Growth of the *Desulfomicrobium* sp. strains, their sulfate- and lactate usage, production of sulfide and acetate by the strains isolated from the human large intestine

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Abstract

The aim and background of the study were to investigate bacterial growth of the *Desulfomicrobium* sp. strains from human large intestine, and to study the processes usage of sulfate- and lactate, production of sulfide and acetate by the obtained bacteria as well as to carry out cluster and correlation analysis of these processes. Microbiology methods of the study for bacterial strains cultivation and photometric methods for determination of bacterial biomass and hydrogen sulfide concentration were used, sulfate ions concentration was determined by turbidymetric method, lactate concentration was carried out by lactate dehydrogenase. Acetate ions accumulation by the strains was determined by titration. Using the experimental data, the methods of statistical analysis have been also used. The highest biomass (3.34 g/l) has been accumulated by the *Desulfomicrobium* sp. strain Rod-9 on the sixth day of cultivation, in comparison to the other strains. Studied strains of the *Desulfomicrobium* sp. actively reduced the sulfate ions and produced hydrogen sulfide. The highest concentration of hydrogen sulfide (3.14 mM) was produced by the SRB Rod-9 strain on the eighth day of cultivation; while, the strain used about 98% of the sulfate compared with its initial concentration. The strains used lactate fully on the sixth day of cultivation and produced acetate. Clustering of the parameters of the bacterial growth, the sulfate and lactate usage as well as hydrogen sulfide and acetate production by the various *Desulfomicrobium* sp. strains has been carried out. The correlation coefficients between the parameters have been determined.

Keywords: Sulfate-reducing bacteria, *Desulfomicrobium*, intestinal microbiocenosis, sulfates, sulfide, inflammatory bowel diseases

Introduction

The *Desulfomicrobium* genus belongs to sulfate-reducing bacteria (SRB) [3]. The species of the genus is the short, straight rod-or ellipsoidal-shaped cells, with rounded ends, either singularly or in pairs. Their size is 0.5–0.9×1.3–2.9 μm. The bacteria reduce sulfate to hydrogen sulfide [3]. They oxidize organic compounds incompletely, with the formation of acetate as an end product [15]. The bacteria can grow using sulfate as an electron acceptor during sulfate anaerobic respiration [2]. The *Desulfomicrobium* bacteria use various nutrients in the human intestine. The bacteria use simple organic compounds (lactate, pyruvate, ethanol, formate and hydrogen) that act as electron donors during sulfate respiration [3,12].

In recent years, the bacteria are often isolated from the oral cavity and intestines of both humans and animals. Van der Hoeven et al., and Willis et al., isolated SRB species from human oral cavity [25,30]. The SRB were also found in subjects with good oral hygiene and subjects who refrained from oral hygiene procedures [19,20,25,29,30]. These bacteria detected on buccal mucosa, vestibulum, palate, tongue, and with a higher frequency in saliva and in plaque [19,20,30]. Therefore, SRB are commensals belonging to the normal oral microbiota. They grow developing biofilms in sites with little access of oxygen [20,22].

The acute inflammation of the intestinal mucosa, increasing its permeability, bloody diarrhea, abdominal pain, frequent need for a bowel movement, weight loss, arthritis, discomfort and general malaise often found in a lot of various people. During these diseases an increased number of SRB in the gut was often detected [5,8,16,21,23,24].

The presence of sulfate in the intestine stimulates the growth of SRB that reduce sulfate to H₂S. Hydrogen sulfide affects the lining of the intestines, it is toxic for epithelial cells, specifically inhibits the growth colonocytes [2], phagocytosis, causes death of other intestinal bacteria, induces hyperproliferation and metabolic abnormalities of epithelial cells [16,23,24,31]. It was found that the concentration of this metabolite and the number of SRB significantly increased during inflammatory bowel processes [21,23,24]. The use of antibiotics (metronidazole or ciprofloxacin) leads to decrease the SRB number and consequently, to the formation of hydrogen sulfide. Inhibition of growth of intestinal SRB causes a decrease in hydrogen sulfide concentration in the human intestinal lumen [5,16].

