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Original Research Article

Sequence analysis of the 16s rRNA gene of sulfate-reducing bacteria isolated from human intestine

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A B S T R A C T

The sulfate-reducing bacteria from the human intestine have been isolated. These bacteria have been identified as the *Desulfovibrio piger* by sequence analysis of the 16S rRNA gene. Comparative sequence analysis has shown the identity of DNA sequences which encode for the 16S rRNA gene of the studied sulfate-reducing bacteria strain with similar sequences as those from the GenBank database. The nucleotide sequence of isolated sulfate-reducing bacteria has 99% homology with sequences *Desulfovibrio piger* ATCC 29098 deposited in the GenBank.

Keywords

Sulfate-reducing bacteria; micro-biocenosis; inflammatory bowel diseases.

Introduction

Sulfate-reducing bacteria (SRB) belong to the intestine microbiocenosis of both humans and animals (Barton *et al.*, 2007; Brenner *et al.*, 2005; Dzierzewicz *et al.*, 2003). The species *Desulfovibrio* genus are dominant SRB in the intestine (Gibson *et al.*, 1991; 1993). They constitute 67–91% of all SRB genera. There are bacteria of the genera *Desulfobacter* (9–16%), *Desulfobulbus* (5–8%) and *Desulfotomaculum* (2%) found in diseased intestines (Gibson *et al.*, 1993; Zinkevich *et al.*, 2000).

Various species of the *Desulfovibrio* genus are often isolated during different illnesses. These species can cause a variety of diseases (cholecystitis, abscesses of the brain and abdomen etc.) in the presence of other infections (Cummings *et al.*, 2003; Loubinoux *et al.*, 2000; 2002). This genus of bacteria has the most pathogenic role compared to other SRB genera and their species [Loubinoux *et al.*, 2000; Rowan *et al.*, 2009]. Similar species, bacterium *Desulfovibrio fairfieldensis* was isolated in mono- and polymicrobial infections of the gastrointestinal tract (Barton *et al.*, 2007; Macfarlane *et al.*, 2007). SRB are also found in the human oral cavity (Langendijk *et al.*, 2001). Dissimilatory sulfate reduction by SRB and the formation of hydrogen sulfide in the intestinal lumen can cause a variety of inflammatory processes (Kushkevych 2013; Pitcher *et al.*, 1996). SRB, which

produces the largest amount of hydrogen sulfide, were isolated from the feces of a distal human colon. It is probably due to a reaction medium where the proximal part of the colon is acidic (pH<5.5), and distal part is neutral (Pitcher *et al.*, 1996; Rowan *et al.*, 2009).

Loubinoux . et al. have isolated bacteria *Desulfovibrio* genera from the human intestine (Loubinoux *et al.*, 2000; 2002). It was established that 12 of 100 samples of purulent peritoneal and pleural cavities contained *Desulfovibrio piger*, *D. fairfieldensis* or *D. desulfuricans* (Loubinoux *et al.*, 2002). Bacteria *D. desulfuricans* were also isolated from the colon during bleeding microvilli, causing bacteremia (Loubinoux *et al.*, 2000). These studies confirm that the main way of SRB penetration in the blood is going through the damaged intestinal microvilli then bacteria can cause infection.

It is of vital importance to obtain new strains of the SRB from different people, identify them by molecular methods and then study their physiological, biochemical and genetic properties. Aside from that, process of dissimilatory sulfate reduction by SRB and the production of hydrogen sulfide should be investigated in order to clarify the etiological role of these bacteria in the development of various diseases. Such studies might help in predicting the development of diseases in the gastrointestinal tract, by providing further details on the etiology of bowel diseases which are very important for the clinical diagnosis of these disease types.

Currently the *D. desulfuricans* strains are the most studied among bowel SRB. However, the physiological and biochemical characteristics of bacteria *Desulfovibrio piger* are unexplored because they are difficult to isolate from among a large number of human intestinal SRB.

In previous studies, the sulfate-reducing bacteria were isolated from human intestine and identified by their morphological, physiological and biochemical characteristics as *Desulfovibrio* sp. according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). The physiological and biochemical properties of obtained bacteria were studied (Kushkevych, 2013). The isolated strain *Desulfovibrio* sp. Vib-7, which produces the largest amount of hydrogen sulfide and acetate, was selected for sequence analysis because it is the most interesting and promising for future study.

