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ORIGINAL RESEARCH ARTICLE

Molecular Cloning *cysK* Gene from *Escherichia coli* Genome, Transferring in the Intestinal Sulfate-Reducing Bacteria and the Expression Analysis of *O*-acetylserine(thiol)lyase

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ABSTRACT

Sulfate-reducing bacteria produce hydrogen sulfide which is toxic and carcinogenic for intestinal epithelial cells and can cause the development of the inflammatory bowel disease and ulcerative colitis in the humans and animals. Enzyme *O*-acetylserine(thiol)lyase, localized in *Escherichia coli* genome, use sulfide as substrate in the cysteine synthesis pathway. In this paper, the molecular cloning *cysK* gene from *E. coli*, its genetic transferring in the intestinal sulfate-reducing bacterium *Desulfovibrio piger* Vib-7 and the expression analysis of the enzyme was studied. Cysteine synthesis from hydrogen sulfide as substrate in the *D. piger* Vib-7 strain at the first time was demonstrated and characterized. The bacterial growth, sulfate and lactate consumption, accumulation of sulfide, acetate and cysteine synthesis in both *D. piger* Vib-7 wild-type and mutant-type were tested. The mutant-strain consumed much faster sulfate and lactate producing cysteine in the cultivation medium. The expression of the *cysK* gene in the mutant-type was studied by the formation of the final reaction product (cysteine) and the activity of *O*-acetylserine(thiol)lyase enzyme. Cysteine level was directly proportional to consumption of sulfate in the mutant-type and accumulation of sulfide in the wild-type. The *D. piger* Vib-7 mutant-type completely used sulfate the 48th hour of cultivation, thereafter additional sulfite and sulfide doses from the medium were also consumed and converted to cysteine. The obtained genetically constructed mutant strain bacterium *D. piger* Vib-7 for therapeutic strategy could be applied as a probiotic substance for subjects with inflammatory bowel disease and ulcerative colitis. This strain can compete with other intestinal sulfate-reducing bacteria, actively growth consuming sulfate and lactate much faster, and converting the toxic sulfide to untoxic cysteine in the gut.

Key Words: Sulfate-Reducing Bacteria, Hydrogen Sulfide, Toxicity, Ulcerative Colitis, Cysteine

Introduction

Sulfate-reducing bacteria *Desulfovibrio* genera belong to the intestinal microbiota of humans and animals (Gibson *et al*., 1991; Barton and Hamilton, 2010). They consume sulfate as an electron acceptor, and the organic compounds as an electron donor and carbon source in the process of dissimilatory sulfate reduction (Kushkevych, 2012a,b). The lactate is the most common substrate used by the species belonging to the intestinal sulfate-reducing bacteria (Gibson *et al*., 1991; Kushkevych, 2012a). The species of *Desulfovibrio* genera oxidize lactate incompletely to acetate (Barton and Hamilton, 2010). Lactate oxidation to acetate occurs together with the concurrent reduction of sulfate to hydrogen sulfide (Rowan *et al*., 2009; Kushkevych, 2012a).

Sulfide and acetate, the final products of sulfate-reducing bacteria *Desulfovibrio* genus, play an important etiologic role in the inflammatory bowel disease and ulcerative colitis (Cummings *et al*., 2003; Kushkevych, 2012b; Levine *et al*., 1998; Loubinoux *et al*., 2000, 2002). The intensive growth of the sulfate-reducing bacteria and their sulfate reduction in the human and animal colon contributes to accumulation of these final products in high concentration (Loubinoux *et al*., 2002; Pitcher and Cummings, 1996; Rowan *et al*., 2009; Ohge *et al*., 2003). These compounds are the highly toxic, mutagenic and carcinogenic to the intestinal epithelial cells, and can cause damage to the integrity of the epithelial barrier cells and inflammation of the colon epithelium (Roediger *et al*., 1993a,b; Rowan *et al*., 2009). Hydrogen sulfide can also cause inhibition of cytochrome oxidase, colonocytes oxidation of butyrate, destruction of the cells, development of ulcers and inflammation with a subsequent development of colon cancer (Roediger *et al*., 1986; Pitcher and Cummings, 1996; Chapman *et al*., 1994; Cummings *et al*., 2003). Thus, the increased number of the sulfate-reducing bacteria and intensive of dissimilatory sulfate reduction in the gut can cause inflammatory bowel diseases of humans and animals (Kushkevych, 2012b).