In patients with ulcerative colitis, SRB were found during the peak of the disease accounting for 95% of the total quantity of intestinal microbiota and then during the recovery stage at a reduced quantity of 55% of the total microbiota [8,9,10]. A viable SRB number is three orders of magnitude larger

by the active phase of the disease than in remission [8,23]. Obviously, it suggests an important role of these bacteria in the development of colitis.

A detailed study of the intestinal SRB along with their metabolism and patterns of forming hydrogen sulfide in the gut is needed to research all the possible mechanisms of its toxic effect on epithelial cells. This will help to develop new approaches to the treatment of ulcerative colitis and other intestinal diseases.

The aim of the research was to study bacterial growth of the *Desulfomicrobium* sp. strains, their sulfate- and lactate usage, production of sulfide and acetate and to carry out cluster and correlation analysis of these processes.

Material and methods

The objects of the study were sulfate-reducing bacteria of the *Desulfomicrobium* sp. strains (SRB Rod-1, SRB Rod-2, SRB Rod-3, SRB Rod-4, SRB Rod-5, SRB Rod-6, SRB Rod-7, SRB Rod-8, SRB Rod-9, SRB Rod-10) isolated from the human large intestine [17]. The strains are kept in the collection of microorganisms at the Biotechnology Laboratory of the Pharmacy Faculty at the University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic).

Medium and cultivation

The bacterial strains were grown in nutrition modified Kravtsov-Sorokin's liquid medium [13] of such composition (g/l): Na_2SO_4 –0.5; KH_2PO_4 –0.3; K_2HPO_4 –0.5; $(\text{NH}_4)_2\text{SO}_4$ –0.2; NH_4Cl –1.0; $\text{CaCl}_2 \times 6\text{H}_2\text{O}$ –0.06; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ –0.1; $\text{C}_3\text{H}_5\text{O}_3\text{Na}$ –2.0; yeast extract–1.0; $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ –0.004; sodium citrate $\times 2\text{H}_2\text{O}$ –0.3. Before bacteria seeding in the medium, 0.05 ml/l of sterile solution of $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$ (1%) was added. To provide pH 7.2 of medium sterile 10N solution of NaOH (0.9 ml/l) was used. The medium was heated in boiling water for 30 min in order to obtain an oxygen-free medium, and cooled to +25°C temperature. The bacteria were grown for 10 days at +35°C under anaerobic conditions. The tube was brim-filled with medium and closed by rubber plug to provide anaerobic conditions.

Measuring of bacterial biomass

Biomass accumulation of the various strains of sulfate-reducing bacteria in liquid medium by photometric method (nutritional medium was without Mohr's salt) has been examined [28].

Measuring of sulfate ions concentration

The sulfate ions concentration in the medium was determined by turbidimetric method after it had been precipitated by barium chloride. To stabilize the suspension, glycerol was used [14].

Measuring of hydrogen sulfide concentration

Hydrogen sulfide concentration in the culture medium was determined by photometric method based on reaction of

sulfide and *p*-aminodimethylaniline with methylene blue formation [27]. The concentration of hydrogen sulfide by calibration curve was established.

Measuring of lactate concentration

Determination of lactate concentration was carried out through a dehydrogenation reaction of lactate by lactate dehydrogenase in the presence of NAD, with formation of pyruvate and NADH [26]. For determination of lactate content the following reagents were used: hydrazine-glycine buffer pH 9.0 (glycine–0.1 M solution containing 0.1 M hydrazine); NAD^+ –0.03 M solution, pH 6.0; lactate dehydrogenase solution (protein content was about 2 mg/ml). The samples of glycine and hydrazine were dissolved in a small amount of distilled water, the pH of the mixture was stabilized by 2 N NaOH solution and it became 9.0, then was diluted in the distilled water to 100 ml. Solutions of NAD^+ and lactate dehydrogenase were kept in ice. The tubes content was thoroughly mixed then were placed inside a thermostat at +25°C for 60 min. After the incubation, samples were cooled and then the optical density of the samples was measured at 340 nm. Taking into account all the dilutions, the quantity of the lactic acid was calculated.

Measuring of acetate ions concentration

Acetate ions accumulation by the bacteria cultures during their growth in the medium was determined by titration [4].