The aim of our work was to carry out sequence analysis of the 16S rRNA genes from the *Desulfovibrio* sp. Vib-7 which was isolated from human intestine in preparation for future studies.

The aim was accomplished using the methods of PCR, gel electrophoresis and sequence analysis of the 16S rRNA gene of the sulfate-reducing bacteria of the human intestine, and after statistical processing of the results, the obtained data were compared with those from literature.

Materials and Methods

Object of the study

Strain *Desulfovibrio* sp. Vib-7 was obtained from the human large intestine (Kushkevych, 2013). The strains are kept in the collection of microorganisms at the Biotechnology laboratory of Pharmacy Faculty at the University of Veterinary and

Pharmaceutical Sciences Brno (Czech Republic).

Cultivating of the bacterial cultures

The bacteria were grown in nutrition modified Kravtsov-Sorokin's liquid medium of the following composition (g/l): Na_2SO_4 – 0.5; KH_2PO_4 – 0.3; K_2HPO_4 – 0.5; $(NH_4)_2SO_4$ – 0.2; NH_4Cl – 1.0; $CaCl_2 \times 6H_2O$ – 0.06; $MgSO_4 \times 7H_2O$ – 0.1; $C_3H_5O_3Na$ – 2.0; yeast extract – 1.0; $FeSO_4 \times 7H_2O$ – 0.004; sodium citrate$\times 2H_2O$ – 0.3. Before bacteria seeding in the medium, 0.05 ml/l of sterile solution of $Na_2S \times 9H_2O$ (1%) was added. The sterile 10N solution of NaOH (0.9 ml/l) in the medium was used to provide the final pH 7.2. The medium was heated in boiling water for 30 min in order to obtain an oxygen-free medium, and cooled to +30°C temperature. The bacteria were grown for 72 hours at +37°C under anaerobic conditions. The tubes were brim-filled with medium and closed to provide anaerobic conditions.

DNA isolation

Isolation and purification of DNA was carried out with three day old culture of sulfate-reducing bacteria using a "QIAmp DNA Mini Kit (QIAGEN), Cat. No 51304". One single bacterial colony was taken from the Kravtsov-Sorokin's medium and suspended in 50 µl of deionised water in a screw cap micro-centrifuge tube. The samples were boiled at 98°C for 5 min prior to being centrifuged for 5 min/14000g to settle cell debris. Two microliters of supernatant, containing the genomic DNA, was used for PCR amplification.

Amplification of gene fragments

Amplification of 16S rRNA gene fragments was carried out using the universal primers (according to Weisburg et al. 1991 (Weisburg *et al*., 1991), and Pershing book [Persing, 2011]): 8FPL 5´-AGT - TTG- ATC- CTG- GCT- CAG-3´ position 8–27, 1492RPL 5´-GGT-TAC-CTT-GTT-ACG-ACT-T-3´ position 1510–1492 (amplicon length approximate 1500 bp) and 8FPL 5´-AGT-TTG-ATC-CTG-GCT-CAG-3´ position 8–27, 806R 5´-GGA-CTA-CCA-GGG-TAT-CTA-AT-3´ position 806–787 (amplicon length approximate 800 bp).

PCR procedure

PCR was carried out on DNA isolated from sulfate-reducing bacteria cells in a final volume of 20 µl consisting of 10.0 µl Taq PCR Master Mix Kit (Cat. No 201445), 0.1 µl of each primer, 0.1 µl uracil D-glycosylase (Cat. No. M0280L), 2.0 µl of DNA supernatant, and 7.7 µl deionised water.

The amplicons were amplified by a preliminary incubation at 94ºC for 5 min (initial denaturation) then 34 cycles of 94ºC for 1 min (denaturation), 55ºC for 1 min (annealing of primers), and 72ºC for 2 min (polymerization), using a Thermocycler (model MJ Research PTC-200, USA). After the last amplification cycle, the samples were incubated further at 72ºC for 2 min for complete elongation of the final PCR products and cooled at 10ºC.

