ANIMAL HUSBANDRY

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MOLECULAR GENETIC TYPING AS A TOOL IN EPIZOOTOLOGICAL CONTROL IN ANIMAL HUSBANDRY

Discovery notes

Abstract

The potential of using molecular genetic methods in the prevention of infectious diseases by fast and accurate identifying the pathogen are described. The problem of bacteria tracking is solved by genotyping a microorganism, in which the genetic profile of bacterial isolates are compared. Another important area of genotyping application in animal husbandry is the identification of genes that determine antibiotic resistance. The developed method of genotyping allows us to identify the routes of pathogenic *E.coli* strains transmission in chickens in the environmental settings. The method can be recommended to address the issues of epidemiology and epizootology, genetic certification of bacterial strains. The proposed method is universal in nature, as it can be adapted to almost any microorganism. In this case, it is necessary to select a pair of restriction enzymes that would be compatible in one reaction buffer and ensure the detection of a small number of DNA fragments on the filter.

Keywords: *E.coli*, isolates, genotyping technique, pathogenic strains.

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МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКОЕ ТИПИРОВАНИЕ КАК ИНСТРУМЕНТ ЭПИЗООТОЛОГИЧЕСКОГО КОНТРОЛЯ В ЖИВОТНОВОДСТВЕ

Исследовательские заметки

Аннотация

Изложены возможности использования молекулярно-генетических методов в профилактике инфекционных заболеваний путем быстрой и точной идентификации возбудителя. Данная задача решается генотипированием микроорганизма, при котором сравниваются генотипы бактериальных изолятов. Другим важным направлением использования генотипирования в животноводстве является выявление генов, определяющих антибиотикорезистентность. Разработанный метод генотипирования позволяет выявить пути распространения патогенных штаммов *E.coli* у кур в полевых условиях. Метод можно рекомендовать для решения вопросов эпидемиологии и эпизоотологии, генетической паспортизации бактериальных штаммов. Предложенный метод носит универсальный характер, так как может быть адаптирован практически к любому микроорганизме. В этом случае необходимо подобрать пару ферментов рестрикции, которые были бы совместимы в одном реакционном буфере и обеспечили детекцию небольшого числа фрагментов ДНК на фильтре.

Ключевые слова: кишечная палочка, изоляты, метод генотипирования, патогенные штаммы.

1. Introduction

Infectious diseases in chickens, despite the great attention to this problem, remain an urgent topic in modern veterinary microbiology. At present, opportunistic microorganisms are of particular importance. In a number of such microorganisms,

Escherichia coli (hereinafter - *E. coli*) occupies a special place. This is due to its wide distribution in the external environment. In addition, the bacterium is a normal component of the intestinal microflora. The ability to quickly mutate and acquire pathogenicity properties through genetic exchange with other strains allows bacteria to colonize not only the intestines, but also other organs, causing diseases that cause significant economic losses to the poultry industry. The use of genotyping allows one to uniquely identify bacterial strains and, thus, determine the routes of transmission of the pathogen and identify the sources of infection. This knowledge makes it possible to scientifically plan veterinary and sanitary measures aimed at breaking up the epizootological chain.

Attention is paid not only to the identification of a pathogen in biological material at the species level, but also to its identification at the strain level (genotyping). Information on the circulation of strains is invaluable in elucidating the pathways of transmission of the pathogen and finding the source of infection. It is known that many pathogens have a very large genetic diversity, which can be easily detected by modern methods. If the same genetic variant (strain) is detected in two sampled animals, in this case it can be argued with a high degree of probability that there is a case of pathogen transmission between individuals.

The variety of genotyping methods reflexes the limited use of each of them. Currently, it has been unequivocally proven that methods based on genomic DNA polymorphism (genotyping) are the most sensitive and reproducible. One of the most common methods of genotyping is the pulsed-field gel electrophoresis method. The disadvantages of the method include the duration of the analysis, which reaches several days, low typability in relation to strains expressing endogenous nucleases. The multilocus sequencing method (MLST) has a low discriminatory ability and is very expensive to apply on numerous bacterial isolates.

2. Materian and methods

The study material was genomic DNA isolated from cultures of 18 isolates of *Escherichia coli*. Isolates of *E. coli* were descended from sick and fallen Hisex brown chickens, kept in separate poultry houses in one of the Russian poultry farms. An overnight culture of the microorganism in a volume of 1 ml was centrifuged for 5 minutes at 8000g. The bacterial cell pellet was used to isolate genomic DNA by the standard method using phenol-chloroform extraction.