Statistical analysis

Using the experimental data, the basic statistical parameters (M – mean, m – standard error, $M \pm m$) have been calculated. For the estimation of the reliability between the statistical characteristics, Student's *t*-test was used. The difference was reliable when $P > 0.95$ [1]. Statistical processing of the results has been performed using packet Excel, Origin [11], and Statistica programs.

Results and discussion

The results of the research have showed that all the *Desulfomicrobium* sp. strains actively grow using sulfate as an electron acceptor and accumulate hydrogen sulfide in the medium (Figures 1-3). These bacterial strains use lactate as the electron donor; the lactate is actively oxidized by the bacteria incompletely, to acetate (Figures 4 and 5).

The studied strains use sulfate and lactate, producing hydrogen sulfide and acetate with varying intensity. The intense growth of these bacteria depended on the use of sulfate and lactate as well as the accumulation of hydrogen sulphide and acetate. The strains of the *Desulfomicrobium* sp. have accumulated a different biomass during 10 days of cultivation. Different growth rates of various strains of *Desulfomicrobium* sp. in the modified Kravtsov-Sorokin's medium have been studied.

Among all the strains the highest biomass (up to 3.34 gram per liter) was accumulated by the SRB Rod-9 strain on the sixth day of cultivation, in comparison to the other strains;

Figure 1. Growth of the various *Desulfomicrobium* sp. strains in the Kravtsov-Sorokin's medium on first, second, third, fourth, fifth, sixth, eighth and tenth day of cultivation.

Figure 2. Sulfate usage by the various *Desulfomicrobium* sp. strains in the Kravtsov-Sorokin's medium on first, second, third, fourth, fifth, sixth, eighth and tenth day of cultivation.

Figure 3. The production of hydrogen sulfide by the various *Desulfomicrobium* sp. strains in the Kravtsov-Sorokin's medium on first, second, third, fourth, fifth, sixth, eighth and tenth day of cultivation.

Figure 4. Lactate usage by the various *Desulfomicrobium* sp. strains in the Kravtsov-Sorokin's medium on first, second, third, fourth, fifth, sixth, eighth and tenth day of cultivation.

Figure 5. The production of acetate by the various *Desulfomicrobium* sp. strains in the Kravtsov-Sorokin's medium on first, second, third, fourth, fifth, sixth, eighth and tenth day of cultivation.

while the SRB Rod-2 strain accumulated the lowest biomass (up to 2.64 gram per liter) on the eighth day of cultivation. Having used all the sulfate and lactate in the medium, the bacteria stopped growing and the stationary growth phase began (Figures 1 and 2). The limiting factor of the growth of these bacteria was hydrogen sulfide and acetate, produced by the bacteria in the process of dissimilatory sulfate reduction and lactate oxidation respectively.

Studied bacterial strains of the *Desulfomicrobium* sp. actively reduce the sulfate ions and used these ions as an electron acceptor. The intensity and time of sulfate reduction is different in each of the strains. All the studied strains had fully used sulfate on the sixth day of cultivation (Figure 2).

Among the *Desulfomicrobium* sp. strains the highest concentration of hydrogen sulfide (up to 3.14 mM) was produced by the SRB Rod-9 strain on the eighth day of cultivation (Figure 3).

At this time, the strain used about 98% of the sulfate in the medium. The SRB Rod-2 strains produced the lowest concentration of hydrogen sulfide (up to 1.91 mM) on the sixth day of cultivation; they used about 96% of the sulfate in the medium, compared to its initial concentration.

The results of the studies have showed that the presence of lactate and sulfate in the medium stimulates growth of the various studied strains of the sulfate-reducing bacteria *Desulfomicrobium* sp. Lactate is an electron donor during dissimilatory sulfate reduction. All studied strains use lactate fully on the sixth day of cultivation. The bacterial *Desulfomicrobium* sp. strains used all the lactate for the dissimilation of sulfate (Figure 4).

Among the various *Desulfomicrobium* sp. strains the highest concentration of acetate ions (up to 16.24 mM) produced by the SRB Rod-8 strain was on the sixth day of cultivation (Figure 5); at this time the strain used about 98% of lactate in the medium. The bacterial SRB Rod-5 strains produced the lowest concentration of acetate ions; they used about 97% of lactate in the medium, compared to its initial concentration. Thus, studied *Desulfomicrobium* sp. strains of the sulfate-reducing bacteria are able to grow actively and utilize substrate of the intestine including sulfate and lactate. The intensity of their growth will depend on which substrates people eat. It can be the cause of intense sulfate reduction by the SRB, producing hydrogen sulfide and acetate in the human gut if foods rich in sulfates are eaten.