Analysis of PCR products

Analysis of PCR products was carried out by electrophoresis in 1.5% agarose gel, with a field strength of 5V/cm. Electrophoresis time was 40 min. The 100 bp ladder (Malamité, Czech Republic) was used as a size standard and molecular weight marker. Isolation and purification of fragments from agarose was performed

by centrifugation of gel strips containing DNA through aerosol filters. For purification *Desulfovibrio* amplicons the commercial kit from QIAGEN „MinElute Gel Extraction Kit" was used.

Sequence was carried out using "Genetic Analyzer" and reagents "BigDye Terminator v3.1 Cycle Sequencing Kit". Search for homologous deposited in GenBank nucleotide sequence encoding the 16S rRNA gene was performed using BLASTN and Blast2 programs.

Results and Discussion

The isolated strain *Desulfovibrio* sp. Vib-7 producing the largest amount of hydrogen sulfide and acetate for sequence analysis was selected. The 16S rRNA gene amplicons which were used for sequence analysis were obtained by using PCR method. The PCR products were separated by electrophoresis (fig. 1). Before sequence analysis the absorbance of amplicons (8FPL/806R, amplicon I was about 800 bp; 8FPL/1492RPL, amplicon I was about 1500 bp; 8FPL/806R, amplicon II was about 800 bp; 8FPL/1492RPL, amplicon II was about 1500 bp) were determined. Comparison identity of the PCR products are shown in table 1. The obtained sequence BLASTN was analysed. The highest homology of the *Desulfovibrio piger* ATCC 29098 strain was obtained.

BLAST search

The *Desulfovibrio piger* ATCC 29098 strain ATCC29098 16S ribosomal RNA, complete sequence. Identities: 1352/1368 (99%).

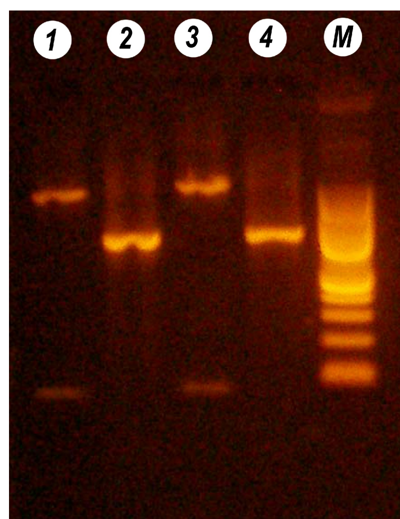
The obtained sequence of results from the isolated sulfate-reducing bacteria were also compared by BLASTN analysis with the nucleotide sequences of 16S rRNA gene of other strains found in table 2.

Thus, the nucleotide sequence of the 16S rRNA gene of studied sulfate-reducing bacteria has the highest 99% homology compared to deposited nucleotide sequence *Desulfovibrio piger* ATCC 29098 (AF192152) in the GenBank database.

The obtained *Desulfovibrio piger* strain belongs to sulfate-reducing bacteria which are usually considered a commensal bacteria in humans [Holt *et al.*, 1994; Moore *et al.*, 1976]. More recently, *Desulfovibrio piger* has attracted more interest as it was found to be the most prevalent species of SRB in faeces of patients with inflammatory bowel disease [Loubinoux *et al.*, 2000; 2002]. The obtained bacterial strains have such phenotypic features as the presence of desulfoviridin, cytochrome c_3 and menaquinone MK-6. They oxidize organic compounds incompletely to acetate

In 1976 Moore W.E. found SRB for the first time in people feces and identified as *Desulfomonas pigra* [Moore *et al.*, 1976], which subsequently reclassified to *Desulfovibrio piger* [Loubinoux *et al.*, 2002]. The described bacterial strain is similar to that of Moore et al. (1976) except for the G–C content of the DNA, which is 64 mol%. Obligate anaerobic, sulfate-reducing, non-saccharolytic, non-proteolytic, non-spore-forming, Gram-negative bacteria that are straight, vibrio-like and have rounded ends (0.8–1.0×2.5–10.0 µm). These microorganisms use lactate, pyruvate, ethanol and hydrogen as electron donors for sulfate reduction.

Fig.1 The results of electrophoresis 16S rRNA PCR products.



