PRACA ORYGINALNA

ARDRA studies of the ribosomal RNA operon within the Desulfovibrio desulfuricans strains.

Joanna Szczerba[1], Ilona Bednarek[2], Zofia Dzierżewicz[1]

ABSTRACT

BACKGROUND

Desulfovibrio desulfuricans belong to the heterogeneous group of anaerobic, sulphate-reducing bacteria (SRB), widely distributed in various environments. As a result of dissimilatory sulphate reduction, they release hydrogen sulphide (H_2S), which has a cytotoxic effect in human and animal organisms. It has been shown by many authors, that *Desulfovibrio* was the genus predominating in patients with *ulcerative colitis*. Some of these bacteria can act as opportunistic pathogens associated with primary bacteremia and abdominal infections such as abscesses.

MATERIAL AND METHODS

Fifteen (soil and intestinal) strains of *Desulfovibrio desulfuricans* species were cultured in modified sulphate-free Postgate's liquid medium with pyruvate for 10 days. Bacterial DNA was extracted by using a commercially available kit and DNA was used as a template for amplification of the full-length 16S, 23S rDNA and the intergenic spacer region. Digested with restriction enzymes (*Alu*I, *Eco*RI, *Hae*III, *Hind*III, *Hinf*I, *Mbo*I and *Pst*I) PCR amplicons were resolved by electrophoresis on 2% agarose gels.

RESULTS

Digestion of *rrn* operon of *Desulfovibrio desulfuricans* by seven restriction enzymes allowed to obtain the characteristic restriction profiles for all 15 investigated strains. The results allow us to suggest three of used enzymes: *Hinf*I, *Alu*I and *Hae*III as a useful for confirmation of the similarity within of *rrn* operon of isolates belonging to this species. Considering the restriction profiles received with *Hind*III, and *Eco*R1 enzymes it seems that their application is insufficient, but *Pst*I enzyme is not acceptable for the analysis of *rrn* operon of these bacteria.

CONCLUSIONS

The obtained data have shown that ARDRA can be used for establishment of phylogenetic relations among isolates of *Desulfovibrio desulfuricans* species, providing the appropriate restriction enzyme is used.

KEY WORDS

Desulfovibrio desulfuricans, restriction enzymes, ribotyping, sulphate-reducing bacteria.

[1]Department of Biopharmacy, Medical University of Silesia, Poland
[2]Department of Biotechnology and Genetic Engineering, Medical University of Silesia, Poland

ADRES DO KORESPONDENCJI:
Joanna Szczerba
Department of Biopharmacy
Medical University of Silesia
41-200 Sosnowiec, Poland
Narcyzów 1 St.
tel:(+48 32) 364 10 61
E-mail: joszczerba@poczta.onet.pl

Ann.Acad.Med.Siles. 2009, 63, 1, 7-13

ISSN 0208-5607

STRESZCZENIE

WSTĘP:
Bakterie *Desulfovibrio desulfuricans* należą do szerokiej grupy beztlenowych bakterii redukujących siarczany (BRS), rozpowszechnionej w różnych środowiskach. Jako rezultat dysymilacyjnej redukcji siarczanów uwalniają do środowiska siarkowodór (H_2S), który wpływa cytotoksycznie na organizm ludzi i zwierząt. Wielu autorów wykazało, iż rodzaj *Desulfovibrio* jest dominujący u osób cierpiących z powodu wrzodziejącego zapalenia okrężnicy. Ponadto niektóre gatunki tego rodzaju odgrywają role oportunistycznych patogenów wywołując bakteriemię, infekcje w obrębie jamy brzusznej oraz wrzody.

MATERIAŁY I METODY:
Piętnaście szczepów *Desulfovibrio desulfuricans* (glebowych i jelitowych) hodowanych było na modyfikowanym podłożu Postgata z dodatkiem pirogronianu przez 10 dni. Po wyizolowaniu genomowego DNA tych bakterii przeprowadzono reakcje PCR w celu namnożenia fragmentu operonu *rrn* obejmującego geny 16S, 23S oraz odcinek zmienny pomiędzy nimi. Otrzymane amplikony poddane zostały trawieniu enzymami restrykcyjnymi (*Alu*I, *Eco*RI, *Hae*III, *Hind*III, *Hinf*I, *Mbo*I and *Pst*I), a otrzymane fragmenty rozdzielono w 2% żelu agarozowym.

REZULTATY:
Przeprowadzona analiza restrykcyjna operonu *rrn* bakterii *Desulfovibrio desulfuricans* pozwoliła na otrzymanie charakterystycznych profili restrykcyjnych dla wszystkich piętnastu badanych szczepów. Uzyskane rezultaty pozwoliły uznać trzy z zastosowanych enzymów (*Hinf*I, *Alu*I oraz *Hae*III) za odpowiednie do potwierdzenia podobieństw w obrębie operonu *rrn* bakterii tego gatunku. Biorąc pod uwagę profile otrzymane po trawieniu enzymami *Hind*III, *Eco*R1, możemy stwierdzić ich małą użyteczność w analizach operonu *rrn* gatunku *Desulfovibrio desulfuricans*, zaś enzym *Pst*I w ogóle nie nadaje się do tego celu.

INTRODUCTION

Desulfovibrio desulfuricans bacteria represent Gram negative, motile, non-spore forming curved rod shaped cells that contain desulfoviridin. Members of *Desulfovibrio* genus have been isolated from the soil and aquatic habitats of natural and anthropogenic environmental sources (1-4). These bacteria have also been isolated from the anaerobic flora of the digestive tract of humans and animals such as sheep, dogs, pigs, hamsters, ferrets, mouse, cats, beetles, termites (5-10). In spite of common occurrence in the large bowel, the role of *Desulfovibrio* genus in the gastrointestinal tract has not been elucidated yet. There are some suggestions that the pathological changes in colon including *ulcerative colitis* and inflammatory bowel disease may be associated with relatively high concentrations of *Desulfovibrio* spp. (11-14). Moreover, these bacteria have been isolated from appendices as well as peritoneal fluid from patients with acute perforating appendicitis (15), from abdominal and brain abscesses, blood, urine and pyogenic liver abscess (16-21). Cases of bacteremia (associated with fever), bacteriuria (associated with meningitis), liver abscess, human thoracoabdominal pus and septicemia caused by *D. fairfieldensis* have been described by several research groups (16, 18, 21-23). Possible pathological influence of *Desulfovibrio* genus on a human body suggests advisability of applying the most appropriate diagnostic methods for identification of this genus strains.

