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Occurrence of sulfate-reducing bacteria in well water: identification of anaerobic sulfidogenic bacterial enrichment cultures

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ABSTRACT

Bacteriological studies of well water mainly focus on aerobic and facultative aerobic coliform bacteria. However, the presence of obligate anaerobic bacteria in well water, especially sulfate-reducing bacteria (SRB), possible causative agents of some diseases, is often ignored. In this study, the presence of SRB and coexisting anaerobic bacteria with SRB in sulfate-reducing enrichment cultures obtained from 10 well water samples in Istanbul was investigated. A nested polymerase chain reaction-denaturing gradient gel electrophoresis strategy was performed to characterize the bacterial community structure of the enrichments. The most probable number method was used to determine SRB number. Out of 10, SRB growth was observed in only one (10%) enrichment culture and the SRB number was low (<10 cells/mL). Community members were identified as *Desulfolutivibrio sulfodismutans* and *Anaerosinus* sp. The results show that SRB coexist with *Anaerosinus* sp., and this may indicate poor water quality, posing a risk to public health. Furthermore, *Anaerosinus* sp., found in the human intestinal tract, may be used as an alternative anaerobic fecal indicator. It is worth noting that the detection of bacteria using molecular analyzes following enrichment culture techniques can bring new perspectives to determine the possible origin and presence of alternative microbial indicators in aquatic environments.

Key words: anaerobic bacteria, *Anaerosinus*, *Desulfolutivibrio sulfodismutans*, enrichment cultures, groundwater

HIGHLIGHTS

- *Anaerosinus* sp. can be found together with SRB.
- *Anaerosinus* genus may be used as an alternative indicator for fecal contamination.

INTRODUCTION

While approximately 97% of the fresh water on earth consists of groundwater, the remainder is composed of lakes, rivers, wetlands, and soil moisture (Quevauviller 2007). Among these freshwater sources, a tiny fraction (1%) is readily accessible to people (Vörösmarty *et al.* 2005). For this reason, different types of technologies have been developed to increase water availability such as digging wells for groundwater harvesting.

The microbial content of groundwater ecosystems including viruses, bacteria, archaea, protozoa, and fungi is relatively diverse (Griebler & Lueders 2009). The presence and activities of some microbial groups could be problematic with respect to water quality. Many countries consider the World Health Organization (WHO) Guidelines for setting national drinking water quality regulations and standards (WHO 2017). Groundwater management approaches focus on the entry of microorganisms into the groundwater sources. In this context, contamination of groundwater by pathogenic microorganisms is of particular concern because they are potentially harmful to human health, leading to outbreaks of waterborne diseases not only worldwide but also in Türkiye. A total of 14 waterborne outbreaks were reported in Türkiye between 2010 and 2020 (Akgül *et al.* 2023). These outbreaks include a gastroenteritis outbreak, affecting more than 1,000 people, which occurred due to bacterial (*Shigella*) and viral contamination of groundwater (Sezen *et al.* 2015). Unfortunately, very limited studies dealt with such microbial quality of groundwater in Türkiye.

Common bacterial pathogens distributed by the fecal–oral route include species of *Vibrio*, *Shigella*, and *Salmonella*. In this respect, fecal coliform bacteria (mainly *Escherichia coli* and enterococci) are used as water quality indicator organisms for fecal contamination and the possible presence of other pathogens in groundwater (Krauss & Griebler 2011). The microbiological quality of groundwater in Türkiye has also been assessed by this approach. The detected bacteria in groundwater, located in different geographical regions of Türkiye, were reported as *E. coli*, *Enterococcus* spp., *Fecal streptococci*, *Salmonella* sp., *Staphylococcus* spp., and *Pseudomonas aeruginosa* (Özler & Aydın 2008; Yolcubal *et al.* 2016; Gunes 2023). However, the absence of coliform bacteria in the water may not always indicate a safe water supply for humans. For instance, *E. coli*, proposed as the best indicator, becomes inactivated in chlorinated water, while the most resistant pathogens may survive for several hours. At this point, obligately anaerobic *Clostridium perfringens* is used as a water quality indicator, because *C. perfringens* spores are less affected by the chlorine (Cabral 2010). In addition to being resistant to water treatment, the longevity of spores makes *C. perfringens* a useful indicator for remote fecal contamination (Stelma 2018). In contrast to traditional aerobic indicators (*E. coli* and enterococci), apart from *C. perfringens*, different fecal anaerobes in the healthy gut microbiome may also be more reliable candidates for alternative indicators in groundwater because their survival is directly linked to the anaerobic environment in the gut (McLellan & Eren 2014).

Anaerobic bacteria are the residents of the groundwater environment, such as deep aquifers (Griebler & Lueders 2009). Due to the low redox potential of the water and the low presence or absence of oxygen in the water, anaerobiosis is highly favored over aerobiosis in an anaerobic zone of the water well. For instance, anaerobic *Desulfovibrio africanus*, a sulfate-reducing bacterium, was reported to be isolated from well water (Campbell *et al.* 1966). Anoxic subsurface of aquatic environments such as groundwater are typical habitats of sulfate-reducing bacteria (SRB) (Miao *et al.* 2012). However, the presence and activity of SRB may also influence well water quality and safety negatively, potentially leading to serious problems.

