expressed by the Bar gene, can disappear from the digestive juices and has no homology with known toxalbumin. Moreover, PAT does not have any features of allergen, such as heat stability, digestive stability, absent glycosylation site, and so on. Thus far, no study has reported on the toxicity of homologs in the acetyltransferase family and PAT on human beings and animals.

3) The amount of PAT expressed in plants is very low. Thus, PAT is relatively safe compared with other allergens [27].

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REFERENCES

[1] Liu, L.F., Cao, J.S., Wu, W.H., *et al*. (2009) Safety assessment of transgenic food and the present situation of management in China. *Acta Agriculturae Jiangxi*, **21**, 155-158.

[2] Kuipera, H.A., Konig, A., Kletera, G.A., *et al*. (2004) Concluding remarks. *Food and Chemical Toxicology*, **42**, 1195-1202. doi:10.1016/j.fct.2004.02.004

[3] Anthony, J.C. and Jeanne, M.E.J. (1999) Genetic engineering of crops as potential source of genetic hazard in the human diet. *Mutation Research*, **443**, 223-234.

[4] Wang, Z.H., Wang, Y., Cui, H.R., *et al*. (2002) Toxicological evaluation of transgenic rice flour with a synthetic cry1Ab gene from *Bacillus thuringiensis*. *Journal of the Science of Food and Agriculture*, **82**, 738-744. doi:10.1002/jsfa.1105

[5] Wang, Y., Lai, W.Q., Chen, J.G., *et al*. (2000) Toxicity of anti-herbicide gene (Bar) transgenic rice. *Journal of Hygiene Research*, **29**, 141-142.

[6] Zhao, Z.H., Yang, L.T., Ai, X.J., *et al*. (2005) Influence of genetically modified rice containing *cod*A gene on physiological metabolism and genetic horizontal transformation in fed rats. *Journal of Agricultural Biotechnology*, **13**, 341-346.

[7] Momma, K., Hashimoto, W., Yoon, H., *et al*. (2000) Safety assessment of rice genetically modified with soybean glycinin by feeding studies rats. *Bioscience Biotechnology and Biochemistry*, **64**, 1881-1886. doi:10.1271/bbb.64.1881

[8] Cromwell, G.L., Henry, B.J., Scott, A.L., *et al*. (2005) Glufosinate herbicide-tolerant (liberty link) rice vs. conventional rice indiets for growing-finishing swine. *Journal of Animal Science*, **83**, 1068-1074.

[9] Wang, Z.H., Wang, Y., Cui, H.R., *et al*. (2002) A Preliminary study on toxicological evaluation of transgenic rice flour with a synthetic cry1Ab gene from bacillus thuringiensis. *Scientia Agricultura Sinica*, **35**, 1487-1492.

[10] Li, Y.H., Piao, J.H., Zhuo, Q., *et al*. (2004) Subchronic toxicity test of Xa21 transgenic rice. *Journal of Hygiene Research*, **33**, 575-578.

[11] Li, Y.H., Piao, J.H., Zhuo, Q., *et al*. (2004) Study on the teratogenicity effects of genetically modified rice with Xa21 on rats. *Journal of Hygiene Research*, **33**, 710-712.

[12] Zhuo, Q., Chen, X.P., Piao, J.H., *et al*. (2004) Study on

the teratogenicity effects of genetically modified rice which expressed cowpea trypsin inhibitor on rats. *Journal of Hygiene Research*, **33**, 74-77.

[13] Wang, Z.H., Wang, Y., Shu, Q.Y., *et al*. (2004) Study on mutagenicity of transgenic rice flour with a synthetic cry1Ab gene from *Bacillus thuringiensis*. *Scientia Agricultura Sinica*, **37**, 2043-2046.

[14] Mackie, R.I., White, B.A. and Isaacson, R.E. (1977) Gastrointestinal microbes and host interactions. Gastrointestinal microbiology. Chapman & Hall, New York.

[15] Savory, C.J. (1992) Enzyme supplementation degradation and metabolism of three U-14C-labelled cell-wall substrates in the fowl. *British Journal of Nutrition*, **67**, 91-102. doi:10.1079/BJN19920012

[16] Reid, C.A. and Hillman, K. (1999) The effects of retrogradation and amylose/amylopectin ratio of starches on carbohydrate fermentation and microbial populations in the porcine colon. *Animal Science*, **68**, 503-510.

[17] Wang, H.F., Zhu, W.Y., Yao, W., *et al*. (2007) DGGE and 16S rDNA sequencing analysis of bacterial communities in colon content and feces of pigs fed whole crop rice. *Anaerobe*, **13**, 127-133. doi:10.1016/j.anaerobe.2007.03.001

[18] Zhu, W.Y., Yao, W. and Mao, S.Y. (2003) Development of bacterial community in feces of weaning piglets as revealed by denaturing gradient gel electrophoresis. *Acta Microbiologica Sinica*, **43**, 503-508.

[19] Ni, X.Q., Gong, J., Hai, Y., *et al*. (2008) The Bacterial Community and Diversity in the layer gastrointestinal tract: From crop to cecum analyzed by PCR-DGGE. *Chinese Journal of Animal and Veterinary Sciences*, **39**, 955-961.

[20] Ministry of Agriculture of the People's Republic of China (2006) Safety assessment of genetically modified plant and derived products 90-day feeding test on rats.

[21] Gong, J.H., Si, W., Forster, R.J., *et al*. (2007) 16S rRNA genebased analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. *FEMS Microbiology Ecology*, **59**, 147-157. doi:10.1111/j.1574-6941.2006.00193.x

[22] Yu, Z. and Morrison, M. (2004) Improved extraction of PCR 2 quality community DNA from digesta and fecal samples. *Bio-Techniques*, **36**, 808-812.

[23] Muyzer, G., de Waal, E.C. and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, **59**, 695-700.

[24] Simpson, J.M., McCracken, V.J., Gaskins, H.R., *et al*. (2000) Denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA amplicons to monitor changes in fecal bacterial populations of weaning pigs after introduction of Lactobacillus reuteri strain MM53. *Applied and Environmental Microbiology*, **66**, 4705-4714. doi:10.1128/AEM.66.11.4705-4714.2000

[25] Zhu, X.F., Xiong, D.X., Li, X.Y., *et al*. (1995) Comparison of membrane microflora and intestinal microflora in several animals. *Chinese Journal of Laboratory Animal Science*, **5**, 30-32.

[26] Wohlleben, W., Amold, W., Broer, I., *et al*. (1988) Nucleotide sequence of the phosphinothricin N-acetyltransferase gene from the *Streptomyces* hygroscopicus Tu 494 and its expression in *Nicotiana tobacum*. *Gene*, **70**, 25-37. doi:10.1016/0378-1119(88)90101-1

[27] Liu, H.Y., Mi, X.J. and Cui, J.Z. (2007) Characteristics and safety of bar gene, PAT proteins and glufosinate. *Chinese Journal of Ecology*, **26**, 938-942.