Shukla and Reed (24) point out that isolation and identification of fastidious, slowly multiplied anaerobic bacteria like *Desulfovibrio* by traditional microbiological methods is extremely difficult. That is why molecular identification of *Desulfovibrio* species is postulated

to be more appropriate method for analyzing *D. desulfovibrio*. Moreover, the methods based on DNA analysis are reliable and they do not require the bacteria culturing steps.

Over the last years, techniques involving the analyses of genes coding for the rRNA are the best nucleic acid-based methods for reconstructions of phylogenetic relationships of prokaryotes. It is due to the high conservation of rRNA gene sequence.

Amplified ribosomal DNA restriction analysis (ARDRA) was recently reported to be a rapid and efficient method of bacteria identification even at the species level (25-30). ARDRA appeared as an appropriate method for phylogenetic and taxonomic studies, especially of large sets of strains where DNA sequencing is expensive and time-consuming method. For these reasons in our understanding, it was reasonable and interesting to evaluate ARDRA method for genotyping strains of *D. desulfuricans* species.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Fifteen (soil and intestinal) strains of *Desulfovibrio desulfuricans* species were investigated (tab. 1.).

The strains were cultured in modified sulphate-free Postgate's liquid medium with pyruvate (31). The cultures were grown for 10 days in an anaerobic chamber (MK 3 anaerobic workstation, dW Scientific, West Yorkshire, England) under the following

Table 1. Investigated bacterial strains.

strains of Desulfovibrio desulfuricans		samples
the wild soil strains	DV-1	excavations
	DV-2	mud deposits
	DV-3	excavations
	DV-4	excavations
	DV-5	excavations
	DV-6	mud deposits
	DV-7	excavations
	DV-8	mud deposits
the wild intestinal strains	DV-A	faeces
	DV-B	faeces
	DV-C	faeces
	DV-H	faeces
	DV-I	faeces
	DV-I/1	biopsy specimen from caecum
the reference strain	La2226	the Swiss National Collection of Type Cultures, Lausanne, Switzerland and was isolated from clay, sand and tar mixtures in a corroded gas main in South Essex, England

conditions: 80% N_2, 10% H_2 and 10% CO_2 at 30°C.

DNA isolation. After 10 days of incubation, the bacterial DNA was extracted by using a commercially available kit (A&A Biotechnology, Poland) and the isolated DNA was used as a template for PCR.

Amplification PCR and restriction analysis (rrn-ARDRA). To amplify the full-length (4500 bp.) 16S, 23S rDNA and the intergenic spacer region we used primers which sequences were as follow: forward 5'-GAGTTTGATCCTGGCTCA-3' and reverse 5'-CCGGTCCTCTCGTACT-3' (32).

The reaction mixture of the total volume of 25 µl contained 2,5 µl of 10x reaction buffer with $MgCl_2$ (MBI Fermentas), 20 pmol of each of the primers, 100 ng of genomic DNA, 200 µM dNTP and 2U of Taq polymerase (MBI Fermentas). Samples were amplified in an automated thermal cycler (Gene Amp PCR system 9600 Perkin Elmer). Thermal cycling conditions included an initial denaturation at 94şC for 3 min, followed by 35 cycles consisting of denaturation at 94şC for 1 min each. Temperature of annealing was 52şC for 1 min and extension at 72şC for 4-min. Final extension cycle was performed at 72şC for 10 min.

Obtained PCR products were veryfied on the basis of their molecular masses (4500 bp.) calculated by useing gel dokumentation system and programm LabWorks 4.0.

The aliquots of 10 µl of the remaining PCR amplificate were digested using one of the enzymes *Alu*I, *Eco*RI, *Hae*III, *Hind*III, *Hinf*I, *Mbo*I and *Pst*I according to the manufacturers' directions (MBI Fermentas).

Analysis of products after enzymatic digestion (*rrn*-ARDRA). Digested with restriction enzymes PCR amplicons were resolved by electrophoresis on 2% agarose gels and visualized using ethidium bromide staining.

Computer and statistical analysis. Gel images were normalized, and the bands were identified and calculated using the UPV Biolmagin gel documentation system in the program LabWorks 4.0. As a molecular weight standard the Gene Ruler™ 100bp (MBI Fermentas) was used. The amplified DNA restriction patterns were statistically analyzed by using SYSTAT for Windows version 5 (SAS Institute Inc., Evanston, I11) by the complete linkage method (furthest neighbour).

RESULTS

The amplicons were subjected to restriction analysis with enzyme *Alu*I and revealed 18 different bands of molecular weight ranging from 126 to 1110 bp (fig.1a). Among these bands, 12 were common for all investigated isolates (836, 723, 600, 480, 392, 263, 242, 205, 195, 183, 147, 126 bp). The strains DV-1, DV-2, DV-H, DV-I were characterized by the highest number (16) of bands and the isolates DV-4, DV-5, DV-6 and DV-I/1, by the least number (13) of bands (fig.1a).

After digestion of the analyzed fragment of *rrn* operon with enzyme *Alu*I, four pairs (DV-H with DV-I; DV-1 with DV-2; DV-8 with DV-B; DV-A with DV-C) showed 100% similarities in their restriction profiles (fig. 5a). Moreover, the restriction patterns obtained with the enzyme *Alu*I were the same for three strains: DV-I/1, DV-4 and DV-6 (fig. 5a). The results confirmed those we presented in our earlier reports, where two of the strains named DV-4 and DV-6, indicated very similar DNA fingerprints after analyses carried out following REP-, ERIC- and AP-PCR (33, 34). Three remaining strains, i.e. DV-5, DV-7 and La2226 were characterized by individual restriction patterns after *Alu*I digestion (fig. 5a).

After digestion of PCR amplicons with the *Hinf*I 20 different bands have been detected on the agarose gel. The molecular weight of *Hinf*I restriction products showed the pattern in range from 152 to 1322 bp. The 12 common bands were found for all strains, the size of bands were as follow: 1322, 967, 800, 661, 534, 513, 476, 376, 335, 235, 205, 152 bp (fig. 1b). The same restriction profiles have been observed for two pairs of isolates: DV-5 with DV-6 and DV-I with DVI/1, as well as for the group of three strains: DV-2, DV-8 and DV-A (fig. 5b).

The restriction analysis with *Hae*III allowed to find 22 different bands for all analyzed strains. In reference to the number of electrophoretic bands, the profile of DV-4 soil strain was the most complex, with 21 restriction fragments. Contrary, the strains DV-8, DV-I and DV-I /1 represented bacterial isolates with the lowest number of restriction fragments (15 electrophoretic bands). Molecular weight of the obtained restriction products were found in the

Figure 1. The restriction profiles of fragment rrn operon (16S, 23S genes and ITS) of D. desulfuricans strains after digestion with a) AluI b) HinfI. (symbols indicate the identical restriction profile).