In anoxic well water environments, SRB use sulfate as an electron acceptor and generate hydrogen sulfide (H_2S), an acidic and toxic product. Released H_2S cause esthetic problems such as 'rotten egg' taste and odor in well water (Cullimore 1999). Exposure to high concentrations of H_2S gas or prolonged exposure at low concentrations may pose a great danger in terms of the health and safety of the people who use the well water for domestic purposes (Chou 2003). In addition, this gas can react with iron to generate iron sulfide (FeS) deposits in well water systems that causes colored water problems. The formation of SRB biofilms on the surface of metals may lead to clogging of the well and also corrosion of ferrous pipes or other materials in the well water system (Cullimore 1999). The occurrence of SRB in the human intestine has been recognized for a long time (Macfarlane *et al.* 2007). Although intestinal SRB are not considered as direct pathogens, recent studies suggest that SRB are associated with health problems, such as sepsis, liver abscess, and inflammatory bowel diseases (IBD) (e.g., ulcerative colitis) (Goldstein *et al.* 2003; Koyano *et al.* 2015; Kushkevych *et al.* 2020). Therefore, SRB should be evaluated as possible agents, and moreover, SRB detection in well water may be carried out routinely. For this reason, this study was first aimed to detect and identify SRB in well water. In the literature, the studies about the detection of SRB in groundwater were performed by only culture-independent molecular techniques or cultivation methods (Wargin *et al.* 2007; Keesari *et al.* 2015; Yang *et al.* 2015; An *et al.* 2016). However, in this study, the composition of the anaerobic bacterial community composed of SRB in sulfate-reducing enrichments obtained from well water samples was investigated using culture-dependent molecular analysis.

Apart from commonly used anaerobes, anaerobic partners of SRB can also be proposed as an alternative fecal contamination anaerobic indicator in water. In this respect, secondly, this study focused on the anaerobic bacteria that coexist with SRB in well water. Thus, the knowledge of anaerobic bacteria coexisting with SRB may be used for SRB indicators in well water. To the best of our knowledge, in this study, SRB and anaerobic bacteria coexisting with SRB in the enrichment culture obtained from a well water sample were identified for the first time.

MATERIALS AND METHODS

Sampling procedure

All water samples were taken from the wells that are mainly used for irrigation in residential areas of Istanbul which is located in the northwestern region of Türkiye on the coast of the Marmara Sea at an average altitude of 40 m. It lies between the longitudes 27° 58′ 17″ E – 29° 57′ 31″ E and the latitudes 40° 48′ 10″ N – 41° 35′ 02″ N. Figure 1 shows the geographic location and the sampling points. Samplings were carried out between September and November 2021. The water is extracted to the surface using a submersible pump from the wells with a depth of 100–250 m and a diameter of 6–7 inches. After extraction, well water is stored in water tanks and distributed to all points of use by a hydrophore pump. The depth of the

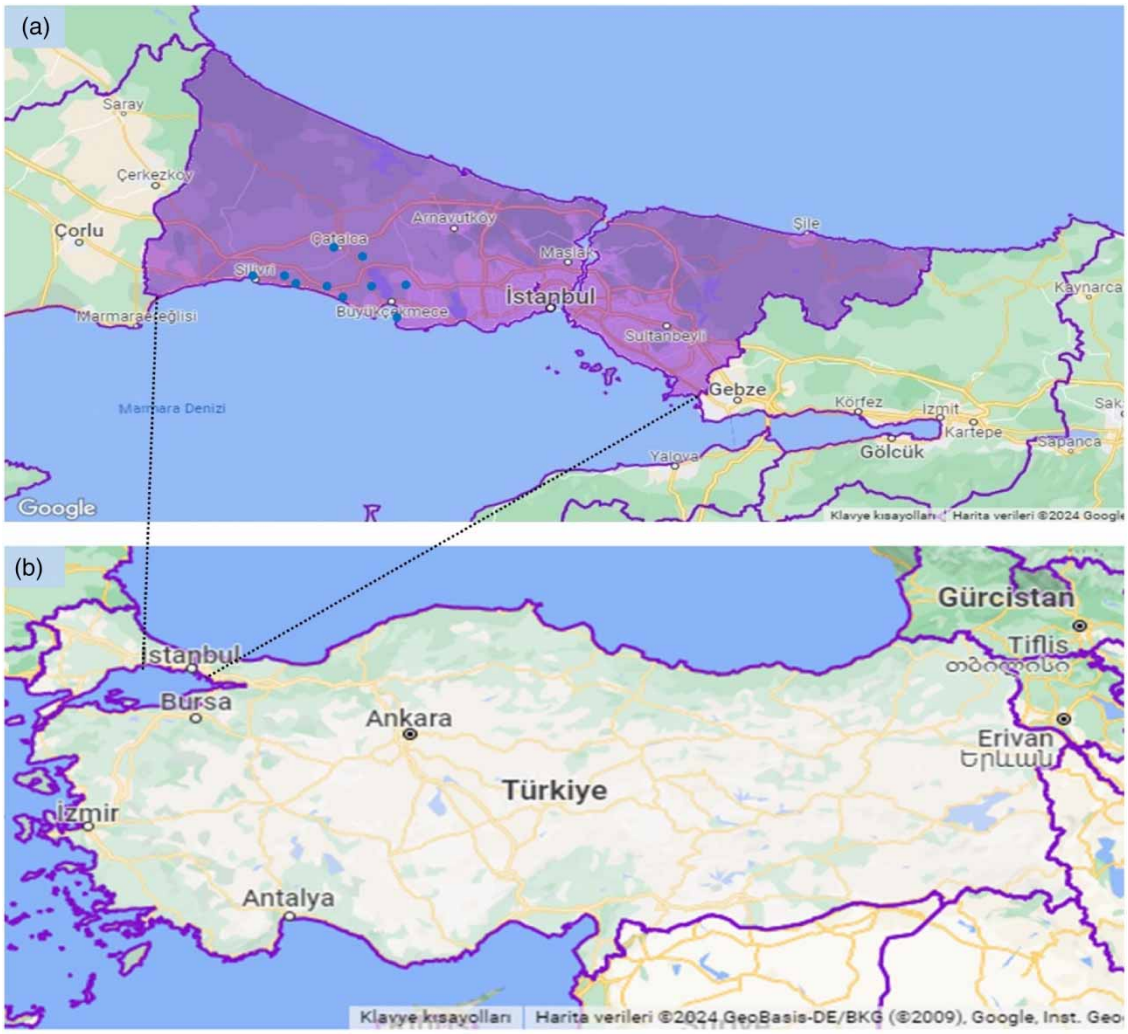

Figure 1 | Location of the sampling points. (a) Istanbul; (b) Türkiye (Source: Google Maps).

rectangular-shaped water storage tanks is approximately 2 m. Well yield is a maximum of 120 m^3/day. Water sampling was carried out from 10 different wells with a depth of 100–250 m depths. The average temperature was 24.4 $\pm$ 2. The pH ranged from 7 to 8. Free chlorine was detected as 0 ppm for all the well water samples. For each sampling point, 500 mL of water sample was collected in a dark-colored sterile glass bottle aseptically, sealed tightly to maintain anoxic conditions, transported to the laboratory at ambient temperature, and processed within 24 h of sample collection.