1 – amplicon 8FPL/1492RPL (1 500 bp); ***2*** – amplicon 8FPL/806R (800 bp); ***3*** – amplicon 8FPL/1492RPL (1 500 bp); ***4***– amplicon 8FPL/806R (800 bp); ***M*** – marker (100 bp ladder)

Table.1 Identity of the samples by BLASTN results

Number	Amplicon	Primer	Identities*	Identity (%)
1	8FPL/806R	8FPL	657/678	97
2	8FPL/806R	806R	723/742	97
3	8FPL/1492RPL	8FPL	908/927	98
4	8FPL/1492RPL	1492RPL	985/999	99
5	8FPL/1492RPL	806R	740/747	99

*Notes. Complete sequence compared with *Desulfovibrio piger* ATCC 29098 strain ATCC29098 16S rRNA.

The following identities were determined by Blast2 analysis. The results received by the Blast2 analysis were used to fix the results then compared again. The results of the comparison:

- Comparison of amplicon 1 with amplicon 2: Identity is 634/648 (98%)
- Comparison of amplicon 1 with amplicon 3: Identity is 667/677 (99%)
- Comparison of amplicon 2 with amplicon 3: Identity is 679/682 (99%)
- Comparison of amplicon 1 with amplicon 5: Identity is 645/653 (99%)
- Comparison of amplicon 2 with amplicon 5: Identity is 738/741 (99%)
- Comparison of amplicon 3 with amplicon 5: Identity is 684/685 (99%)
- Comparison of amplicon 3 with amplicon 4: Identity is 547/559 (98%)

By comparision of individual sequencing data from the amplicons 1–5 the following gene for the 16S rRNA sequence with a total length of 1370 bp was compiled.

TTCGGTCCCGAGTAAAGTGGCGCACGGGTGAGTAACACGTGGATGATCTGCCTCTATGA
TGGGGATAACAGTTGGAAACGACTGCTAATACCGAATACGCTCATGATGAACGTTGTGA
GGAAAGGTGGCCTCTGCTTGCAAGCTATCGCATAGAGATGAGTCCGCGTCCCATTAGCTC
GTTGGTGGGGTAACGGCCTACCAAGGCAACGATGGGTAGCCGATCTGAGAGGATGATCG
GCCACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCGCAATGGGCGAAAGCCTGACGCAGCGACGCCGCGTGAGGGATGAAGGTCTTCGGATC
GTAAACCTCTGTCAGAAGGGAAGAAACTGGGGTGTTCTAATCAGCATCCCACTGACGGT
ACCTTCAAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
AAGCGTTAATCGGAATCACTGGGCGTAAAGCGCACGTAGGCTGTTATGTAAGTCAGGGG
TGAAAGCCCACGGCTCAACCGTGGAACTGCCCTTGATACTGCACGACTCGAATCCGGGA
GAGGGTGGCGGAATTCCAGGTGTAGGAGTGAAATCCGTAGATATCTGGAGGAACATCAG
TGGCGAAGGCGGCCACCTGGACCGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGATGCTAGATGTCGGGAT
GTATGTCTCGGTGTCGTAGTCAACGCGTTAAGCATCCCGCCTGGGGAGTACGGTCGCAA
GGCTGAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAAT
TCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATCTGGGGAATCCTCCCGAAAAGG
AGGAGTGCCCTTCGGGGAGCCCCAAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATGCATAGTTGCCAGCAGG
TAGAAGCTGGGCACTCTATGCAGACTGCCCGGGTTAACCGGGAGGAAGGTGGGGACGAC
GTCAAGTCATCATGGCCCTTACACCCAGGGCTACACACGTACTACAATGGCACGCACAA
AGGGCAGCGATACCGTGAGGTGGAGCCAATCCCAAAAAACGTGTCCCAGTCCGGATTGC
AGTCTGCAACTCGACTGCATGAAGTCGGAATCGCTAGTAATTCGAGGTCAGCATACTCG
GGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAAAGTCGGTTTACC
CGAAGCCGGGGG

Black nucleotides are the summary of 1, 2, 3 and 5 which are the best sequence data (a total of four sequences). Green nucleotides are the summary of 3 and 4 which are good sequence data (two sequences). Red nucleotides are the rest of 4 which are the worse sequence data because they were received from one sequence only but its quality was excellent

Figure.2 *Desulfovibrio piger* ATCC 29098 strain ATCC29098 16S ribosomal RNA, complete sequence. Identities: 1352/1368 (99%).