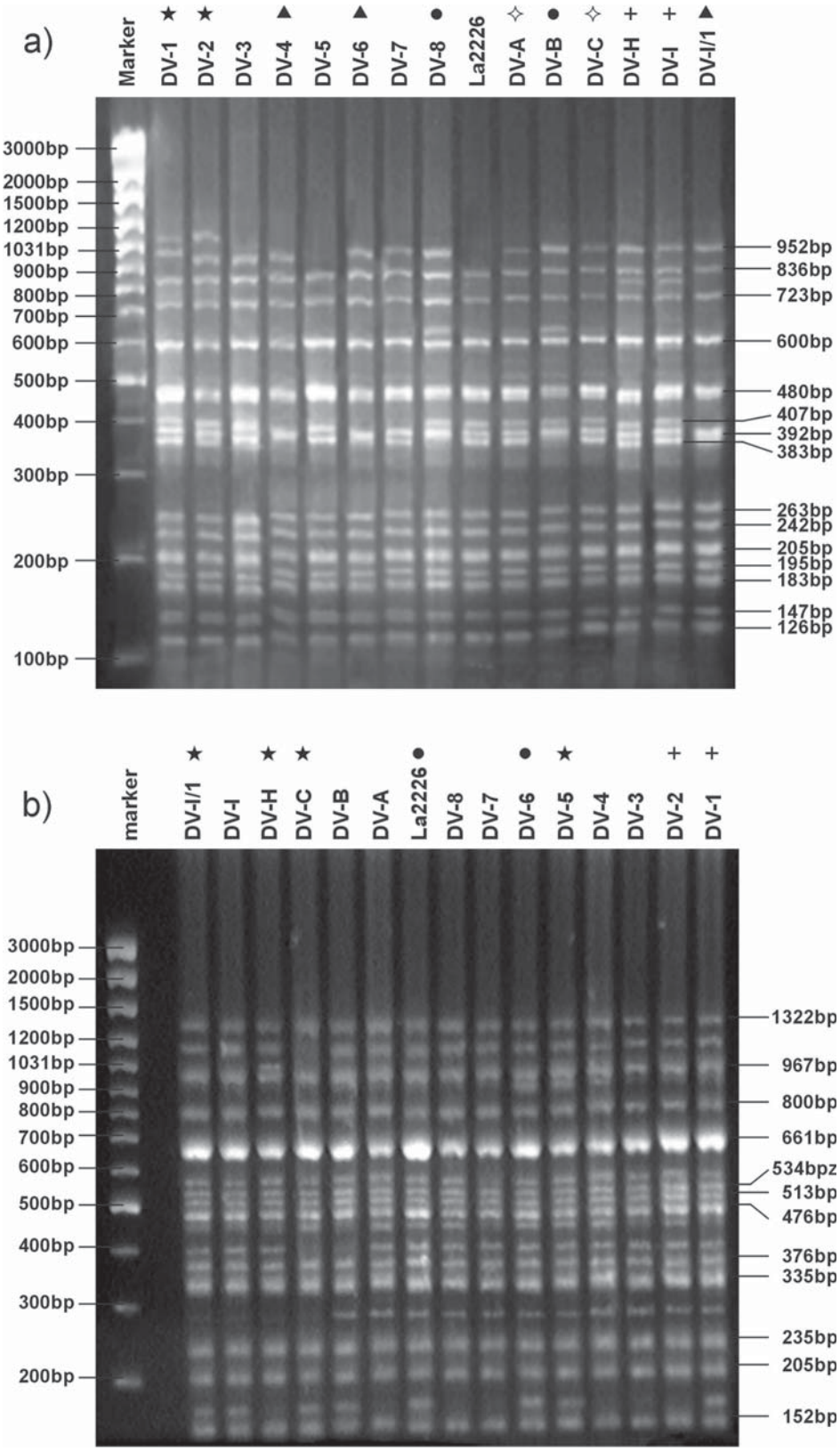


Figure 2. The restriction profiles of fragment rrn operon (16S, 23S genes and ITS) of D. desulfuricans strains after digestion with a) HaeIII b) MboI (symbols indicate the identical restriction profile).