The next stage of the study was to perform cluster analysis of the parameters of the bacterial growth, the sulfate and lactate usage as well as hydrogen sulfide and acetate production by the studied strains (Figures 6-10).

As a result of the clustering of the growth parameters is formed three independent clusters. A dendrogram shows the greatest similarity between strains *Desulfomicrobium* sp. strains Rod-9 with *Desulfomicrobium* sp. strains Rod-4 and *Desulfomicrobium* sp. strains Rod-3 by the tested parameter (Figure 6). The strains *Desulfomicrobium* sp. strains Rod-8 with *Desulfomicrobium* sp. strains Rod-7 as well as *Desulfomicrobium* sp. strains Rod-6 and *Desulfomicrobium* sp. strains Rod-10 are the most varied by tested parameter compared to strains *Desulfomicrobium* sp. strains Rod-1 with *Desulfomicrobium* sp. strains Rod-2 and *Desulfomicrobium* sp. strains Rod-5, and they form two separate isolated clusters.

The parameters of intensity of utilization sulfate by the studied strains are most similar to each other, allowing combining them in the clusters. In this case, there is also the formation of three clusters, which the combining of strains *Desulfomicrobium* sp. strains Rod-7 with *Desulfomicrobium* sp. strains Rod-3 and *Desulfomicrobium* sp. strains Rod-4 with *Desulfomicrobium* sp. strains Rod-9 in one cluster. The strains *Desulfomicrobium* sp. strains Rod-5 with *Desulfomicrobium* sp. strains Rod-1 and *Desulfomicrobium* sp. strains Rod-2 still formed a stable isolated cluster which was observed in the previous series of experiments. The similar isolated cluster is formed by the

Desulfomicrobium sp. strains Rod-8 with *Desulfomicrobium* sp. strains Rod-10 and *Desulfomicrobium* sp. strains Rod-6 (Figure 7).

The results clustering of quantitative formation of hydrogen sulfide have shown form several isolated clusters. In this case, the *Desulfomicrobium* sp. strains Rod-3 with *Desulfomicrobium* sp. strains Rod-5 as well as *Desulfomicrobium* sp. strains Rod-6 with *Desulfomicrobium* sp. strains Rod-1 and *Desulfomicrobium* sp. strains Rod-2 were formed into a stable cluster by tested parameter. The *Desulfomicrobium* sp. strains Rod-7 with *Desulfomicrobium* sp. strains Rod-9 and *Desulfomicrobium* sp. strains Rod-4 as well as *Desulfomicrobium* sp. strains Rod-8 with *Desulfomicrobium* sp. strains Rod-10 were combined

into other cluster. These two clusters were most isolated from each other (**Figure 8**).

The clustering of the parameters of lactate usage by the studied strains has showed that the strains strains *Desulfomicrobium* sp. strains Rod-7 with *Desulfomicrobium* sp. strains Rod-3 and *Desulfomicrobium* sp. strains Rod-4 with *Desulfomicrobium* sp. strains Rod-9 form a cluster cluster by tested parameter. Similar results were observed in the previous series of experiments of utilization sulfate. Moreover, the strains *Desulfomicrobium* sp. strains Rod-5 with *Desulfomicrobium* sp. strains Rod-2 and *Desulfomicrobium* sp. strains Rod-1 were still formed a stable isolated cluster. This cluster was is the most distant to cluster which was formed by the *Desulfomicrobium* sp. strains Rod-8 with *Desulfomicrobium* sp. strains Rod-6 and *Desulfomicrobium* sp. strains Rod-10. In this case, there are formed a clear division into two isolated cluster on the dendrogram (**Figure 9**).