The species of *Desulfovibrio* genera most commonly were found in the intestines of humans and animals during various inflammatory bowel diseases (Cummings *et al*., 2003; Pitcher and Cummings, 1996; Gibson *et al*., 1991; Kushkevych, 2012b). These bacteria were also often found in persons with rheumatic diseases, and with ankylosing spondylitis, etc. (Barton and Hamilton, 2010). The species of *Desulfovibrio* genus also caused the bloody diarrhea, weight loss, anorexia, epithelial hyperplasia, abscesses and inflammatory infiltrates in animals and humans (Cummings *et al*., 2003; Loubinoux *et al*., 2000, 2002; Kushkevych, 2012a). The injection of these bacteria in hamster intestine caused infection is clinically similar to human colitis (Low *et al*., 2013). The increased number of sulfate-reducing microorganisms was in feces from people with ulcerative colitis (Pitcher and Cummings, 1996). There was also assumption that sulfate-reducing bacteria can cause some forms of cancer of the rectum through the formation of hydrogen sulfide (Cummings *et al*., 2003).

The enzyme of *O*-acetylserine(thiol)lyase (OASTL, EC 4.2.99.8) catalyzes cysteine biosynthesis from hydrogen sulfide in *Escherichia coli* (Boronat *et al*., 1984). The enzyme (previously known as *O*-acetylserine sulphydrylase A, OASS-A), isolated from *Salmonella typhimurium* by Becker *et al.* (1969), is a protein of M_r 68000 and composed of two identical subunits of M_r 34000. The subunit of OASTL is the product of the *cysK* gene (Becker *et al*., 1969). Addition

of cysteine to cultures of *S. typhimurium* and *E. coli* represses the synthesis of OASTL and the expression of the cysteine biosynthetic enzymes requires a positive regulator that is specified by the *cysB*$^+$ gene (Jones-Mortimer, 1968). The *cysK* gene maps at about 52 min on the genomes of *E. coli* (Fimmel and Loughlin, 1977; Wiater and Hulanicka, 1979; Boronat *et al*., 1984).

In literature, there are a lot of data on the role of the intestinal microorganisms in the development of inflammatory bowel diseases and ulcerative colitis in animals and humans (Cummings *et al*., 2003; Pitcher and Cummings, 1996; Gibson *et al*., 1991; Levine *et al*., 1998; Loubinoux *et al*., 2000, 2002; Roediger *et al*., 1993a,b; Rowan *et al*., 2009). However, the solution to this important problem has not been well-characterized. The treatment of these diseases is often to apply the toxic antibiotics and various antimicrobial agents which are inhibiting not only sulfate-reducing bacteria but also other intestinal microorganisms. As far as it is aware, these conditions often cause the intestinal dysbiosis.

The aim of this work was to clone the gene of *O*-acetylserine(thiol)lyase from *E. coli* K-12 and transfer of this gene to the intestinal sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 cells constructing the mutant strain which could be to synthesize cysteine from sulfide as well as to carry out the expression analysis of the enzyme.

Materials and Methods

The object of the study was the sulfate-reducing bacteria of the *Desulfovibrio piger* strain Vib-7 isolated from the human large intestine (Kushkevych, 2013) and identified by the sequence analysis of the 16S rRNA gene (Kushkevych *et al.*, 2014). The strain has been kept in the collection of microorganisms at the Laboratory of Biotechnology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic).

Cultivation of the sulfate-reducing bacteria

The bacteria were grown in a nutrition-modified Kravtsov-Sorokin's liquid medium (Kushkevych, 2013) 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%) to initiate bacterial growth was added. A sterile 10N solution of NaOH (0.9 ml/l) in the medium (for the final pH 7.2) was used. The medium was heated in boiling water for 30 min in order to obtain an oxygen-free medium, and then cooled to +30°C. The bacteria were grown for 72 hours at +37°C under anaerobic conditions. The tubes (volume 1.5 ml) were brim-filled with medium containing bacteria and closed to provide anaerobic conditions.

Genetic procedure

CysK gene was cloned from *E. coli* K-12 and transferred into the *D. piger* Vib-7. The presence of the CysK gene in *D. piger* Vib-7 was verified using the BLAST algorithm in the GenBank database. According to the obtained gene sequence, the specific primers for the polymerase chain reaction were used. Genomic DNA from *E. coli* K-12 was obtained from single bacterial colony, which was triturated in sterile water and heated to +90°C for five minutes. Free DNA was centrifuged at 14,600 g for three minutes to separate and remove the cells debris. DNA remaining in the supernatant was used to amplify the CysK gene using gradient PCR. HotStar Master Mix Taq polymerase (QIAGEN), UDG-glycosylase (New England Biolabs) and primers CysK-R and CysK-F (Generi-Biotech) for cloning were used:

Forward Primer: (cgaggcagatct tag); cysK-F:
cgaggcagatctAATTAATTGGGAATACGC.
Reverse Primer: (cgaggcagatctctcgag tag); cysK-R:
cgaggcagatctctcgagGCTCAGCACCATATGCGC.