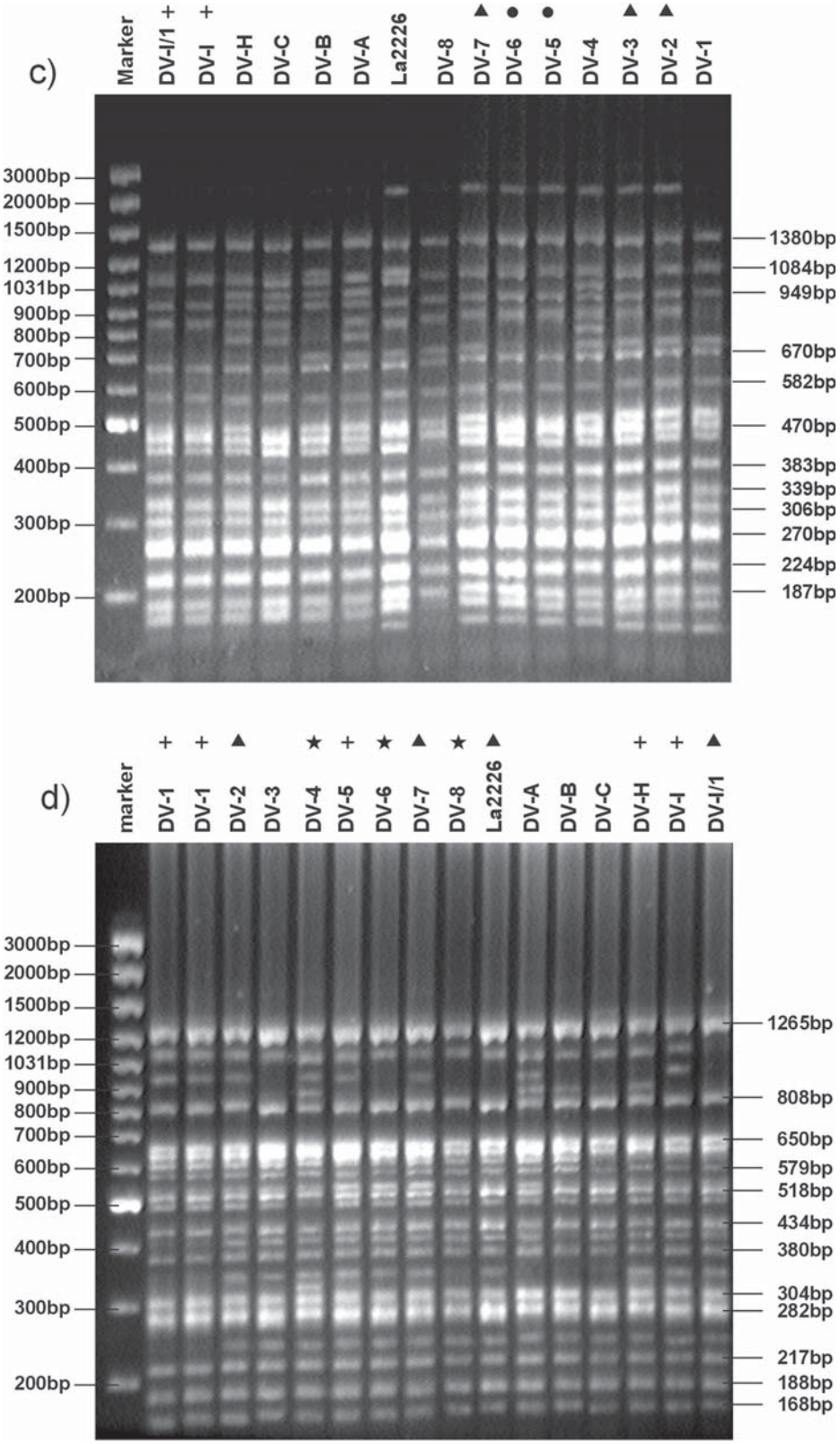


Figure 3. The restriction profiles of fragment rrn operon (16S, 23S genes and ITS) of D. desulfuricans strains after digestion with a) HindIII b) EcoR1 (symbols indicate the identical restriction profile).

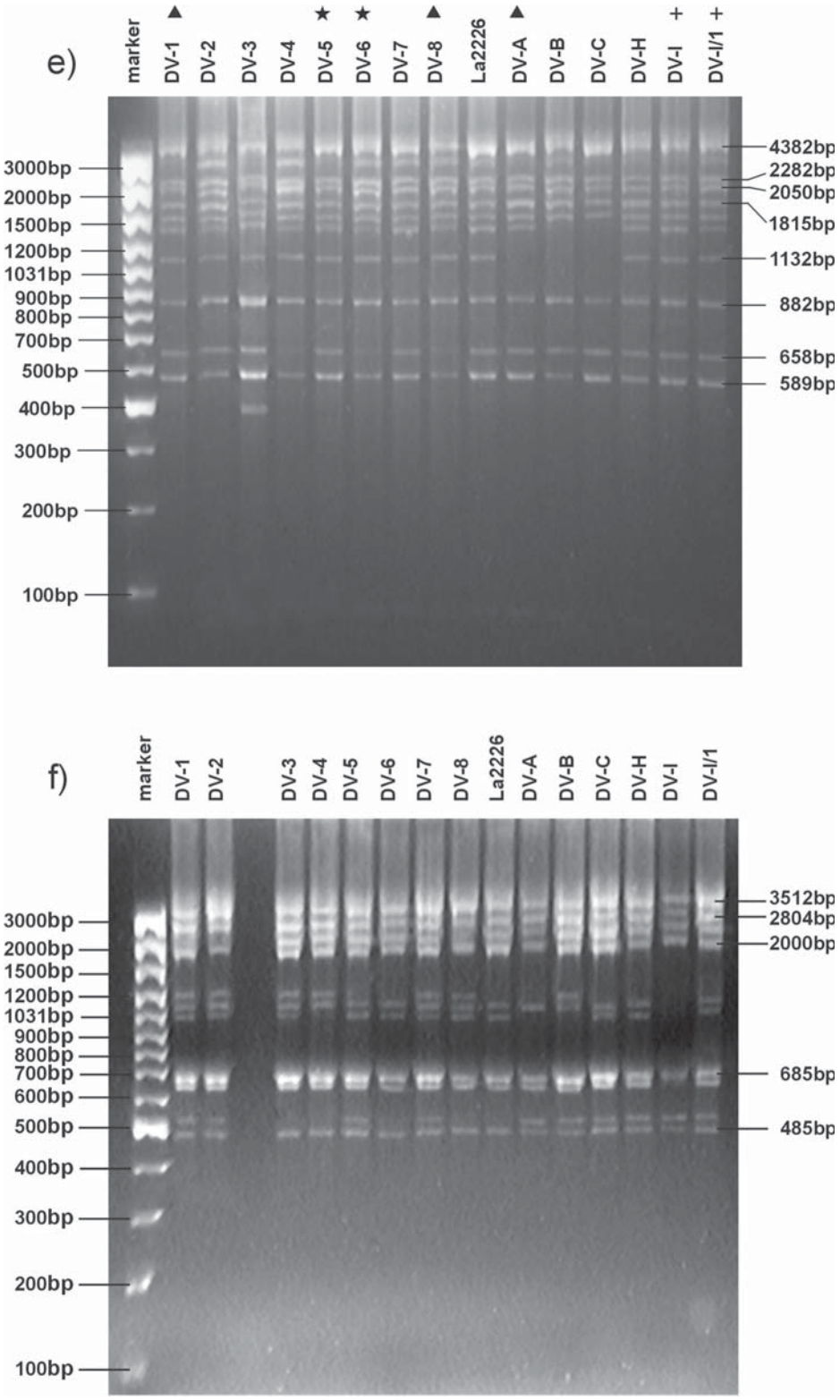
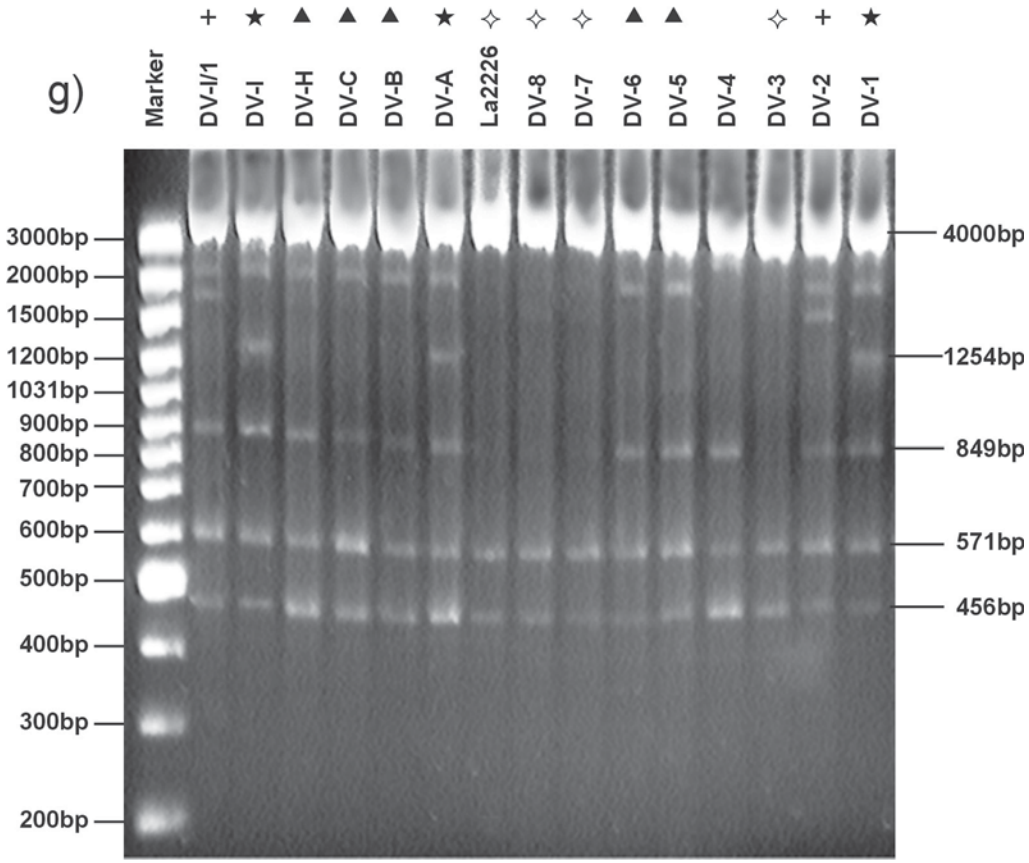