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Query  1     TTCGGTCCCGAGTAAAGTGGCGCACGGGTGAGTAACACGTGGATGATCTGCCTCTATGAT  60
             |||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  73    TTCGGTCCCGAGTAAAGTGGCGCACGGGTGAGTAACACGTGGATAATCTGCCTCTATGAT  132

Query  61    GGGGATAACAGTTGGAAACGACTGCTAATACCGAATACGCTCATGATGAACGTTGTGAGG  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  133   GGGGATAACAGTTGGAAACGACTGCTAATACCGAATACGCTCATGATGAACTTTGTGAGG  192

Query  121   AAAGGTGGCCTCTGCTTGCAAGCTATCGCATAGAGATGAGTCCGCGTCCCATTAGCTCGT  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  193   AAAGGTGGCCTCTGCTTGCAAGCTATCGCATAGAGATGAGTCCGCGTCCCATTAGCTAGT  252

Query  181   TGGTGGGGTAACGGCCTACCAAGGCAACGATGGGTAGCCGATCTGAGAGGATGATCGGCC  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  253   TGGTGGGGTAACGGCCTACCAAGGCAACGATGGGTAGCCGATCTGAGAGGATGATCGGCC  312

Query  241   ACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  313   ACACTGGAACTGAAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGC  372

Query  301   AATGGGCGAAAGCCTGACGCAGCGACGCCGCGTGAGGGATGAAGGTCTTCGGATCGTAAA  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  373   AATGGGCGAAAGCCTGACGCAGCGACGCCGCGTGAGGGATGAAGGTCTTCGGATCGTAAA  432

Query  361   CCTCTGTCAGAAGGGAAGAAACTGGGGTGTTCTAATCAGCATCCCACTGACGGTACCTTC  420
             ||||||||||||||||||||||| ||||| |||||||| ||||| |||||||||||||||
Sbjct  433   CCTCTGTCAGAAGGGAAGAAACTAGGGTGCTCTAATCATCATCCTACTGACGGTACCTTC  492

Query  421   AAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTT  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  493   AAAGGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTT  552

Query  481   AATCGGAATCACTGGGCGTAAAGCGCACGTAGGCTGTTATGTAAGTCAGGGGTGAAAGCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  553   AATCGGAATCACTGGGCGTAAAGCGCACGTAGGCTGTTATGTAAGTCAGGGGTGAAATCC  612

Query  541   CACGGCTCAACCGTGGAACTGCCCTTGATACTGCACGACTCGAATCCGGGAGAGGGTGGC  600
             |||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct  613   CACGGCTCAACCGTGGAACTGCCCTTGATACTGCACGACTTGAATCCGGGAGAGGGTGGC  672

Query  601   GGAATTCCAGGTGTAGGAGTGAAATCCGTAGATATCTGGAGGAACATCAGTGGCGAAGGC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673   GGAATTCCAGGTGTAGGAGTGAAATCCGTAGATATCTGGAGGAACATCAGTGGCGAAGGC  732

Query  661   GGCCACCTGGACCGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGA  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  733   GGCCACCTGGACCGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGA  792

Query  721   TACCCTGGTAGTCCACGCCGTAAACGATGGATGCTAGATGTCGGGATGTATGTCTCGGTG  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  793   TACCCTGGTAGTCCACGCCGTAAACGATGGATGCTAGATGTCGGGATGTATGTCTCGGTG  852

Query  781   TCGTAGTCAACGCGTTAAGCATCCCGCCTGGGGAGTACGGTCGCAAGGCTGAAACTCAAA  840
             ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  853   TCGTAGTTAACGCGTTAAGCATCCCGCCTGGGGAGTACGGTCGCAAGGCTGAAACTCAAA  912

Query  841   GAAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAA  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  913   GAAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAA  972

Query  901   GAACCTTACCTGGGTTTGACATCTGGGGAATCCTCCCGAAAAGGAGGAGTGCCCTTCGGG  960
             ||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  973   GAACCTTACCTAGGTTTGACATCTGGGGAATCCTCCCGAAAAGGAGGAGTGCCCTTCGGG  1032

Query  961   GAGCCCCAAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA  1020
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Sbjct  1033  GAACCCCAAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA  1092

Query  1021  GTCCCGCAACGAGCGCAACCCCTATGCATAGTTGCCAGCAGGTAGAAGCTGGGCACTCTA  1080
             |||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  1093  GTCCCGCAACGAGCGCAACCCCTATGCATAGTTGCCAGCAGGTA-AAGCTGGGCACTCTA  1151

