MOLECULAR CHARACTERISATION OF PASTEURELLA MULTOCIDA STRAINS ISOLATED FROM CALVES BY PULSED-FIELD GEL ELECTROPHORESIS

AGNIESZKA KĘDRAK-JABŁOŃSKA, AND BOGNA BORKOWSKA-OPACKA

Department of Microbiology, National Veterinary Research Institute, 24-100 Pulawy, Poland
akedrak@piwet.pulawy.pl

Received for publication October 22, 2007

Abstract

Seventy-two Pasteurella multocida strains collected from calves with bronchopneumonia were examined. An analysis of the polymorphism of DNA restrictive fragments with the use of the pulsed-field gel electrophoresis technique of contour-clamped homogeneous electric field electrophoresis was made. Philogenetic relatedness among the examined strains was calculated by the unweighted pair-group method using arithmetic averages. The Polish strains originating from the cases of enzootic bronchopneumonia in calves demonstrated a marked diversity of the genomic DNA. The restrictive patterns of all taxonomic groups of P. multocida occurring in calves in Poland were characteristic for the groups containing several isolates or for single strains. Only P. canis biotype 2 isolates revealed a close relatedness at a genotype level.

Key words: calves, Pasteurella multocida, genetic relatedness, PFGE.

The phenotype characteristic of Pasteurella multocida strains isolated from cattle reveals marked differences in the morphology of a colony, biochemical activity, antigenic structure and pathogenicity. Imperfect bacteria classification according to the phenotype characteristics was a reason to use the methods based on the genetic information that is the most fundamental feature of microorganisms. The genotype characteristic is more universal than the traditional phenotyping methods, because the nucleic acid analysis permits identification and faster microorganism detection, determination of taxonomic position and examination of species genetic relatedness. The genotyping techniques focusing on chromosomal and extrachromosomal DNA analysis provided methods to study pathogenesis and epidemiology of bacterial infection at a molecular level (14, 15, 17).

In our previous work, the pulsed-field gel electrophoresis (PFGE) was a genotype method that complemented the phenotype determination of P. multocida subsp. multocida isolated from the cases of cattle haemorrhagic septicaemia (9). In the present studies the comparison of DNA restrictive patterns obtained by PFGE was also made to determine genetic and epidemiologic relatedness in field strains of P. multocida isolates from calves.

Material and Methods

Bacterial strains. Seventy-two strains isolated in 1985-2003 from the nasal cavity swabs of calves with signs of respiratory system diseases or internal organs of dead animals with the lesions typical for bronchopneumonia (7).

Pulsed-field gel electrophoresis. Preparation of genomic DNA, digestion with restrictive endonuclease Apal and pulsed-field gel electrophoresis technique of contour-clamped homogeneous electric field electrophoresis (CHEF) was carried out as described earlier (9).

The electrophoretic analysis of Pasteurella rods was carried out taking into account their classification according to the criteria of Mutter et al. (11, 12) and Bisgaard et al. (2).

Data analysis. The philogenetic relatedness of the strains examined was calculated by the unweighted pair-group method using arithmetic averages (UPGMA) with the Dice similarity coefficient and the band position tolerance of 1%.

The discriminatory power is the average probability that the typing system will assign a different type to two unrelated strains randomly sampled from the test population. This probability was calculated by Simpson’s index of diversity (13).

Results

The isolates collected from calves have been classified as follows: 16 strains (22.2%) – P. multocida
subsp. multocida; 38 strains (52.8%) – *P. multocida* subsp. *multocida* ornityno–; 2 strains (2.8%) – *P. multocida* subsp. *septica*; 11 strains (15.3%) – *P. multocida* subsp. *septica* ornityno– and 5 strains (6.9%) – *P. canis* biotype 2.

The electrophoretic separation of 16 isolates of *P. multocida* subsp. *multocida* resulted in the electrophoretic patterns comprising 8 to 11 fragments in the range of 51 kb to 510 kb. (Fig. 1). The examined strains revealed the occurrence of six electrophoretic profiles. The rate of phylogenetic relatedness among individual restrictive patterns ranged from 44% to 67% (Fig. 3). Among the examined strains, two main clusters showing the genetic relatedness at a level higher than 50% were singled out (10). The first group involved nearly all the strains belonging to *P. multocida* subsp. *multocida* isolated from various regions of Poland. The second group constituted two strains representing the same profile and originating from the same region.