Figure 4. The restriction profiles of fragment rrn operon (16S, 23S genes and ITS) of D. desulfuricans strains after digestion with PstI (symbols indicate the identical restriction profile).



range from 167 bp to 2611 bp. All 15 studied isolates showed 12 identical electrophoretic bands (1380, 1084, 949, 670, 582, 470, 383, 339, 306, 270, 224, 187 bp) (fig. 2a).

After digestion PCR products with *Hinf*I, the restriction patterns two pairs of strains (DV-5 and DV-6) and (DV-I and DV-I/1) were identical (fig. 5b,c). The group including the strains DV-2, DV-3 and DV-7 indicated as well 100% similarity in their restriction profiles (fig 5c).

*Mbo*I DNA fingerprints for all 15 analyzed bacterial strains contain the 23 different fragments ranging in size from 168 to 1265 bp. The restriction profiles were the most complex for the DV-2, DV-4 and DV-A strains (20 fragments), whereas the profiles of the DV-1, DV-B, DV-C, DV-I/1 strains were limited to 17 bands (fig. 2b). In presented *Mbo*I restriction analyses for all 15 strains, the common bands included fragments: 1265, 806, 650, 579, 518, 434, 380, 304, 282, 217, 188, and 168 bp. Besides of these fragments, three other bands (1040, 480, 230 bp) were present for 14 investigated isolates (fig. 2b).

On the other hand, the analyses performed with the *MboI* allowed to obtain the unique restriction profiles for all 15 investigated isolates (fig. 2b, 5d) easily visualised on an electrophoretic gels.

The banding patterns obtained with *Hind*III restriction analysis visualized maximally 12 different fragments depending on the strain (ranging from 8 to 11 DNA bands) from 400 to 4382 bp. Seven fragments (4382, 2282, 2050, 1815, 1565, 882, 589 bp) were common for all analyzed isolates, in addition five of them had relatively high molecular weight (fig. 3a).

The *Hind*III restriction profiles of *rrn* operon fragments were identical for the strains DV-4, DV-6, DV-8 as well as for

Figure 5. The concordance of DNA profiles of 15 strains of Desulfovibrio desulfuricans determined by restriction analysis performed with a) AluI b) HinfI c) HaeIII d) MboI. e) HindIII f) EcoR1 g) PstI

