# ANIMAL HUSBANDRY

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# MOLECULAR GENETIC IDENTIFICATION OF BACTERIAL STRAINS OF THREE SPECIES ISOLATED FROM TURKEY AND CHICKEN

Discovery notes

**Abstract**

The material presents data on the identification of bacterial strains isolated from turkeys and chicken. We used isolates of *Pseudomonas*, *Acinetobacter* and *Escherichia coli*. The latter were isolated from turkeys, and two previous ones from chickens. *In-silico* search made it possible to propose a number of enzymes for genotyping isolates of *Acinetobacter*, and genotyping of isolates of *Pseudomonas* and *E. coli* was carried out using previously selected enzymes. The enzymes *Sgs*I and *Eco*47III, which show 100% activity in one buffer, have been shown to have optimal combination for *Acinetobacter*. *Pseudomonas* were genotyped using a pair of *Bcu*I/*Stu*I enzymes, two genetically different strains were identified, two isolates were identical (organs of one individual). In 21 isolates of *E.coli*, 17 strains were identified using the *Xba*I/*Pst*I pair of enzymes, and 16 strains were identified using the *Xho*I/*Bsu*RI pair. The discrimination index showing the ability of the method to separate strains was not used, since it can only be used on isolates that are not epidemiologically related. In this case, turkeys were kept in the same farm facilities, the sampling was carried out at the same time, therefore, the possibility of re-infection of the bird cannot be ruled out.

**Keywords:** strain identification, pathogenic bacteria, *E.coli, Acinetobacter, Pseudomonas,* preventive veterinary medicine.

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# МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКАЯ ИДЕНТИФИКАЦИЯ ШТАММОВ БАКТЕРИЙ ТРЕХ ВИДОВ, ВЫДЕЛЕННЫХ ОТ ИНДЕЕК И КУР

Исследовательские заметки

**Аннотация**

В материале представлены данные об идентификации штаммов бактерий, выделенных у индеек и кур. В работе были использованы изоляты псевдомонад, асинетобактерий и кишечной палочки. Последние были выделены у индеек, а предыдущие у кур. Поиск *in-silico* дал возможность предложить ряд ферментов для генотипирования изолятов асинетобактерий, а генотпирование изолятов псевдомонад и кишечной палочки проводилось с использованием ранее подобранных ферментов. Показано, что для асинетобактерий оптимальной сочетанностью обладают ферменты *Sgs*I и *Eco*47III, которые показывают 100% активность в одном буфере. Псевдомонады генотипировали с помощью пары ферментов *Bcu*I/*Stu*I, были выявлены два генетически отличающихся штамма, два изолята были идентичными (органы одной особи). У 21 изолята кишечнгой палочки с использованием пары ферментов *Xba*I/*Pst*I выявили 17 штаммов, а при использовании пары *Xho*I/*Bsu*RI – 16 штаммов. Индекс дискриминации, показывающий способность метода разделять штаммы, не применяли, т.к. он может использоваться

исключительно на эпидемиологически не родственных изолятах. В данном случае, индейки содержались в условиях одного хозяйства, отбор проб проводился в одно время, поэтому нельзя исключить возможность перезаражения птицы.

**Ключевые слова:** идентификация штаммов, патогенные бактерии, кишечная палочка, асинетобактерии, псевдомонады, превентивная ветеринария.

## 1. Introduction

The circulation of pathogenic and opportunistic microorganisms in the environment and in animals has long attracted the attention of scientists [1]. Despite this, many questions remain unresolved, for example, why epidemics (epizootics) periodically occur and according to which laws the infection develops. The relevance of the research topic is emphasized by the social aspect of the problem associated with the circulation of a number of pathogens not only in birds, but also in humans (anthropozoonoses).

In many poultry farms, there is an acute issue of infectious diseases of a bacterial nature caused by *Escherichia coli*, *Acinetobacter* and *Pseudomonas*. The problem is aggrevated by the increasing prevalence of antibiotic-resistant bacterial strains. When planning preventive measures aimed at reducing the infection propagation, it is important to accurately find the source of the infection and determine the routes of transmission [2], [3]. In leading foreign countries, the genotyping of pathogens is given great attention in preventive medicine. Medical centers (hospitals) have specialized microbiological laboratories that regularly carry out genotyping of the main pathogens. Initially, the method of double digest and selective label (DDSL) was developed in foreign medical institutions. It can be used to check the effectiveness of therapeutic measures (compare strains before and after treatment), the effectiveness of vaccination (compare vaccine and pathogenic strains), identify routes of infection spreading, etc. The aim of the work was to genotype a group of bacterial isolates and to discuss the potential of the DDSL method in molecular epidemiology under the conditions of poultry enterprises engaged in raising turkeys and chickens.