The gradient PCR reaction was initially heated to +37°C for 2 minutes, then at +95°C for 10 minutes, followed by 35 cycles of +95°C for 15 seconds, the temperature gradient from +45°C to +69°C for 30 seconds, and +72° C for 30 seconds, followed by a step for 7 minutes at +72°C and the final cooling to +10°C.

The PCR products were analyzed by electrophoresis continued for 45 minutes at 10 V / cm to 2% electrophoresis gel in buffer 0.5x of TBE, the staining was performed using a fluorescent substance GelRed (Labmark). Before electrophoresis, the PCR products were mixed with the GLB buffer (5 µl of buffer to 25 µl PCR) in an amount of 5 µl applied to an electrophoretic gel. As the size standard, 100 bp ladder (Malamité) was used.

Specific amplicons of 600 bp were cut out from the gel and purified commercial MinElute Gel Extraction Kit (QIAGEN) according to manufacturer's instructions. The ligation mixture contained 4 µl of the PCR product (the concentration was estimated at 1.75 ng/µl), 1 µl of vector pSTVib-7 AccepTor^{TM} (concentration 50 ng/µl) and 5 µl Clonable^{TM} 2x Ligation Premix (Novagen). Ligation was carried out for 2 hours at +14°C.

The ligation products were transformed into *D. piger* Vib-7 using AccepTor^{TM} Vector Kit (Novagen). Before that, the bacterial cells were thawed on ice and 1 µl of the ligation mixture was added. After incubation on ice lasting 5 minutes, the mixture was immersed in a water bath at +42°C for exactly 30 seconds, and moved back to ice. To the mixture, 250 µl of regeneration medium was added and left horizontally shaken at 220 rpm and +37°C for 30 minutes. In total 50 µl of the transformed cells were smeared on a bacteriological stick and passed in warm (+30°C) nutrition-modified Kravtsov-Sorokin's agar medium (Kushkevych, 2013) containing IPTG (c=2 µl/ml), X-GAL (c=4 µl/ml), kanamycin (c= 1 µl/ml) and tetracycline (c=15 µl/ml). After 48 hours of cultivation at +37°C, the grown colonies in thickness of the medium on the Petri dishes were observed and the selection of transformed cells by the below mentioned components was carried out.

Plasmid DNA from the selected transformants *D. piger* Vib-7 (pSTVib-7-CysK) was isolated using QIAprep Spin Miniprep Kit (QIAGEN). The presence of the inserted CysK gene in the plasmid was confirmed by polymerase chain reaction using Maxima™ Probe qPCR Master Mix 2X, „FERMENTAS" and universal primers M13R and M13F20. The reaction was according to the following scheme: 2 minutes at +37°C, 15 minutes at +95°C, 35 cycles of 10 seconds at +95°C, 20 seconds at +50°C and 30 seconds at +72°C, 5 minutes at +72°C and final cooling to +10°C. The PCR products were analyzed by electrophoresis in a 1.5% gel. The sequence of the cloned gene plasmids CysK was confirmed by sequencing.

Isolation of CysK mutants

The CysK mutants were isolated by spreading 0.2 ml of overnight culture on the Kravtsov-Sorokin's agar medium containing IPTG (c=0.5 µl/ml) and X-GAL (c=2 µl/ml) and on the 10 mM of triazole Kravtsov-Sorokin's agar plates or on the plates containing 4.5 mg/ml of azaserine and 0.1 mM of L-cysteine. Mutants arised spontaneously; all colonies which appeared after a few days on plates containing inhibitors were resistant to the inhibitor used. In same case, triazole plates were supplemented with L-methionine to a final concentration of 0.1 mM. The method was applied to the strains of *D. piger* Vib-7 (pSTVib-7-CysK) (test, mutant strain) and *D. piger* Vib-7 (pSTVib-7) (control, wild-type).

Assay of *O*-acetylserine(thiol)lyase

Strains were grown in the Kravtsov-Sorokin's media supplemented with 10 mM of sodium lactate and ampicillin (100 mg/ml) with or without cystine (100 mg/ml) at +37°C overnight. Samples (20 mg dry mass/ml) were sonicated in an ultrasonic bath (Megason) for 10 min, centrifuged and the supernatants assayed for the production of cystcine

from O-acctylscrine and hydrogen sulfide according to the method of Fimmel and Loughlin (1977). Protein concentration in the cell-free extracts was determined by the Lowry method (Lowry *et al*., 1951).

Assay of cysteine production in *D. piger* Vib-7 mutant-type

A cysteine production in the Kravtsov-Sorokin's liquid medium by the *D. piger* Vib-7 mutant-type was studied. The mutant-type was also cultivated in the medium with sulfate+sulfite or sulfate+sulfide as a sulfur source, 3+3 mM of sodium sulfate+sulfite or 3+3 mM of sodium sulfate+sulfide, respectively. The culture was grown at 37°C for 84 h, and the amount of cysteine accumulated during this time in the medium was quantified. The quantification of cysteine was performed by the method described by Gaitonde (1967). The experiment was performed in five-plicate, and averages and standard deviations of the accumulated cysteine amounts were calculated.