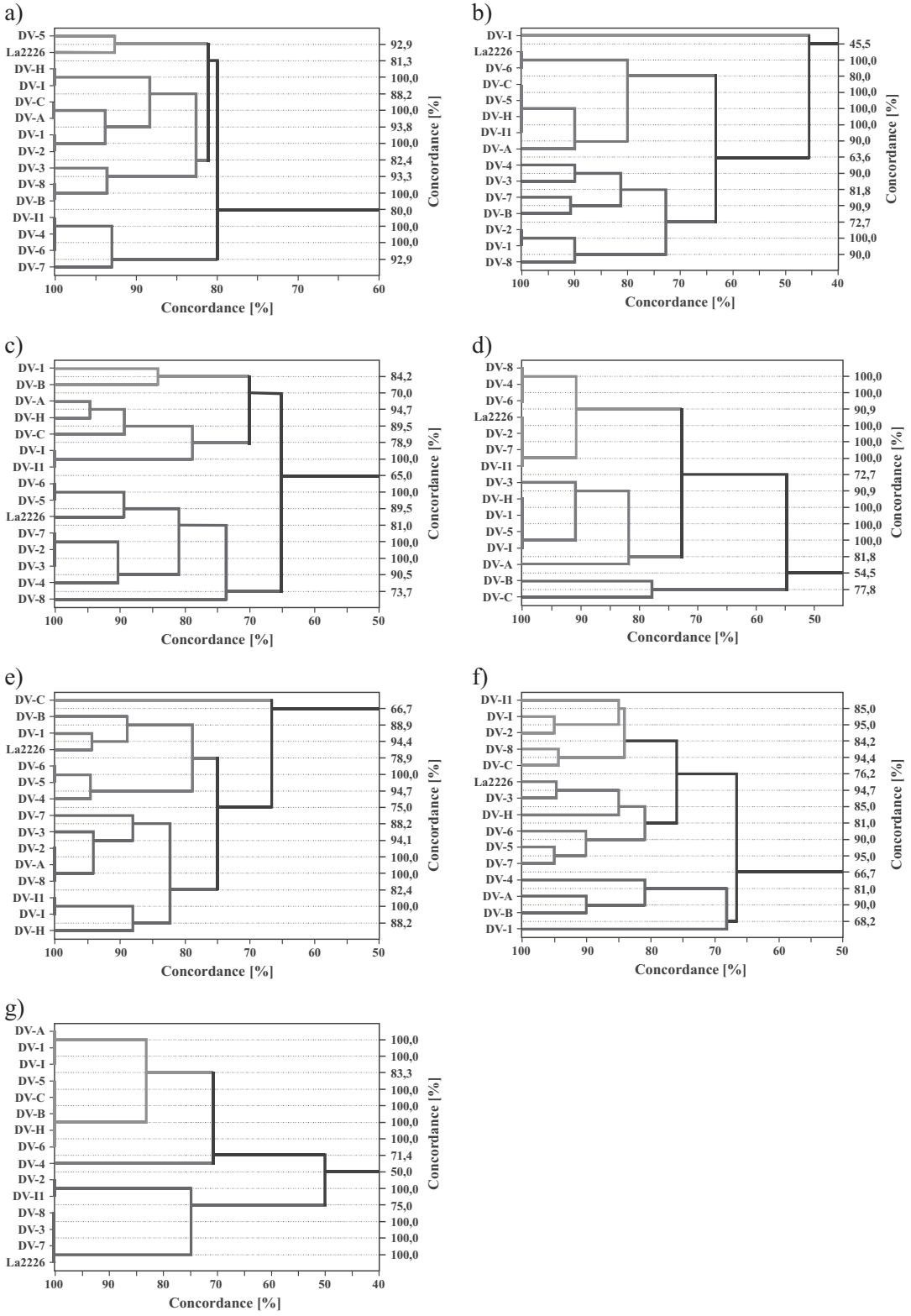


Table 1. Percent of similarity calcualted by the statistical complete linkage method on the base restriction patterns obtained with use enzymes AluI, HinfI and MboI. Similarity was calculated between wild isolates and the type strain La2226.

Strains	Enzyme		
	*Alu*I	*Hinf*I	*Mbo*I
DV-1	87 %	94 %	76 %
DV-2	87 %	94 %	95 %
DV-3	80 %	89 %	95 %
DV-4	80 %	89 %	78 %
DV-5	93 %	95 %	86 %
DV-6	80 %	95 %	90 %
DV-7	87 %	83 %	90 %
DV-8	81 %	94 %	95 %
DV-A	93 %	94 %	82 %
DV-B	91 %	89 %	86 %
DV-C	93 %	83 %	90 %
DV-H	87 %	79 %	86 %
DV-I	87 %	89 %	90 %
DV-I/1	80 %	89 %	90 %

DV-2, DV-7, DV-I/1, La2226 and DV-1, DV-5, DV-H, DV-I (fig. 5e).

The least complex fingerprints were obtained after digestion with *Eco*R1 and *Pst*I enzymes. With *Eco*R1 enzyme analysis, 11 different fragments ranging from 485 to 3512 bp in size, were observed. The profile of strain DV-7 have been characterized only by the highest number of bands (11), contrary to the DNA fingerprint of strain DV-I (only 7 DNA fragments). Five electrophoretic bands of approximately 485, 685, 2000, 2804, 3512 bp were present in all isolates (fig. 3b). The group of 4 isolates DV-5, DV-C, DV-H, DV-I/1 was characterized by the same restriction profiles as well as two pairs DV-6, La2226 and DV-1, DV-2 (fig. 5f).

Treatment with the enzyme *Pst*I produced the least number of fragments with three common ones for all analysed strains (4000, 571 and 456 bp in size, (fig. 4)). In that case, the genetic profiles consisted of 3 to 6 bands per isolate. The size of the *Pst*I digestion products was ranging from 456 to 4000 bp in size (fig. 4)

The restriction profiles of 16S-23S rDNA fragments after *Pst*I treatment were not much differentiated. Five strains DV-5, DV-6, DV-B, DV-C, DV-H were characterized by the identical fingerprints, the same as the restriction patterns of four soil strains DV-3, DV-7, DV-8, La2226 and isolates DV-1, DV-A, DV-I. Also the restriction patterns of pair DV-2 and DV-I/1 were the same (fig. 5g).

DISCUSSION

Advances in molecular biology lead to development of culture-independent approaches for describing bacterial communities and identification of individual species. The techniques involving the analysis of the genes coding for rRNA (rDNA) have been revolutionised prokaryotic taxonomy. The rRNA operon is of broad interest for evolutionary studies. The reason is that, the rRNA genes are essential for the survival of all organisms and sufficiently conserved to establish of evolutionary relations.