Query  1081  TGCAGACTGCCCGGGTTAACCGGGAGGAAGGTGGGGACGACGTCAAGTCATCATGGCCCT  1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1152  TGCAGACTGCCCGGGTTAACCGGGAGGAAGGTGGGGACGACGTCAAGTCATCATGGCCCT  1211

Query  1141  TACACCCAGGGCTACACACGTACTACAATGGCACGCACAAAGGGCAGCGATACCGTGAGG  1200
             |||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1212  TACACCTAGGGCTACACACGTACTACAATGGCACGCACAAAGGGCAGCGATACCGTGAGG  1271

Query  1201  TGGAGCCAATCCCAAAAAACGTGTCCCAGTCCGGATTGCAGTCTGCAACTCGACTGCATG  1260
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1272  TGGAGCCAATCCCAAAAAACGTGTCCCAGTCCGGATTGCAGTCTGCAACTCGACTGCATG  1331

Query  1261  AAGTCGGAATCGCTAGTAATTCGAGGTCAGCATACTCGGGTGAATGCGTTCCCGGGCCTT  1320
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1332  AAGTCGGAATCGCTAGTAATTCGAGGTCAGCATACTCGGGTGAATGCGTTCCCGGGCCTT  1391

Query  1321  GTACACACCGCCCGTCACACCACGAAAGTCGG-TTTACCCGAAGCCGG  1367
             |||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  1392  GTACACACCGCCCGTCACACCACGAAAGTCGGTTTTACCCGAAGCCGG  1439
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Table.2 Comparison of the resulting sequence of 16S rRNA gene with other *Desulfovibrio* species

Name	Acc. No	Identities	Identity (%)
Desulfovibrio piger ATCC 29098	AF192152	1352/1368	99
Desulfovibrio fairfieldensis ATCC700045	U42221	1313/1374	96
Desulfovibrio desulfuricans Essex 6	AF192153	1299/1369	95
Desulfovibrio intestinalis DSM 11275	Y12254	1289/1373	94
Desulfovibrio desulfuricans MB	AF192154	1293/1373	94
Bilophila wadsworthia ATCC49260	L35148	1242/1369	91
Desulfovibrio vulgaris subsp. *Oxamicus* DSM 1925	AJ295677	1089/1195	91
Desulfovibrio longreachensis ACM 3958	Z24450	1253/1374	91
Desulfovibrio termitidis DSM 5308	X87409	1237/1372	90
Lawsonia intracellularis NCTC 12656	U30147	1215/1374	88
Desulfovibrio vulgaris subsp. *vulgaris* DSM 644	M34399	726/834	87

They oxidize lactate and pyruvate incompletely to acetate. The optimum temperature for growth is +37°C. Growth is not affected by 20% bile. Colonies on anaerobic blood agar are translucent, 2 mm in diameter, circular and non-haemolytic. Cells contain desulfoviridin and cytochrome c_3. These bacteria are isolated from human specimens (faeces, peritoneal fluids and intra-abdominal collections). The strain type was isolated from human faeces, is ATCC 29098 (Brenner *et al.*, 2005; Holt *et al.*, 1994).

According to the Bergey's manual of Systematic Bacteriology (2005), the obtained and identified sulfate-reducing bacteria strain belong to Class IV. *Deltaproteobacteria*; Order II. *Desulfovibrionales*; Family I. *Desulfovibrionaceae* (Genus I. *Desulfovibrio*) (Brenner *et al.*, 2005).

Taking into consideration all of the obtained results, which was obtained using the methods of PCR and sequence analysis of the 16S rRNA genes, the isolated sulfate-reducing bacteria strain has a 99% homology identity with nucleotide sequences *Desulfovibrio piger* ATCC 29098 deposited in the GenBank database.

The isolated sulfate-reducing bacteria strain is identified as *Desulfovibrio piger* Vib-7 by the methods of PCR and sequence analysis of the 16S rRNA genes.

The presence and activity of these microorganisms in the human intestine might be considerably responsible for the development of very serious pathological conditions of the human intestine, causing various human intestinal diseases and inflammatory bowel processes. Therefore the isolated bacteria are very interesting and promising for further studies.

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