The results of the cluster analysis of the parameters of the acetate production by the strains have shown the formation of separate clusters on the dendrogram (**Figure 10**). Bacterial strains *Desulfomicrobium* sp. strains Rod-7 with *Desulfomicrobium* sp. strains Rod-3 and *Desulfomicrobium* sp. strains Rod-5 as well as *Desulfomicrobium* sp. strains Rod-9 with *Desulfomicrobium* sp. strains Rod-10 and *Desulfomicrobium* sp. strains Rod-8 have formed two stable clusters by tested parameter. The strains *Desulfomicrobium* sp. strains Rod-2 with *Desulfomicrobium* sp. strains Rod-1 were the most varied by tested parameter compared to strains *Desulfomicrobium* sp. strains Rod-4 with *Desulfomicrobium* sp. strains Rod-6, and they form two separate isolated clusters on the dendrogram by this parameters.

Thus, the cluster analysis on the basis of the different

parameters of the bacterial growth, the sulfate and lactate usage and hydrogen sulfide and acetate production by the various *Desulfomicrobium* sp. strains has been carried out. Cluster analysis suggests the selection of compacted and distant groups of objects; it finds «natural» decomposition all clusters objects. It is used when the original data is represented as matrices of proximity or distance between objects or as points in a multidimensional space. The most common type of data in the second group, for which cluster analysis is focused on, is the selection of some distant groups within which objects are similar. The cluster analysis has helped classify a large amount of data to establish relations between different strains of the *Desulfomicrobium* sp. genus of sulfate-reducing bacteria of human intestine.

Table 1. Correlation coefficients between dissimilatory sulfate reduction parameters by the various *Desulfomicrobium* sp. strains.

SRB Rod-1						SRB Rod-2					
	Biomass	Sulfate	Sulfide	Lactate	Acetate		Biomass	Sulfate	Sulfide	Lactate	Acetate
Biomass	1	-0.953	0.955	-0.916	0.823	Biomass	1	-0.972	0.95	-0.939	0.941
Sulfate	-0.953	1	-0.851	0.993	-0.927	Sulfate	-0.972	1	-0.873	0.987	-0.985
Sulfide	0.955	-0.851	1	-0.789	0.651	Sulfide	0.95	-0.873	1	-0.814	0.818
Lactate	-0.916	0.993	-0.789	1	-0.956	Lactate	-0.939	0.987	-0.814	1	-0.986
Acetate	0.823	-0.927	0.651	-0.956	1	Acetate	0.941	-0.985	0.818	-0.986	1
SRB Rod-3						SRB Rod-4					
	Biomass	Sulfate	Sulfide	Lactate	Acetate		Biomass	Sulfate	Sulfide	Lactate	Acetate
Biomass	1	-0.995	0.95	-0.982	0.954	Biomass	1	-0.99	0.995	-0.976	0.933
Sulfate	-0.995	1	-0.968	0.991	-0.966	Sulfate	-0.99	1	-0.991	0.991	-0.955
Sulfide	0.95	-0.968	1	-0.987	0.994	Sulfide	0.995	-0.991	1	-0.967	0.924
Lactate	-0.982	0.991	-0.987	1	-0.991	Lactate	-0.976	0.991	-0.967	1	-0.966
Acetate	0.954	-0.966	0.994	-0.991	1	Acetate	0.933	-0.955	0.924	-0.966	1
SRB Rod-5						SRB Rod-6					
	Biomass	Sulfate	Sulfide	Lactate	Acetate		Biomass	Sulfate	Sulfide	Lactate	Acetate
Biomass	1	-0.985	0.995	-0.96	0.946	Biomass	1	-0.99	0.984	-0.966	0.82
Sulfate	-0.985	1	-0.985	0.985	-0.967	Sulfate	-0.99	1	-0.982	0.985	-0.852
Sulfide	0.995	-0.985	1	-0.95	0.93	Sulfide	0.984	-0.982	1	-0.946	0.795
Lactate	-0.96	0.985	-0.95	1	-0.993	Lactate	-0.966	0.985	-0.946	1	-0.9
Acetate	0.946	-0.967	0.93	-0.993	1	Acetate	0.82	-0.852	0.795	-0.9	1
SRB Rod-7						SRB Rod-8					
	Biomass	Sulfate	Sulfide	Lactate	Acetate		Biomass	Sulfate	Sulfide	Lactate	Acetate
Biomass	1	-0.965	0.937	-0.931	0.81	Biomass	1	-0.991	0.969	-0.97	0.833
Sulfate	-0.965	1	-0.99	0.993	-0.927	Sulfate	-0.991	1	-0.979	0.974	-0.84
Sulfide	0.937	-0.99	1	-0.991	0.948	Sulfide	0.969	-0.979	1	-0.988	0.878
Lactate	-0.931	0.993	-0.991	1	-0.955	Lactate	-0.97	0.974	-0.988	1	-0.925
Acetate	0.81	-0.927	0.948	-0.955	1	Acetate	0.833	-0.84	0.878	-0.925	1
SRB Rod-9						SRB Rod-10					
	Biomass	Sulfate	Sulfide	Lactate	Acetate		Biomass	Sulfate	Sulfide	Lactate	Acetate
Biomass	1	-0.991	0.987	-0.959	0.927	Biomass	1	-0.995	0.963	-0.983	0.823
Sulfate	-0.991	1	-0.994	0.967	-0.936	Sulfate	-0.995	1	-0.972	0.986	-0.845
Sulfide	0.987	-0.994	1	-0.989	0.967	Sulfide	0.963	-0.972	1	-0.988	0.94
Lactate	-0.959	0.967	-0.989	1	-0.992	Lactate	-0.983	0.986	-0.988	1	-0.906
Acetate	0.927	-0.936	0.967	-0.992	1	Acetate	0.823	-0.845	0.94	-0.906	1