SRB enrichment culture and enumeration

Four mL of water samples were inoculated into 36 mL of Postgate's B (PB) medium under anaerobic conditions for obtaining SRB enrichment (Postgate 1984). PB medium of the following composition was prepared (per liter deionized water): KH_2PO_4 (0.5 g), NH_4Cl (1.0 g), $CaSO_4$ (1.0 g), $MgSO_4 \times 7\ H_2O$ (2.0 g), yeast extract (1.0 g), $FeSO_4 \times 7\ H_2O$ (0.5 g), sodium lactate (3.5 g), sodium acetate (2.46 g), sodium ascorbate, (0.1 g), sodium thioglycolate (0.1 g) and resazurin (0.001 g). The pH was adjusted to 7.2. The medium was heated to boiling point and purged with high-purity N_2 for 15 min, and autoclave-sterilized at 120 °C for 20 min. The sterile medium was cooled under a stream of N_2 gas. Ten mL of a vitamin solution containing biotin (2.0 mg), folic acid (2.0 mg), pyridoxine HCl (10.0 mg), thiamin (5.0 mg), riboflavin (5.0 mg), nicotinic acid (5.0 mg), calcium D-(þ)-pantothenate (5.0 mg), vitamin B12 (0.1 mg), p-aminobenzoic acid (5.0 mg), and lipoic acid (5.0 mg) was added to the sterile medium. The water samples were inoculated into serum bottles (capped with rubber stoppers and crimped with aluminum seals) containing PB medium. All manipulations were done in an anaerobic chamber (System One Glovebox, Innovative

Technology, Amesbury, MA, USA) under a strict and controlled oxygen-free environment. The cultures were incubated for 2 months in the dark at 30 °C. SRB growth was monitored by observing the formation of a black FeS precipitate. SRB counts were determined by the most probable number (MPN) technique using PB medium. Standard MPN evaluation tables and 95% confidence intervals were used. MPN tubes were incubated in the dark at 30 °C for 2 months (The Institute of Petroleum 1995). In each inoculated tube, the growth of sulfate reducers was indicated by the formation of a black FeS precipitate and by turbidity.

DNA extraction

Prior to DNA extraction, the internal method was performed. Three mL of culture sample was placed in a centrifuge tube and centrifuged at 10,000 rpm for 10 min, and then the supernatant was discarded. The pellet was used for DNA extraction, performed by Powersoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The final volume of DNA was ~ 100 μL. The yield and quality of the extracted DNA were evaluated using gel electrophoresis on 1% (w/v) agarose gel stained with GelRed (Biotium, Hayward, CA, USA), visualized on a UV transilluminator and photographed.

Polymerase chain reaction

Bacterial 16S rDNA fragments were amplified by a two-step nested polymerase chain reaction (PCR) using primer sets, 27F/1495R and 341FGC/907R. The sequences of primers are listed in Table 1.

For the first step, PCR universal bacterial primers, 27F and 1495R, were used to amplify a fragment of about 1,400 bp in length. Each PCR reaction mix, with a final volume 25 μL, containe 1 μL genomic DNA (undiluted and diluted), 1.0 μL of each primer, 15.75 μL of Molecular Biology Grade Water (HiMedia, India), 5.0 μL of 5× PCR Dye Master Mix II (GeneMark, Taichung, Taiwan), and 1.25 μL dimethyl sulfoxide (DMSO) (Biomatik, Canada). Amplification was performed in a T100 thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) under the following conditions: initial denaturation for 5 min at 95%, followed by 25 cycles: denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min, followed by a final extension at 72 °C for 5 min. After the first step, PCR products were analyzed by electrophoresis on a 1.5% agarose gel.

For the second-step PCR, 341F-GC and 907R primers were used to amplify a fragment of about 500 bp in length. The first PCR product was used as the template for the second PCR. Each PCR reaction mix, with a final volume of 25 μL, contained 1 μL DNA template (undiluted and diluted), 0.76 μL of each primer, 16.23 μL of Molecular Biology Grade Water (HiMedia, India), 5.0 μL of 5X PCR Dye Master Mix II (GeneMark, Taichung, Taiwan), and 1.25 μL DMSO (Biomatik, Canada). PCR amplification was performed on the same thermal cycler as mentioned earlier. This PCR was carried out under a touchdown protocol consisting of 5 min at 94 °C, followed by 10 cycles of 1 min at 94 °C, 1 min at 65 °C to 55 °C with a touchdown decrease of −1.0 °C cycle^{-1}, and 3 min at 72 °C, followed by 20 cycles of 1 min at 94 °C, 1 min at 55 °C, and 3 min at 72 °C, and was concluded with a final extension of 5 min at 72 °C. After the second step, PCR products were analyzed by electrophoresis on a 1.5% agarose gel.

Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis (DGGE) analysis was performed using the Dcode Universal Mutation System (Bio-Rad Laboratories Inc., Hercules, CA, USA). The PCR product of the second step was applied directly onto 1-mm-thick, 6% polyacrylamide (37.5: 1 acrylamide/bis-acrylamide) gel with a denaturing gradient ranging from 20 to 80% (w/v) (100% (w/v) denaturing solution containing 7 M urea and 40% (v/v) deionized formamide. Electrophoresis was run for 14 h at 80 V and

Table 1 | Primers used in this study

Primer	Sequence	Reference
27F	5′-AGA GTT TGA TCC TGG CTC AG-3′	Lane (1991)
1495R	5′-CTA CGG CTA CCT TGT TAC GA-3′	Lane (1991)
341F	5′-CCT ACG GGA GGC AGC AG-3′	Muyzer *et al.* (1993)
341F-GC	40-base GC clamp connected to the 5′ end of 314F	Muyzer *et al.* (1993)
907R	5′-CCG TCA ATT CMT TTG AGT TT-3′	Muyzer *et al.* (1995)

60 °C in 1X TAE (Tris-Acetate-EDTA) buffer. After electrophoresis, the gel was stained with SYBR Gold (Invitrogen, Carlsbad, CA, USA) for 20 min and photographed under UV transilluminator.

The DNA bands of interest were excised from the DGGE gel under UV transilluminator using sterile razor blades. The pieces of gel were placed in 40 μL of 1× Tris buffer (pH 8) and stored for 2 days at 4 °C to allow DNA diffusion. Re-amplification of the eluted DNA was performed in a 25-μL reaction volume containing 1.0 μL of template, 0.76 μL of primer 341F, 0.76 μL of primer 907R, 5.0 μL of 5× PCR Dye Master Mix II (GeneMark, Taichung, Taiwan) 16.23 μL of Molecular Biology Grade Water (HiMedia, India), and 1.25 μL DMSO (Biomatik, Canada). The PCR was run with an initial 2 min denaturation at 94 °C, 30 cycles of 60 s at 94 °C, 120 s at 55 °C, 120 s at 72 °C, and a final 5 min extension at 72 °C. After re-amplification, PCR products were verified by electrophoresis on a 1.5% agarose gel.

The resulting PCR products were sent to a commercial company (Eurofins Genomics, Constance, Germany) for purification and sequencing.

Sequence analysis

A consensus sequence was compiled for each DNA fragment obtained from both strands and consensus sequences were compared to published sequences deposited in GenBank using NCBI Nucleotide BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Benson *et al.* 2005). Multiple sequence alignments were performed using ClustalX (Thompson *et al.* 1997). Sequence data were processed with the GeneDoc sequence editor (Nicholas *et al.* 1997).

RESULTS AND DISCUSSION

Enrichment cultures

Sulfate-reducing enrichment cultures were prepared to investigate the presence of SRB and anaerobic bacteria that coexist with SRB in the water wells. The cultures were incubated at 30 °C due to mesophilic conditions of the water wells. Among the 10 different well water samples, blackening of PB medium was observed in only one (10%) enrichment culture (Figure 2). The number of SRB in the well water was low (<10 cells/mL). There is not always a correlation between the bacterial cell number and the activity. For this reason, this result may indicate active SRB in the culture environment despite being in low numbers. Gram-negative bacteria with different morphologies were observed in this enrichment culture. Subsequently, the anaerobic bacterial community in this enrichment culture was investigated by PCR-DGGE strategy.

DGGE and sequence analysis

A total of 10 bands were excised from the gel and all bands yielded a high-quality sequence (Figure 3). A comparative analysis of the sequences showed high sequence similarities, with members belonging to the *Firmicutes* and *Proteobacteria*.

Figure 2 | Positive enrichment culture of SRB. Blackening of PB medium is a typical sign of the growth of SRB.

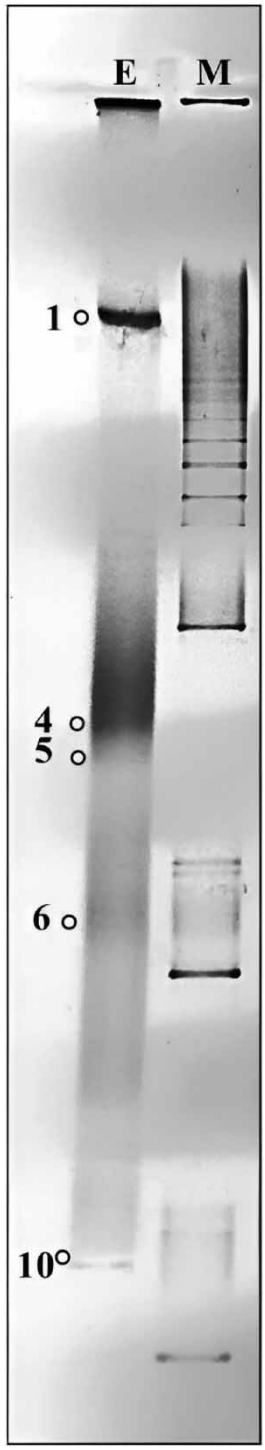

Figure 3 | DGGE profiles of 16S rRNA gene fragments amplified from the positive enrichment culture of SRB. E, enrichment and M, marker.

The sequences from DGGE bands 6 and 10 were identified (98.63 and 98.80% similarities, respectively) as *Desulfolutivibrio sulfodismutans* strain DSM 3696 (MN596860), a mesophilic sulfate-reducing bacterium isolated from freshwater mud (Thiel *et al.* 2020). *D. sulfodismutans* was known as *Desulfovibrio sulfodismutans* until Thiel *et al.* (2020) proposed the reclassification of *Desulfovibrio sulfodismutans* to *Desulfolutivibrio sulfodismutans*. The sequence of *Desulfolutivibrio sulfodismutans* was deposited in the GenBank database under the accession number OR647565.