Genotyping of field isolates makes it possible to trace the transmission routes and find the source of infection [2]. If the genetic profiles of the compared isolates coincide, this indicates the transmission of infection (re-infection of individuals). In this regard, rapid methods for identifying bacterial strains have acquired particular relevance. Comparison of bacterial genetic profiles can distinguish epidemic strains (all isolates will be identical) from sporadic outbreaks of infection (isolates differ). In the same way, it is possible to identify the pathways of transmission of the pathogen, to identify the source of infection, which, when planning veterinary and sanitary measures, allows you to interrupt the epizootic chain and, thus, prevent the spread of the disease. This knowledge allows specialists to break the epizootic chain, i.e. prevent further spread of the disease. Thus, genotyping is a modern tool for molecular preventive epizootology. Currently, a whole group of genotyping methods is used (pulse-field gel electrophoresis, AFLP, ribotyping, multilocus sequencing (MLST), sequencing of individual genes, PCR genotyping [4], [5]. All these methods have both advantages and disadvantages [6], [7]. As part of the implementation of foreign projects in previous years, we developed and validated a new genotyping method based on the idea of double digest and selective label of genomic DNA fragments - DDSL on large samples of isolates [2].

## 2. Methods

The method is based on our earlier idea of double digest and selective label of DNA restriction fragments (DDSL). The genomic DNA of a microorganism is digested simultaneously by two restriction enzymes and specifically labeled with biotinylated deoxycytosinetriphosphate (Bio-dCTP) using *Taq*-DNA polymerase. Enzymes are selected *in-silico* for each type of microorganism in such a way as to obtain a limited number of labeled DNA fragments that can be easily separated in a common agarose gel. The result of genotyping by the DDSL method is a group of DNA fragments in the form of bands on a filter, the distribution of which is specific for each strain. The accuracy of strain identification calculated by the discrimination index (D) exceeds the accuracy of the current "gold standard" of genotyping (pulse-field gel electrophoresis) and reaches 0.98 for Pseudomonas species and 0.96 for *Salmonella* spp.

The reaction was carried out in one microtube, where the test DNA, two restriction endonucleases, *Taq* polymerase, Bio-14-dCTP was mixed. The total volume of the mixture was 20 microliters. The incubation was carried out for 1-2 hours at 37°C. Then the obtained DNA fragments were separated by size in electrophoresis using standardl 0.8% agarose gel in tris-acetate buffer (TAE). 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 ™). The DNA fragments on the filter were then visualized in color reaction using streptavidin-alkaline phosphatase conjugate (Streptavidin-AP, Bio-Rad ™) to detect alkaline phosphatase activity. This color reaction was based on the use of two commercially available dyes NBT and BCIP (Thermo Fisher Scientific ™). After the development of DNA fragments, the filter was washed several times in distilled water to remove nonspecifically bound dyes. The final stage of the work is the analysis of the distribution of DNA fragments to identify identical, closely related and genetically distant bacterial genotypes (strains).

## 3. Results

The selection of *in-silico* restriction enzymes using the available on-line http: *in-silico/ehu.es* program made it possible to propose one pair of *Bcu*I/*Stu*I enzymes for genotyping *Pseudomonas* isolates. The number of cleavage sites for the first enzyme is 30-50, for the second - 600-1200, depending on the *Pseudomonas* species. A typical picture of the distribution of DNA fragments is shown in the figure. In total, 3 isolates of *Pseudomonas* were grown, according to the results of genotyping, 2 genetically different strains were identified, and two isolates were identical (organs of one individual).