Total correlation coefficients for all SRB Rod-1-10 bacterial strains

	Biomass	Sulfate	Sulfide	Lactate	Acetate
Biomass	1	-0.983	0.9685	-0.958	0.881
Sulfate	-0.983	1	-0.958	0.9853	-0.92
Sulfide	0.9685	-0.958	1	-0.941	0.8845
Lactate	-0.958	0.9853	-0.941	1	-0.957
Acetate	0.881	-0.92	0.8845	-0.957	1

The results of cluster analysis of tested parameters have shown that studied bacteria form various clusters. The similarity between various *Desulfomicrobium* sp. strains has been established. Similar clusters were obtained by Dzierzewicz Z. et al., for fifteen (soil and intestinal) SRB strains. Distinct fingerprint patterns of two isolates derived from the same patient pointed to the different origin of both strains. There is also cluster analysis showing the presence of three distinct clusters for all intestinal strains. Similarity was evaluated in second group containing six soil strains. The members of this group had similarity with the soil strain. All intestinal strains and soil strains were similar [6].

Dzierzewicz Z. et al., have also shown that the soil bacterial group as well as the intestinal bacterial group formed two different clusters. The soil strains showed greater variability than the intestinal isolates. *D. desulfuricans* strains were differentiated depending on their origin [7].

The correlation coefficients between parameters of dissimilatory sulfate reduction by the *Desulfomicrobium* sp. various strains were defined (Table 1). Between biomass and sulfate (coefficient equal -0.983); biomass and lactate (coefficient equal -0.958); sulfate and sulfide (coefficient equal -0.958); sulfate and acetate (coefficient equal -0.92); lactate and acetate (coefficient equal -0.957); and lactate and sulfide (coefficient equal -0.941) an inverse (negative) strong correlation was established.

Between biomass and sulfide (coefficient equal +0.9685); biomass and acetate (coefficient equal +0.881); lactate and sulfate (coefficient equal +0.9853); acetate and sulfide (coefficient equal +0.8845) a direct (positive) strong correlation was established.

Taking into consideration all the obtained results: studies of bacterial *Desulfomicrobium* sp. strains growth in the medium, their sulfate and lactate usage, the production of hydrogen sulfide and acetate by the strains as well as cluster and correlation analyses of the different parameters of dissimilatory sulfate reduction by the strains.

The isolated *Desulfomicrobium* sp. various strains can cause human intestinal diseases and inflammatory bowel processes. The studied bacteria actively reduce sulfates and produce hydrogen sulphide. Lactate oxidation by the strains was incomplete since acetate ions were accumulated in the medium. Thus, these strains produce dangerous metabolic compounds such as hydrogen sulfide and acetate. Hydrogen sulfide is the main product during the metabolism of these strains and can be accumulated in significant quantities in the intestine. Increased sulfide and acetate concentrations can lead to the inhibition of digestive processes in the human intestine. Hydrogen sulfide is also cytotoxic and can potentially cause a carcinogenic effect along with the development of intestinal diseases [18,23,24]. It is known that hydrogen sulfide has an effect on butyrate oxidation processes and can consequently cause damage to the integrity of the epithelial barrier cells and inflammation of the colon epithelium [2,16,18,21,22,24].