The type strain of *D. sulfodismutans* DSM 3696 has been known to carry out a unique metabolic pathway enabling disproportionation of sulfite or thiosulfate to sulfide and sulfate. *D. sulfodismutans* could also carry out dissimilatory sulfate reduction by using lactate, ethanol, propanol, and butanol like typical sulfate reducers. However, this species is unable to utilize pyruvate as an electron donor and it grows very slowly when hydrogen is the only source (Bak & Pfennig 1987). Furthermore, although sulfate reducers are defined as obligate anaerobes, it was demonstrated that *D. sulfodismutans* is capable of microaerobic respiration (Dilling & Cypionka 1990). This type of respiration enables the sulfate-reducing bacterial species to grow at the oxic–anoxic interfaces of aquatic ecosystems such as marine sediments and oligotrophic freshwater lakes (Cypionka 2000). In this context, the detection of *D. sulfodismutans* in water samples flowing from groundwater to a pumping well in the present study proves that this species maintains its metabolic capacity throughout the well water system consisting of anoxic and oxic zones. It was also reported that *D. sulfodismutans* was able to reduce iron (III) and uranium (VI) (Lovley *et al.* 1993). Due to this metabolic capability, *D. sulfodismutans* is a potential candidate to be used for the remediation of heavy metal pollution such as the destruction of the ecosystem by mine wastes (Ayangbenro *et al.* 2018). On the other hand, the presence of *D. sulfodismutans* in well water may lead to severe issues such as corrosion in the well water system. As a matter of fact, it is known that hydrogen sulfide production and FeS formation due to SRB activity lead to the biocorrosion of the metal (Enning & Garrelfs 2014). Indeed, due to metal biocorrosion caused by SRB, a significant reduction in the operation life of the water well installations was reported previously (Calbo *et al.* 2018). However, the presence of *D. sulfodismutans* in well water has not been reported in the literature so far.

The presence of SRB, predominantly *Desulfovibrio* spp., in the large intestines of humans, has long been reported (Kushkevych *et al.* 2021). Despite being commonly known as nonpathogenic, different members of *Desulfovibrio* genus were reported to be associated with bacteremia (*D. desulfuricans* & *D. fairfieldensis*), incidents of an abdominal abscess (*D. vulgaris*), brain abscess and liver abscess (*D. desulfuricans*), and infections such as periodontitis (*Desulfovibrio* sp.), and IBD including ulcerative colitis and Crohn's disease (*Desulfovibrio* sp.) (Lozniewski *et al.* 1999; Langendijk *et al.* 2000; Goldstein *et al.* 2003; Koyano *et al.* 2015; Kushkevych *et al.* 2019; Kushkevych *et al.* 2020). Moreover, many studies have also reported a positive relationship between *Desulfovibrio* spp. and various human diseases such as Parkinson's disease, autism, obesity, and cancer (Singh *et al.* 2023). However, there is no reported information related to the correlation between the presence of *D. sulfodismutans* and human health.

The sequences of bands 1, 4, and 5 were related (with 99.15, 99.49, and 99.49 sequence similarities, respectively) to that of *Anaerosinus* sp. Jh2 (KX388181), an iron (III)-reducing strain that was detected in coastal riverine sediment (Zheng *et al.* 2017). The sequence of *Anaerosinus* sp. was deposited in the GenBank database under the accession number OR647564.

The genus *Anaerosinus* consists of obligately anaerobic, mesophilic, and chemo-organotrophic bacteria (Strömpl & Hippe 2015). Until now, only one species, *Anaerosinus glycerini*, was classified in this genus. *A. glycerini* was detected by DGGE analysis along with sulfidogenic bacteria, *Clostridium* genus, in the enrichment of groundwater contaminated by chlorinated ethene, a widely used industrial solvent. In that study, it was also reported that this enrichment showed dechlorinating activity and members of *Clostridium* genus were responsible for degrading chlorinated ethene (Arpita *et al.* 2013). On the contrary, the extracellular electron transfer capability *of Anaerosinus* sp. Jh2 in iron cycling was previously reported which makes this isolate a potential candidate for heavy metal bioremediation by reducing the toxicity of heavy metals in the environment (Zheng *et al.* 2017). In addition, the isolation of bacteria belonging to the genus *Anaerosinus* from the sub-surface horizons of a uranium deposit is important in terms of indicating the bioremediation potential of this genus (Babich *et al.* 2021). On the other hand, members of the *Anaerosinus* genus are found also in the human gut microbiome (Lin *et al.* 2018; Oluwagbemigun *et al.* 2019) and skin microbiome (Procopio *et al.* 2021). In this context, it is not surprising that *Anaerosinus* was detected in influent from the municipal wastewater treatment plant where domestic sewage is treated to control pathogenic risks. Although *Anaerosinus* genus are not defined as pathogens, their occurrence in the human sewage microbiome may indicate the presence of potential pathogens that may be co-existing (Cai *et al.* 2014). In other words, the presence of *Anaerosinus* sp. may be used as a potential indicator for pathogens. For example, it has been shown that the gut microbiome of children infected with rotavirus included higher levels of *A. glycerini* than those of healthy children (Sohail *et al.* 2021). It has also been reported that *Anaerosinus*, which is abundant in the gut microbiome, may affect the development of ulcerative colitis (Sahu *et al.* 2021), and colorectal cancer (Qingbo *et al.* 2024).

The genus *Anaerosinus* and *Desulfovibrio* survive in the same natural environments as well. For instance, they were stated as members of the microbial community of sub-surface horizons of a uranium deposit (Babich *et al.* 2021). In that study, while *Anaerosinus* was detected in the culture of aerobic organotrophic bacteria, *Desulfovibrio* was found in the medium for SRB.

The coexistence of *Anaerosinus* and *Desulfovibrio* genera in the laboratory-scale environment has also been previously reported. They were both detected in the sulfidogenic upstream anaerobic sludge blanket reactor inoculated with methanogenic granular sludge after 110 days of operation (Mora *et al.* 2020).

In the literature, there are limited studies about the investigation of SRB in groundwater and groundwater associated environments, and in these studies, analyses were performed either by using culture-independent molecular technique or the cultivation method (Wargin *et al.* 2007; Yang *et al.* 2015; An *et al.* 2016). However, no study was found in which SRB was investigated in these environments by culture-dependent molecular methodology. In the present study, the presence of SRB in well water was investigated for the first time by cultivation based molecular fingerprinting technique in Türkiye. In this context, the bacterial community of the SRB enrichment was analyzed by a two-step nested PCR-DGGE because this approach makes it possible to detect even low numbers of SRB in complex microbial communities from natural environments (Dar *et al.* 2005).