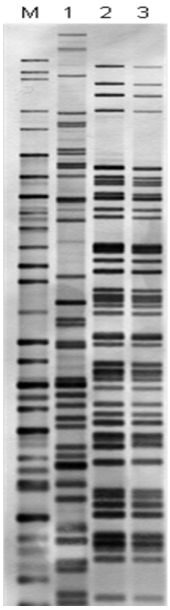


Figure 1 – Genotyping three *Pseudomonas* isolates by DDSL technique with *Bcu*I/*Stu*I pair of enzymes. M – molecular mass marker

The *in-silico* search for *Acinetobacter* made it possible to propose a number of enzymes that can potentially be used in genotyping of this bacterium by the DDSL method (Table). In particular, the *Sgs*I and *Eco*47III enzymes exhibit optimal combination, which show 100% activity in one buffer - R from Thermo Fischer company. In the absence of these enzymes, the reaction can be carried out using enzymes from Sibenzyme company: *Pal*AI (isoschizomer of *Sgs*I), optimal buffer Y and *Pvu*II with optimal buffer G. The *Pal*AI/*Pvu*II pair will be 40-100% active in one Rose buffer (Sibenzyme).

Table 1 – Suggested pairs of enzymes for DDSL genotyping of *Acinetobacter* bacyerium (first enzyme forms 3'-recessed ends) and second enzyme forms blunt ends) and their main properties

| Enzyme | Number of recognition sites in DNA | Optimal reaction buffer | Compatibility with another buffer |
|---|---|---|---|
| SgsI or PalAI (GG↓CGCG) (first enzyme) | 11-19 | R (Thermo Fischer Scientific ™) | + |
| EcoRV (CAT↓ATC) (second enzyme) | 554-1068 | W (Sibenzyme) | ++ |
| PvuII CAG↓CTG (second enzyme) | 624-1007 | G (Sibenzyme) | +++ |
| Eco47III AGC↓GCT (second enzyme) | 637-801 | R (Sibenzyme) | + |

Identification of *E. coli* strains on the set of 21 isolates isolated from organs of diseased and dead turkeys in which two pairs of enzymes were used - *Xba*I/*Pst*I and *Xho*I/*Bsu*RI in DDSL strain identification. Genotyping made it possible to identify 17 bacterial genotypes (including closely related genotypes under the numbers: 5 and 6; 7 and 8, which differed from each other by only 2-3 DNA fragments. In all cases of closely related genotypes, isolates were isolated from the same individual (different organs). In this case, the appearance of adaptive mutations in a microorganism is possible, allowing it to exist in conditions of different organs and tissues. Isolates isolated from different individuals, as a rule, had completely different genetic profiles, the differences ranged from 10 to 20 DNA fragments, which confirms the natural genetic diversity of *E.coli* bacteria and the circulation of a large number of pathogenic strains.

Similar results of genotyping were obtained on the same bacterial isolates using a pair of *Xho*I/*Bsu*RI enzymes. In total, 16 genotypes were identified (one less than in the pair of *Xba*I/*Pst*I enzymes. In this case, it was not possible to differentiate two isolates numbered 7 and 8 which were closely related when using *Xba*I/*Pst*I enzymes. Nevertheless, typing results produced by these enzyme pairs wre in good correspondence. Thus, the *Xho*I/*Bsu*RI enzyme pair is also highly discriminating and most of the isolates were genetically distinguished.

The discrimination index showing the ability of the method to separate strains was not used, since it can only be used on isolates that are not epidemiologically related. In this case, the turkeys were kept in the same farm, the sampling was carried out at the same time, therefore, the possibility of re-infection of the bird cannot be ruled out. Earlier, we calculated the discrimination index for groups of epidemiologically unrelated isolates of different types of bacteria (pseudomonas, salmonella) isolated from clinically ill patients from different countries and at different periods of time. This work was carried out to prove the effectiveness of the DDSL method; it was shown that the method is not inferior in terms of discrimination index to the widely used method of pulse-gel electrophoresis and multilocus sequencing (MLST).

## 4. Conclusion

Thus, genotyping of microorganisms makes it possible to assess the genetic diversity of pathogens circulating in turkeys and chicken, determine the possibility of transmission of infection, identify the source of infection in the external environment, and suggest the appearance of mutations in bacteria colonizing different organs of one individual. The DDSL technique can be applied for *E.coli, Pseudomonas and Acinetobacter* bacterial species.

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## Conflict of Interest

None declared.

## Конфликт интересов

Не указан.

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