The DDSL genotyping method is based on the simultaneous digestion of the genomic DNA of a microorganism by two restriction endonucleases and the selective labeling of individual DNA fragments with their subsequent visualization. The first of restriction endonucleases, having a small number of recognition sites in the genome, gives 3'-recessed ends of DNA fragments, and the second - blunt or 3'-protruding ends. The presence of Taq polymerase and Bio-dCTP tags (biotinylated deoxycytosine triphosphate, Invitrogen ™) in the reaction mixture provides efficient labeling of DNA fragments with at least one 3'-recessed end in the fill-in reaction. Thus, only a small fraction of DNA fragments will incorporate the label and can subsequently be visualized after conventional agarose gel electrophoresis. A search *in-silico* (Bikandi et al., 2004) made it possible to select restriction enzymes that can be used as a producer of DNA fragments with recessed “sticky ends” capable of incorporating the Bio-dCTP tag. The following enzyme was used for *E.coli*: XbaI. As the second “trimming” restriction endonuclease, the enzyme *Pst*I, turned out to be optimal. It turned out that E. coli DNA digest is possible with the same combination of two enzymes as was for *Salmonella* which were typed earlier. The second restriction enzyme reduces the size of DNA fragments obtained by digestion by the first enzyme, making them suitable for size separation in a conventional 0.8% agarose gel.

The DDSL reaction was carried out in one microtube, where the test DNA (0.5-1 μg) was added, two restriction endonucleases (5 units per reaction), Taq polymerase (0.05 units), Bio-dCTP (0.1 μM). The total volume of the mixture was 20 microliters. Incubation was carried out for 1-2 hours at 37°C. Then, the obtained DNA fragments were size separated by electrophoresis in a conventional 0.8% agarose gel for 16 hours (overnight). Immediately after electrophoresis, the DNA was transferred to a nylon filter. The process lasted 30-60 minutes and was carried out in distilled water on a VacuGene XL Vacuum Blotting System ™ (Amersham Biosciences ™). After that, the DNA fragments on the filter were visualized in a color reaction using the streptavidin-alkaline phosphatase conjugate (Streptavidin-AP, Bio-Rad ™). This color reaction was based on the use of two commercially available dyes, NBT and BCIP (Thermo Fisher Scientific ™).

3. Result and discussion

According to the DRIM genotyping data of 18 E. coli isolates, 7 different genotypes were identified, including closely related variants (Table 1). It was possible to identify several groups of bacterial isolates having an identical profile of all DNA fragments. The largest group of identical strains were isolates 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 13, and 14, which were assigned to genotype 1. All of the 18 isolates listed were obtained from different organs of four chickens kept in one house. The identity of the isolates indicates the possible re-digestion of different chickens from each other within the same house. In some cases, it was possible to identify genetically closely related strains. For example, genotype 1 additionally had variants 1a and 1b. Isolate 15 (genotype 1a) was obtained from the duodenum of the same individual as isolates 1 through 4 (other organs, genotype 1). Differences in profiles amounted to only 2-3 DNA fragments. In this case, we can talk about the occurrence of a mutation in bacteria specializing in reproduction in different organs of the chicken, or about infection of an individual simultaneously with two strains. The origin of the isolate from endogenous microflora living in the duodenum of chickens cannot also be ruled out. The probability of the last two scenarios is small, since the corresponding strains differed slightly from each other, and the endogenous strain or infection with two strains should appear in the form of completely different genotypes.

Table 1 – Genotypes of the field isolates of *E.coli*, identified by DDSL technique

genotypes (strains)	isolates	organs	individual/ house
1	1,2,3,4,	heart, liver, легкие, фолликулы	1/1
1	6,7	liver, follicules	2/1
1	8,10,11	heart, follicules, bone marrow	3/1
1	12,13,14	heart, liver, follicules	4/1
1а	15	duodenum	1/1
1б	5	heart	2/1
2	16	cecum	1/1
3	9	liver	3/1
4	17	duodenum	4/1
5	18	cecum	4/1

4. Conclusion

Thus, the proposed method of genotyping allows one to identify routes of transmission of pathogenic bacterial strains in the field setting. The method can be recommended to address the issues of epidemiology and epizootology and genetic certification of bacterial strains. The proposed method is universal in nature, as it can be adapted to almost any microorganism.

Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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