CONCLUSIONS

The following conclusions can be drawn from the obtained data in this study:

- Even in low numbers, SRB can be found in a well water environment.
- Anaerobic sulfate-reducing *D. sulfodismutans* and anaerobic *Anaerosinus* sp. were isolated from the well water for the first time.
- *Anaerosinus* sp. may coexist with SRB.
- The presence of both SRB and *Anaerosinus* sp. in well water may be used as an indicator of water quality and may also be considered as potential microbial risk factors for public health.
- *Anaerosinus* genus, a member of the human gut microbiota, may be used as an alternative anaerobic indicator for fecal contamination of water quality. Further research is needed to confirm this suggestion.
- It is noteworthy that the detection of bacteria that can live in the same environmental conditions as the bacteria accepted as indicator microorganisms, using molecular analyzes following enrichment culture techniques, can bring new perspectives to evaluate microbial contamination in aquatic environments and to determine the possible origin and presence of alternative microbial indicators.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICTS OF INTEREST

The authors declare there is no conflict.

REFERENCES

Akgül, E., Arslan, H. N. & Terzi, Ö. 2023 A literature review of waterborne outbreaks in the last decade in Türkiye. *Journal of Experimental and Clinical Medicine* **40** (2), 401–409. doi: 10.52142/omujecm.40.4.25.

An, X., Baker, P., Li, H., Su, J., Yu, C. & Cai, C. 2016 The patterns of bacterial community and relationships between sulfate-reducing bacteria and hydrochemistry in sulfate-polluted groundwater of Baogang rare earth tailings. *Environmental Science and Pollution Research* **23** (1), 21766–21779. doi: 10.1007/s11356-016-7381-y.

Arpita, B., Ishimura, K., Nakamura, K. & Takamizawa, K. 2013 Microbial dynamics in the process of restoration of groundwater contaminated by chlorinated ethene in the presence of *Escherichia coli*. *Journal of Material Cycles and Waste Management* **15**, 335–341. doi:10.1007/s10163-013-0124-y.

Ayangbenro, A. S., Olanrewaju, O. S. & Babalola, O. O. 2018 Sulfate-reducing bacteria as an effective tool for sustainable acid mine bioremediation. *Frontiers in Microbiology* **9**, 1986. doi:10.3389/fmicb.2018.01986.

Babich, T. L., Semenova, E. M., Sokolova, D. S., Tourova, T. P., Bidzhieva, S. K., Loiko, N. G., Avdonin, G. I., Lutsenko, N. I. & Nazina, T. N. 2021 Phylogenetic diversity and potential activity of bacteria and fungi in the deep subsurface horizons of an uranium deposit. *Microbiology* **90**, 607–620. doi:10.1134/S0026261721040032.

Bak, F. & Pfennig, N. 1987 Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionation of inorganic sulfur compounds. *Archives of Microbiology* **147**, 184–189. doi:10.1007/BF00415282.

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. & Wheeler, D. L. 2005 Genbank. *Nucleic Acids Research* **33**, 34–38. doi:10.1093/nar/gki063.

Cabral, J. P. S. 2010 Water microbiology, bacterial pathogens and water. *International Journal of Environmental Research and Public Health* **7** (10), 3657–3703. doi:10.3390/ijerph7103657.

Cai, L., Ju, F. & Zhang, T. 2014 Tracking human sewage microbiome in a municipal wastewater treatment plant. *Applied Microbiology and Biotechnology* **98**, 3317–3326. doi:10.1007/s00253-013-5402-z.

Calbo, V., Furlong, O. J. & Julián, S. V. 2018 Metal biocorrosion of a water well: A case study. *KnE Engineering* **3** (2), 301–310. doi:10.18502/keg.v3i1.1435.

Campbell, L. L., Kasprzycki, M. A. & Postgate, J. R. 1966 *Desulfovibrio Africans* sp. n., a new dissimilatory sulfate-reducing bacterium. *Journal of Bacteriology* **92** (4), 1122–1127. doi:10.1128/jb.92.4.1122-1127.1966.

Chou, S.C.H. J. 2003 *Hydrogen Sulfide: Human Health Aspects, Concise International Chemical Assessment, Document 53*, World Health Organization, Geneva.

Cullimore, R. 1999 *Microbiology of Well Biofouling*, 1st edn. CRC Press, Boca Raton.

Cypionka, H. 2000 Oxygen respiration by *Desulfovibrio* species. *Annual Review of Microbiology* **54**, 827–848. doi:10.1146/annurev.micro.54.1.827.

Dar, S. A., Kuenen, J. G. & Muyzer, G. 2005 Nested PCR-denaturing gradient gel electrophoresis approach to determine the diversity of sulfate-reducing bacteria in complex microbial communities. *Applied and Environmental Microbiology* **71** (5), 2325–2330. doi:10.1128/AEM.71.5.2325-2330.2005.

Dilling, W. & Cypionka, H. 1990 Aerobic respiration in sulfate-reducing bacteria. *FEMS Microbiology Letters* **71**, 123–128. doi:10.1111/j.1574-6968.1990.tb03809.x.

Enning, D. & Garrelfs, J. 2014 Corrosion of iron by sulfate-reducing bacteria: New views of an old problem. *Applied and Environmental Microbiology* **80** (4), 1226–1236. doi:10.1128/AEM.02848-13.

Goldstein, E. J., Citron, D. M., Peraino, V. A. & Cross, S. A. 2003 *Desulfovibrio desulfuricans* bacteremia and review of human *Desulfovibrio* infections. *Journal of Clinical Microbiology* **41** (6), 2752–2754. doi:10.1128/JCM.41.6.2752-2754.2003.