The SRB can metabolize dietary supplements containing oxidized sulfur compounds [2,16]. Supplements with sulfate in ruminants causes the increase of hydrogen sulfide concentration in the rumen through the presence of SRB. The hydrogen sulfide toxicity manifested clinically, causing weight loss, fever, bloody diarrhea and intestinal inflammation. A large number of sulfur oxides are present in the food as they are added as a preservative to extend shelf life. These preservatives are sulfur dioxide (E220), sulfites (E221–227) and less so carrageenan (E407) all of which are consumed by 98.6% of the population. The analysis of food and drinks on a sulfate level showed that people who live in more developed western countries, consume over 16.6 mmol sulfate/day. It was established that the small human intestine absorbs sulfate in a concentration of about 7 mmol SO_4^{2-} per day, the rest of sulfate falls into the colon [2]. Consumption of sulfate is a regulatory factor in the reduction of sulfate in the gut [23,24,31]. It can have an important role in the development of ulcerative colitis [5]. Thus, the obtained *Desulfomicrobium* sp. bacterial strains are quite interesting and promising for further studies.

Conclusions

The *Desulfomicrobium* sp. strains actively grow in the Kravtsov-Sorokin's medium using sulfate ions as an electron acceptor and accumulating hydrogen sulfide. They use lactate as the electron donor and actively oxidize it incompletely to acetate. The highest biomass (up to 3.34 gram per liter) was accumulated by the *Desulfomicrobium* sp. strain Rod-9 on the sixth day of cultivation, in comparison to the other strains. Studied bacterial strains actively reduce the sulfate ions. Among the *Desulfomicrobium* sp. strains the highest concentration of hydrogen sulfide (up to 3.14 mM) was produced by the *Desulfomicrobium* sp. strain Rod-9 on the eighth day of cultivation. At this time, the strain used about 98% of the sulfate in the medium. All studied strains use lactate fully on the sixth day of cultivation for the dissimilation of sulfate and produce acetate. The highest concentration of acetate ions (up to 16.24 mM) produced by the *Desulfomicrobium* sp. strain Rod-8 on the sixth day of cultivation; while the strain used about 98% of lactate in the medium compared to its initial concentration.

Clustering of the parameters of the bacterial growth, sulfate and lactate usage along with hydrogen sulfide and acetate production by the studied strains showed that some obtained *Desulfomicrobium* sp. strains were closely related with some strains more separated from the other by the parameters.

The correlation coefficients between parameters of dissimilatory sulfate reduction by the various *Desulfomicrobium* sp. strains were defined. Between these processes an inverse (negative) and a direct (positive) strong correlation has been estimated.

The obtained data on the concentration of hydrogen sulfide and acetate, produced by the isolates is supposed to

help in establishing and assessing a toxicity effect of these substances on the epithelial cells of the human intestine. 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.

Competing interests

The authors declares that he has no competing interests.

Acknowledgement

The author expresses his gratitude to Yuriy J. Beno, PhD student at the Department of Biophysics and Bioinformatics at the Ivan Franko National University of Lviv for his help in conducting the statistical analysis. In addition to, Lucy McLennan and Robyn Cochrane from Forensic Biology, Immunology and Microbiology at University of Strathclyde (Scotland, United Kingdom) for their assistance and reading of the manuscript.

Publication history

Editors: David J. Sanchez, Western University of Health Sciences, USA.

António Pedro Fonseca, University of Porto, Portugal.

EIC: Todd R. Callaway, Food and Feed Safety Research Unit, USA.

Received: 15-Oct-2013 Revised: 15-Jan-2014

Accepted: 23-Jan-2014 Published: 11-Feb-2014

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Citation:

Kushkevych IV. Growth of the *Desulfomicrobium* sp. strains, their sulfate- and lactate usage, production of sulfide and acetate by the strains isolated from the human large intestine. *Microbiol Discov*. 2014; 2:1.
<http://dx.doi.org/10.7243/2052-6180-2-1>