Results of the restrictive analysis of 38 isolates of *P. multocida* subsp. *multocida* ornityno- allowed to obtain 28 electrophoretic patterns comprising 7 to 14 restrictive fragments in the range of 42 kb to 546 kb (Figs 1 and 2). Relatedness between electrophoretic profiles ranged from 33% to 95% (Fig. 4). Among the examined group, 6 primary clusters revealing genotype correlation above 50% were found. The most represented groups 1 and 4 included strains from various regions of Poland.

Two *P. multocida* subsp. *septica* strains isolated in the same area showed the same restrictive pattern, which was not present in the other isolates. The electrophoretic profile revealed 15 lanes ranging from 45 kb to 611 kb.

---

**Fig. 1.** Electrophoretic profiles of the chromosomal DNA of *P. multocida* subsp. *multocida* (P121, P199, P271, and P274) and *P. multocida* subsp. *multocida* ornityno- (P209, P234, and P264) strains. M – marker

**Fig. 2.** Electrophoretic profiles of the chromosomal DNA of *P. multocida* subsp. *multocida* ornityno-strains. M – marker
Fig. 3. Dendrogram of philogenetic relatedness among *P. multocida* subsp. *multocida* strains.

Fig. 4. Dendrogram of philogenetic relatedness among *P. multocida* subsp. *multocida* ornitno-strains.
The restrictive analysis of 11 \( P. \text{multocida} \) subsp. \( \text{septica} \) ornityno-isolates showed the electrophoretic profiles comprising from 8 to 18 fragments ranging from 35 kb to 611 kb. The examined strains revealed seven restrictive patterns. The relatedness rate among the restrictive patterns obtained ranged from 20% to 94% (Fig. 5). Among examined isolates with the relatedness rate above 50%, five main clusters were found. All the strains were from the same area.

Electrophoresis of five \( P. \text{canis} \) biotyp 2 isolates showed three electrophoretic profiles with 8 to 10 lanes weighing from 49 kb to 431 kb. One profile was common for three strains. The two remaining electrophoregrams that differed from the above-mentioned by occurring an additional restrictive fragment or the lack of one fragment were characteristic for individual strains. A 94% relatedness rate was found between electrophoretic patterns (Fig. 6). All the isolates were from the same area in Poland and represented one cluster.

The restrictive pattern of the reference P1059 serotype A:3 strains revealed 12 lanes in the range of 60 kb to 631 kb. The relatedness rate of the electrophoretic profile of the strain in relation to the profiles of field serotype A:3 isolates belonging to various taxonomic groups ranged from 8% to 27%.

The discriminatory power of the typing system described by the index of diversity was 0.984.

**Discussion**

The pulsed-field gel electrophoresis (PFGE) technique is regarded “the gold standard” among the methods of the molecular typing of \( P. \text{multocida} \) strains (1, 4 - 6, 8, 10, 16). The data reported by others indicate that the method is characterised by a high reproducibility of results (17). In the last decade PFGE has became an integral part of bacterial genetic and molecular epidemiology.

Our studies showed that nearly all the Polish strains of \( P. \text{multocida} \) sampled from the cases of enzootic bronchopneumonia in calves revealed a wide variety of genomic DNA. Similarly to our results Gunawardana et al. (8) found 21 electrophoretic profiles among 73 Australian strains isolated from poultry by digestion of chromosomal DNA with Apal endonuclease. All the isolates showed the type A capsule and somatic serotypes 1, 3, 4 and 3, 4 were dominating. In the majority of cases a visible connection between the PFGE profile and geographic origin of strains was noticed. The identical and closely related patterns (differed in 1 to 3 fragments) were found in \( P. \text{multocida} \) isolates sampled from neighbouring areas. The strains originating from different outbreaks of the disease in the same farm, showed relatedness only when the outbreaks occurred in a short time. The \( P. \text{multocida} \) isolates from the same farm isolated within one month showed the identical profiles, whereas isolates obtained...
phenotyping methods. The electrophoresis technique should be used to supplement polymorphism with the use the pulsed-field gel differences. Thus, the analysis of chromosomal DNA phenotype features indicates only the presence of such between individual isolates whereas the analysis of enables evaluation of the rate of genetic diversity comparison to phenotyping methods. The method diversify genotyping method gives a more extensive possibility to profiles.

Altogether, it may be found that the used genotyping method gives a more extensive possibility to diversify *P. multocida* strains isolated from calves in comparison to phenotyping methods. The method enables evaluation of the rate of genetic diversity between individual isolates whereas the analysis of phenotype features indicates only the presence of such differences. Thus, the analysis of chromosomal DNA polymorphism with the use the pulsed-field gel electrophoresis technique should be used to supplement phenotyping methods.

References