Griebler, C. & Lueders, T. 2009 Microbial diversity in groundwater ecosystems. *Freshwater Biology* **54**, 649–677. doi:10.1111/j.1365-2427.2008.02013.x.

Gunes, G. 2023 Evaluation of groundwater quality with microbiological and physicochemical parameters in Bartın, Turkey. *Environmental Monitoring and Assessment* **195** (7), 828. doi:10.1007/s10661-023-11323-5.

Keesari, T., Ramakumar, K. L., Prasad, M. B. K., Chidambaramet, S., Perumal, P., Prakash, D. & Nawani, N. 2015 Microbial evaluation of groundwater and its implications on redox condition of a multi-layer sedimentary aquifer system. *Environmental Processes* **2**, 331–346. doi:10.1007/s40710-015-0067-5.

Koyano, S., Tatsuno, K., Okazaki, M., Ohkusu, K., Sasaki, T., Saito, R., Okugawa, S. & Moriya, K. 2015 A case of liver abscess with *Desulfovibrio desulfuricans* bacteremia. *Case Reports in Infectious Diseases* **2015**, 354168. doi:10.1155/2015/354168.

Krauss, S. & Griebler, C. 2011 Pathogenic microorganisms and viruses in groundwater. *Acatech Materialien* **6**, 1–69.

Kushkevych, I., Leščanová, O., Dordević, D., Jančíková, S., Hošek, J., Vítězová, M., Buňková, L. & Drago, L. 2019 The sulfate-reducing microbial communities and meta-analysis of their occurrence during diseases of small–large intestine axis. *Journal of Clinical Medicine* **8** (10), 1656. doi:10.3390/jcm8101656.

Kushkevych, I., Castro Sangrador, J., Dordević, D., Rozehnalová, M., Černý, M., Fafula, R., Vítězová, M. & Rittmann, S. K. R. 2020 Evaluation of physiological parameters of intestinal sulfate-reducing bacteria isolated from patients suffering from IBD and healthy people. *Journal of Clinical Medicine* **9** (6), 1920. doi: 10.3390/jcm9061920.

Kushkevych, I., Hýžová, B., Víťezová, M. & Rittmann, S. K.-M. R. 2021 Microscopic methods for identification of sulfate-reducing bacteria from various habitats. *International Journal of Molecular Sciences* **22** (8), 4007. doi:10.3390/ijms22084007.

Lane, D. J., 1991 16S/23S rRNA sequencing. In: *Nucleic Acid Techniques in Bacterial Systematics* (Stackebrandt, E. & Goodfellow, M., eds). John Wiley and Sons, Chichester, pp. 115–175.

Langendijk, P. S., Hanssen, J. T. & Van der Hoeven, J. S. 2000 Sulfate-reducing bacteria in association with human periodontitis. *Journal of Clinical Periodontology* **27** (12), 943–950. doi:10.1034/j.1600-051x.2000.027012943.x.

Lin, X. H., Huang, K. H., Chuang, W. H., Luo, J. C., Lin, C. C., Ting, P. H., Young, S. H., Fang, W. L., Hou, M. C. & Lee, F. Y. 2018 The long term effect of metabolic profile and microbiota status in early gastric cancer patients after subtotal gastrectomy. *PLoS One* **13** (11), e0206930. doi:10.1371/journal.pone.0206930.

Lovley, D. R., Roden, E. E., Phillips, E. J. P. & Woodward, J. C. 1993 Enzymatic iron and uranium reduction by sulfate-reducing bacteria. *Marine Geology* **113**, 41–53. doi:10.1016/0025-3227(93)90148-O.

Lozniewski, A., Maurer, P., Schumacher, H., Carlier, J. P. & Mory, F. 1999 First isolation of *Desulfovibrio* species as part of a polymicrobial infection from a brain abscess. *European Journal of Clinical Microbiology & Infectious Diseases* **18** (8), 602–603. doi:10.1007/s100960050357.

Macfarlane, G., Cummings, J., Macfarlane, S., 2007 Sulphate-reducing bacteria and the human large intestine. In: *Sulphate-reducing Bacteria: Environmental and Engineered Systems* (Barton, L. & Hamilton, W., eds). Cambridge University Press, Cambridge, pp. 503–522.

McLellan, S. L. & Eren, A. M. 2014 Discovering new indicators of fecal pollution. *Trends in Microbiology* **22** (12), 697–706. doi:10.1016/j.tim.2014.08.002.

Miao, Z., Brusseau, M. L., Carroll, K. C., Carreón-Diazconti, C. & Johnso, B. 2012 Sulfate reduction in groundwater: Characterization and applications for remediation. *Environmental Geochemistry and Health* **34** (4), 539–550. doi:10.1007/s10653-011-9423-1.

Mora, M., Lafuente, J. & Gabriel, D. 2020 Influence of crude glycerol load and pH shocks on the granulation and microbial diversity of a sulfidogenic upflow anaerobic sludge blanket reactor. *Process Safety and Environmental Protection* **133**, 159–168. doi:10.1016/j.psep.2019.11.005.

Muyzer, G., de Waal, E. C. & Uitterlinden, A. G. 1993 Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* **59**, 695–700. doi:10.1128/aem.59.3.695-700.1993.

Muyzer, G., Teske, A., Wirsen, C. O. & Jannasch, H. W. 1995 Phylogenetic relationship of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Archives of Microbiology* **164**, 165–172. doi:10.1007/BF02529967.

Nicholas, K. B., Nicholas, H. B. & Deerfield, D. W. 1997 Genedoc: Analysis and visualization of genetic variation. *EMBnet News* **4**, 1–4.

Oluwagbemigun, K., Foerster, J., Watkins, C., Fouhy, F., Stanton, C., Bergmann, M. M., Boeing, H. & Nothlings, U. 2019 Dietary patterns are associated with serum metabolite patterns and their associa-tion is influenced by gut bacteria among older German adults. *The Journal of Nutrition* **150** (1), 149–158. doi:10.1093/jn/nxz194.