The one of the rybotyping method - ARDRA (Amplified Ribosmal DNA Restriction Analysis) is considered as a very useful tool for identification of various species (26, 27, 35, 36).
Vaneechoutte et al., (37) showed that ARDRA method is technically less demanding than other molecular biology approaches, secondly, it allows for identification purpose within one day, when starting from a pure culture.
The suggestion about pathogenicity and the fact of the harmful influences bacteria of *Desulfovibrio* genus on human and animal body reported by some authors (11, 13, 14, 16-24) revealed the need for evaluation a rapid method of *Desulfovibrio* identification. Then, the purpose of present study was to evaluate the usefulness of ARDRA method for analyzing of fragments *rrn* operon of 15 *D. desulfuricans* strains in respect *Desulfovibrio* identification.
To evaluate the similarity and differences within the fragments *rrn* operon coding for the 16S and 23S rDNA genes and intergenic spacer region, seven restriction enzymes have been used (*Alu*I, *Hinf*I, *Hae*III, *Mbo*I, *Hind*III, *Eco*RI, *Pst*I). Obtained restriction profiles allowing for suggestion abou usefulness three of enzymes: *Hinf*I, *Alu*I and *Hae*III for confirmation of the similarity within isolates belonging to *D. desulfuricans* species. The restriction profiles characterizing the relatively many common fragments for investigated strains. Moreover, the profiles of wild strain were quite similar to the restriction profiles of the type strain (fig. 1a,b; 2a, tab. 2). In the banding patterns minor variations were observed, but Schlegel et al. (26) suggested that these differences can be strain specific, and they cannot come to conclusion that we have dealt with different species.
The highest similarities between the wild strains and the type strain have been noticed in the fingerprints obtained with the enzyme *Hinf*I. The La2226 strain showed 18 fragments in the banding pattern, which was almost identical comparing with the patterns of strains DV-2, DV-4, DV-5, DV-6, DV-8 and DV-A (fig. 1b, tab. 2). On the other hand, the highest number of the common fragments were present in the restriction profiles received after digestion with *Alu*I (fig. 1a, fig. 5a, tab. 2).
The restriction profiles received after *Hae*III treatment were quite similar to each other, especially in a group of small fragments of DNA (fig. 2a). Similarly, Garcia-Arata and co workers (32) found the Hae*III* enzyme as not optimal for discrimination between all DNA groups of *Acinetobacter calcoaceticus-A. baumanii* complex.
The received results allowed to choose the *Alu*I and *Hinf*I enzymes as those making possible to find the similarities within *rrn* operon of *D. desulfuricans* strain and probably could be used for identification of this species. Contrary, the *Mbo*I enzyme may be used for differentiation of the strains belonging to this species. Therefore, *MboI* could be recommended for the *D. desulfuricans* strains differentiation based on 16S-23S rDNA fragments analysis. Also De Baere et al. (38) used the *Mbo*I for analysing of 16S gene within *Mycobacterium* and they received characteristic electrophoretical profiles, which could be a standard for further identification of the species of this genus.
Considering the restriction profiles received with *Hind*III and *Eco*R1 enzymes seems that their application for analysis of *rrn* operon within *D. desulfuricans* species is insufficient. That conclusion come out of the limited number of obtained restriction fragments for investigated strains (fig. 3a,b).
The least number of DNA fragments were received after treatment with *Pst*I (3 to 6, depending on strain) (fig. 4) and these results showed that the *Pst*I enzyme is not acceptable for analyzing the *rrn* operon of *D. desulfuricans* species.
Based on the consideration, that the received restriction profiles of wild strains were similar and they did not differ essentially from the patterns of type strain, the ARDRA method can be used for identification of *D. desulfovibrio* species. Reasuming, we do not recommend restriction analysis of investigated segment of genome for differentiation these bacteria. In our opinion, the most likely to get that goal, should be the use of the several restriction enzymes at the same time during the restriction analysis and examination of longer region of *rrn* operon, as Katoch et al. suggested as well (39). Our results are in agreement with Vaneechoutte et al., (37) who showed, that the ARDRA method is useful for identification almost every species of bacteria, but usually is not sufficient for differentiation of isolates within the species. The reason is that the number of nucleotide differentiating the sequences of rRNA genes is too low for closely related bacteria. What is more, the scores comparisons of almost identical se-

quences are burdened with large statistical error (40).

Finally it is worth of emphasising that construction of a database of restriction patterns of *Desulfovibrio* genus *rrn* operons seems to be reasonable. The availability of this internet database could be very useful for fast and simple identification of these slow growing and culture demanding bacteria. Especially because *D. desulfiricans* isolation requires a specific or selective growth medium and what is more, identification these bacteria by the phenotyping test at the species level is very difficult (13). The database need to be gradually up-dated, for a better clustering of the restriction patterns around the type strain allowing for more appropriate identification (26).

REFERENCES

1. Boyle A., Phelps C.D., Young L.Y. Isolation from estaurine sediments of a *Desulfovibrio* strain which can grow on lactete coupled to the reductive dehalogenation of 2,4,6-tribromophenol. Appl. Environ. Microbiol. 1999; 65: 1133-1140.

2. Haouari O., Fardeau M.L., Casalot L., Tholozan J.L., Hamdi M., Ollivier B. Isolation of sulfate-reducing bacteria from Tunisian marine sediments and description of *Desulfovibrio bizertensis* sp. nov. Int. J. Syst. Evol. Microbiol. 2006; 56: 2909-2913.

3. Perez-Jimenez J.R., Kerkhof L.J. Phylogeography of sulfate-reducing bacteria among disturbed sediments disclosed by analysis of the dissimilatory sulfite reductase genes (dsrAB). Appl. Environ. Microbiol. 2005; 71: 1004-1011.

4. Webster G., Watt L.C., Rinna J., Fry J.C., Evershed R.P., Parkes R.J., Weightman A.J. A comparison of stable-isotope probing of DNA and phospholipid fatty acids to study prokaryotic functional diversity in sulfate-reducing marine sediment enrichment slurries. Environ. Microbiol. 2006; 8: 1575-1589.

5. Deplancke B., Hristova K. R., Oakley H. A., McCracken V. J., Aminov R., Mackie R. I., Gaskons H. R. Molecular ecological analysis of the succesio and diversity of sulfate – reducing bacteria in the mouse gastrointestinal tract. Appl. Environ. Microbiol. 2000; 66: 2166 – 2174.

6. Droge S., Limper U., Emtiazi F., Schonig I., Pavlus N., Drzyzga O., Fischer U., Konig H. *In vitro* and *in vivo* sulfate reduction in the contents of the termite *Mastotermes darwiniensis* and the rose-chafer *Pachnoda marginata*. J. Gen. Appl. Microbiol. 2005; 51: 57-64.

7. Dzierżewicz Z., Cwalina B., Gawlik B., Wilczok T., Gonciarz Z. Isolation and evaluation of susceptibility to sulphasalazine of *Desulfovibrio desulfuricans* strains from the human digestive tract. Acta Microbiol. Pol. 1997; 46: 175 – 187.

8. Fox J.G., Dewhirst F.E., Fraser G.J., Paster B.J., Shames B., Murphy J.C. Intracellular *Campylobacter* – like organism from ferrets and hamsters with proliferative bowel disease is a *Desulfovibrio* sp.. J. Clin. Microbiol. 1994; 32: 1229 – 1237.

9. Gibson G.R., Cummings J.H., McFarlane G.T. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. FEMS Microbiol. Ecol. 1991; 86: 103-112.

10. Inness V.L., McCartney A.L., Khoo C., Gross K.L., Gibson G.R. Molecular characterization of the gut microflora of healthy and inflammatory bowel disease cats using fluorescence in situ hybridisation with special reference to *Desulfovibrio spp.*: J. Anim. Physiol. Anim. Nutr. 2007; 91: 48-53.

11. Gibson G.R., McFarlane G.T., Cummings J. H. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. J. Appl. Bacteriol. 1988; 65: 103-111.

12. Head K.A., Jurenka J.S. Inflammatory bowel disease. Part 1: *ulcerative colitis* - pathophysiology and conventional and alternative treatment options. Altern. Med. Rev. 2003; 8: 247-283.

13. Loubinoux J., Bronowicki J.P., Pereira I. A. C., Mougenel J.L., Le Faou A.E. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. FEMS Microbiol. Ecol. 2002; 40: 107-112.

14. Pitcher M.C.L., Cummings J.H. Hydrogen sulphide: a bacterial toxin in *ulcerative colitis*? Gut. 1996; 39: 1-4.

15. Baron E.J., Bennion R., Thompson J., Strong C., Summanen P., McTeague M., Finegold S.M. A microbiological comparison between acute and complicated appendicitis. Clin. Infect. Dis. 1992; 14: 227-231.

16. La Scola B., Raoult D. Third human isolate of a *Desulfovibrio* sp. identical to the provisionally named *Desulfovibrio fairfieldensis*. J. Clin. Microbiol. 1999; 37: 3076-3077.

17. Lozniewski A., Maurer P., Schuhmacher H., Carlier J.P., Mory F. First isolation of *Desulfovibrio* sp. as part of a polymicrobial infection from a brain abscess. Eur. J. Clin. Microbiol. Infect. Dis. 1999; 18: 602-603.

18. McDougall R., Robson J., Paterson D., Tee W. Bacteremia caused by a recently described novel *Desulfovibrio* species. J. Clin. Microbiol. 1997; 35: 1805-1808.

19. Porschen R.K., Chan P. Anaerobic vibrio-like organisms cultured from blood: *Desulfovibrio desulfuricans* and *Succinivibrio* species. J. Clin. Microbiol. 1977; 5: 444-447.

20. Schoenborn L., Abdollahi H., Tee W., Dyall-Smith M., Janssen P.H. A member of the delta subgroup of proteobacteria from a pyogenic liver abscess is a typical sulfate reducer of the genus *Desulfovibrio*. J. Clin. Microbiol. 2001; 39: 787-790.

21. Tee W., Dyall-Smith M., Woods W., Eisen D. Probable new species of *Desulfovibrio* isolated from a pyogenic liver abscess. J. Clin. Microbiol. 1996; 34: 1760-1764.

22. Loubinoux J., Mory F., Pereira I.A.C., Le Faou A.E. Bacteremia caused by a strain of *Desulfovibrio* related to the provisionally named *Desulfovibrio fairfieldensis*. J. Clin. Microbiol. 2000; 38: 931-934.

23. Loubinoux J., Jaulhac B., Piemont Y., Monteil H., Le Faou A.E. Isolation of sulfate-reducing bacteria from human thoracoabdominal pus. J. Clin. Microbiol. 2003; 41: 1304-1306.

24. Shukla S.K., Reed K.D. *Desulfovibrio desulfuricans* bacteremia in a dog. J. Clin. Microbiol. 2000; 38: 1701-1702.

25. Olivares-Fuster O., Shoemaker C.A., Klesius P.H., Arias C.R. Molecular typing of isolates of the fish pathogen, *Flavobacterium columnare*, by single-strand conformation polymorphism analysis. FEMS Microbiol. Lett. 2007; 269: 63-69.

26. Schlegel L., Grimont F., Grimont P.A.D., Bouvet A. Identification of major streptococcal species *rrn*-amplified ribosomal DNA restriction analysis. J. Clin. Microbiol. 2003; 41: 657-666.

27. Spergser J., Rosengarten R. Identification and differentiation of canine *Mycoplasma* isolates by 16S-23S rDNA PCR-RFLP. Vet. Microbiol. 2007; *available on line*.

28. Mendoza-Espinoza A., Koga Y., Zavaleta A.I. Amplified 16S ribosomal DNA restriction analysis for identification of *Avibacterium paragallinarum*. Avian Dis. 2008; 52: 54-58.

29. Shkoporov A.N., Khokhlova E.V., Kulagina E.V., Smeianov V.V., Kafarskaia L.I., Efimov B.A. Application of several molecular techniques to study numerically predominant *Bifidobacterium* spp. and *Bacteroidales* order strains in the feces of healthy children. Biosci. Biotechnol. Biochem. 2008; 72: 742-748.

30. Najjari A., Ouzari H., Boudabous A., Zagorec M. Method for reliable isolation of *Lactobacillus sakei* strains originating from Tunisian seafood and meat products. Int. J. Food Microbiol. 2008; 121: 342-351.

31. Postgate J.R. The sulphate-reducing bacteria. 2nd ed. Cambridge United Kingdom: Cambridge University Press. 1984.
32. Garcia-Arata M.I., Gerner-Smidt P., Baquero F., Ibrahim A. PCR-amplified 16S and 23S rDNA restriction analysis for the identification of *Acinetobacter* strains at the DNA group level. Res. Microbiol. 1997; 148: 777-784.
33. Dzierżewicz Z., Szczerba J., Węglarz L., Komarska-Szostak A., Wilczok T. Evaluation of arbitrarily primed PCR for typing of *Desulfovibrio desulfuricans* strains. Microbiol. Res. 2003; 158: 1-6.
34. Dzierżewicz Z., Szczerba J., Węglarz L., Świątkowska L., Jasińska D., Wilczok T. Intraspecies variability of *Desulfovibrio desulfuricans* strains determined by the genetic profiles. FEMS Microbiol. Lett. 2003; 219: 69-74.
35. Fujita S., Senda Y., Iwagami T., Hashimoto T. Rapid identification of staphylococcal strains from positive-testing blood culture bottles by internal transcribed spacer PCR followed by microchip gel electrophoresis. J. Clin. Microbiol. 2005; 43: 1149-1157.
36. Rodas A.M., Ferrer S., Pardo I. 16S-ARDRA, a tool for identification of lactic acid bacteria isolated from grape must and wine. Syst. Appl. Microbiol. 2003; 26: 412-422.
37. Vaneechoutte M., Riegel P., de Briel D., Monteil H., Verschraegen G., De Rouck A., Claeys G. Evaluation of the applicability of amplified rDNA-restriction analysis (ARDRA) to identification of species of the genus *Corynebacterium*. Res. Microbiol. 1995; 146: 633-641.
38. De Baere T., Mendonca R., Claeys G., Verschraegen G., Mijs W., Verhelst R., Rottiers S., Simaey L., Ganck C.D., Vaneechoutte B.M.C. Evaluation of amplified rDNA restriction analysis (ARDRA) for the identification of cultured mycobacteria in a diagnostic laboratory. Microbiol. 2002; 2: 4-15.
39. Katoch V.M., Parashar D., Chauhan D.S., Singh D., Sharma V.D., Ghosh S. Rapid identification of mycobacteria by gene amplification restriction analysis technique targeting 16S-23S ribosomal RNA internal transcribed spacer & flanking region. Indian J. Med. Res. 2007; 125: 155-162.
40. Kur J., Lewandowski K., Krawczyk B., Samet A. Phylogeny of *Acinetobacter* genus. Adv. Microb. (in polish). 2000; 39: 291 – 301.