Özler, H. M. & Aydın, A. 2008 Hydrochemical and microbiological quality of groundwater in West Thrace Region of Turkey. *Environmental Geology* **54**, 355–363. doi:10.1007/s00254-007-0822-7.

Postgate, J. R. 1984 *The Sulphate Reducing Bacteria*. Cambridge University Press, Cambridge.

Procopio, N., Lovisolo, F., Sguazzi, G., Ghignone, S., Voyron, S., Migliario, M., Renò, F., Sellitto, F., D'Angiolella, G., Tozzo, P., Caenazzo, L. & Gino, S. 2021 'Touch microbiome' as a potential tool for forensic investigation: A pilot study. *Journal of Forensic and Legal Medicine* **82**, 102223. doi:10.1016/j.jflm.2021.102223.

Qingbo, L., Jing, Z., Zhanbo, Q., Jian, C., Yifei, S., Wu Yinhang, W. & Shuwen, H. 2024 Identification of enterotype and its predictive value for patients with colorectal cancer. *Gut Pathogens* **16**, 12. doi:10.1186/s13099-024-00606-y.

Quevauviller, P., 2007 General introduction: The need to protect groundwater. In: *Groundwater Science and Policy: An International Overview,* (Quevauviller, P., ed.). The Royal Society of Chemistry, London, pp. 3–18.

Sahu, P., Kedia, S., Vuyyuru, S. K., Bajaj, A., Markandey, M., Singh, N., Singh, M., Kante, B., Kumar, P., Ranjan, M. & Sahni, P. 2021 Randomised clinical trial: Exclusive enteral nutrition versus standard of care for acute severe ulcerative colitis. *Alimentary Pharmacology & Therapeutics* **53** (5), 568–576. doi: 10.1111/apt.16249.

Sezen, F., Aval, E., Ağkurt, T., Yilmaz, Ş., Temel, F., Güleşen, R., Korukluoğlu, G., Sucakli, M. B., Torunoğlu, M. A. & Zhu, B. P. 2015 A large multi-pathogen gastroenteritis outbreak caused by drinking contaminated water from antique neighbourhood fountains, Erzurum city, Türkiye, December 2012. *Epidemiology and Infection* **143** (4), 704–710.

Singh, S. B., Carroll-Portillo, A. & Lin, H. C. 2023 *Desulfovibrio* in the gut: The enemy within? *Microorganisms* **11**, 1772. doi: 10.3390/microorganisms11071772.

Sohail, M. U., Al Khatib, H. A., Al Thani, A. A., Ansari, K. A., Yassine, H. M. & Al-Asmakh, M. 2021 Microbiome profiling of rotavirus infected children suffering from acute gastroenteritis. *Gut Pathogens* **13**, 21. doi:10.1186/s13099-021-00411-x.

Stelma Jr., G. N. 2018 Use of bacterial spores in monitoring water quality and treatment. *Journal of Water & Health* **16** (4), 491–500. doi:10.2166/wh.2018.013.

Strömpl, C. & Hippe, H., 2015 Anaerosinus. In: *Bergey's Manual of Systematics in Archaea and Bacteria* (Whitman, W. B., ed.). John Wiley and Sons, pp. 1–3. doi:10.1002/9781118960608.gbm00692.

The Institute of Petroleum 1995 Determination of the viable microbial content of fuels and fuel components boiling below 390 °C – Filtration and Culture Method, IP Method Number 385/95.

Thiel, J., Spring, S., Tindall, B. J., Spröer, C., Bunk, B., Koeksoy, E., Ngugi, D. K., Schink, B. & Pester, M. 2020 Desulfolutivibrio sulfoxidireducens gen. nov. , sp. nov., isolated from a pyrite-forming enrichment culture and reclassification of *Desulfovibrio sulfodismutans* as *Desulfolutivibrio sulfodismutans* comb. nov. *Systematic and Applied Microbiology* **43** (5), 126105. doi:10.1016/j.syapm.2020.126105.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997 The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25** (24), 4876–4882. doi:10.1093/nar/25.24.4876.

Vörösmarty, C. J., Lévêque, C., Revenga, C., Bos, R., Caudill, C., Chilton, J., Douglas, E. M., Meybeck, M., Prager, D., Balvanera, P., Barker, S., Maas, M., Nilsson, C., Oki, T., Reidy, C. A., 2005 Fresh water. In: *Ecosystems and Human Well-Being: Current State and Trends* (Hassan, R., Scholes, R. & Ash, N., eds). Island Press, Washington, DC, pp. 165–207.

Wargin, A., Olańczuk-Neyman, K. & Skucha, M. 2007 Sulfate-reducing bacteria, their properties and methods of elimination from groundwater. *Polish Journal of Environmental Studies* **16** (4), 639–644.

WHO 2017 *Guidelines for Drinking-Water Quality*. Available from: https://www.who.int/publications/i/item/9789241549950 (accessed 14 April 2023).

Yang, X., Huang, T. L., Guo, L., Xia, C., Zhang, H. H. & Zhou, S. L. 2015 Abundance and diversity of sulfate-reducing bacteria in the sediment of the Zhou Cun drinking water reservoir in eastern China. *Genetics and Molecular Research* **14** (2), 5830–5844. doi:10.4238/2015.May.29.15.

Yolcubal, I., Gündüz, Ö. C. & Sönmez, F. 2016 Assessment of impact of environmental pollution on groundwater and surface water qualities in a heavily industrialized district of Kocaeli (Dilovası), Turkey. *Environmental Earth Sciences* **75**, 1–23. doi: 10.1007/s12665-015-4986-2.

Zheng, S., Wang, B., Li, Y., Liu, F. & Wang, O. 2017 Electrochemically active iron (III)-reducing bacteria in coastal riverine sediments. *Journal of Basic Microbiology* **57** (12), 1045–1054. doi:10.1002/jobm.201700322.

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