Assay of bacterial biomass

Optical density of sulfate-reducing bacteria *D. piger* Vib-7 in the liquid medium (without Mohr's salt) was determined by the dilute suspension of the bacterial cells using the photometric method (Sutton, 2011). The biomass of the cells was calculated by the formula:

$$C = \frac{E \times n}{K} \ ,$$

where C – bacterial biomass (mg cells/ml of medium); E – extinction at λ nm (λ=340 nm); n – dilution factor, times; K – coefficient of conversion, obtained gravimetrically (K=0.19).

Assay of sulfate, lactate sulfide and acetate in cultivation medium

The sulfate ion concentration in the medium was determined by the turbidimetric method after it had been precipitated by barium chloride. To stabilize the suspension, glycerol was used (Kolmert *et al*., 2000). Lactate concentration was measured through the dehydrogenation reaction using Lactate Assay Kit (Sigma-Aldrich, Catalog Number MAK064). Sulfide concentration in the culture medium was assayed by the spectrophotometric method as was described in paper (Cline 1969). Accumulation of acetate ions in process of bacterial growth in the medium was determined using Acetate Assay Kit (Colorimetric, Catalog Number KA3764).

Statistical analysis

The statistical calculations of the results were carried out using the software MS Office and Origin computer programs. The research results were treated by the methods of variation statistics using Student *t*-test. The equation of the straight line that the best approximates the experimental data was calculated by the method of least squares. The absolute value of the correlation coefficient r was from 0.90 to 0.98. The statistical significance of the calculated parameters of line was tested by the Fisher's F-test. The accurate approximation was when $P \leq 0.05$ (Bailey, 1995).

Results and Discussion

Physiological and biochemical properties of the strain of *D. piger* Vib-7 mutant-type were studied (Fig. 1, 2). The results of our studied shown, the accumulated biomass of the mutant-type and wild-type in Kravtsov-Sorokin's liquid medium was quite different. However, the stationary growth phase for both bacterial types at the 60th hour was began; at this time, the accumulated biomass of the *D. piger* Vib-7 mutant-type was 36% higher compared to the wild-type (Fig. 1*A*). These data were consistent to the studies of dynamics of sulfate and lactate consumption as well as accumulation of sulfide/cysteine and acetate (Fig. 1*B*). In this case, the level of sulfate and lactate in the medium at the 60th hour was minimal values. It should also be noted that the mutant-type *D. piger* Vib-7 consumed these substrates much faster than the wild-type. The level of the accumulated sulfide (3.11 ± 0.28 mM) in the wild-type and cysteine (3.26 ± 0.31 mM) in the mutant-type was directly proportional to consumption of sulfate at the 60th hour of cultivation. Similar pattern was observed in the case of lactate consumption and acetate accumulation for both bacterial types . However, the values of consumption and accumulation of these compounds for the mutant- and wild-type were more similar (15.21 ± 1.49 and 15.63 ± 1.54 mM, respectively) (Fig. 1*C*).

As shown in fig 1*B*, *D. piger* Vib-7 mutant-type completely consumed sulfate at the 48th hour of cultivation. Final products of the dissimilatory sulfate reduction in the sulfate-reducing bacteria are sulfite and sulfide (Kushkevych, 2012a). Therefore, the following our task was to study of the potential consummation of sulfite and sulfide for synthesis of cysteine by *D. piger* Vib-7 mutant-type (Fig. 2*A*). As results shown, the mutant-type consumed of both these compounds accumulating cysteine in the medium. The highest cysteine accumulation (5.38±0.52 mM at the 84th hour of cultivation) was observed in the medium with additional sulfide. However, *D. piger* Vib-7 mutant-type after the 84th hour consumed sulfide incompletely. In this case, sulfite consumption was completely. Under these conditions, the level of accumulated cysteine was lower (4.32±0.41 mM) than in case with sulfide. The consumption of sulfite and sulfide in the mutant-type was directly proportional to cysteine accumulation. It should be also noted that the level of cysteine from 48th to 84th hour was almost unchanged in the control medium with sulfate. It confirmed that sulfate at the 48th hour was completely consumed. These data were consistent to enzyme activity of *O*-acetylserine (thiol) lyase under the effect of sulfate, sulfite and sulfide during bacterial growth in the cultivation medium (Fig. 2*B*). The highest enzyme activity (62.12±6.23, 74.31±7.34, 97.56±8.97 U × mg^{-1} protein) for sulfate, sulfite and sulfide, respectively, at the 72th hour was measured.

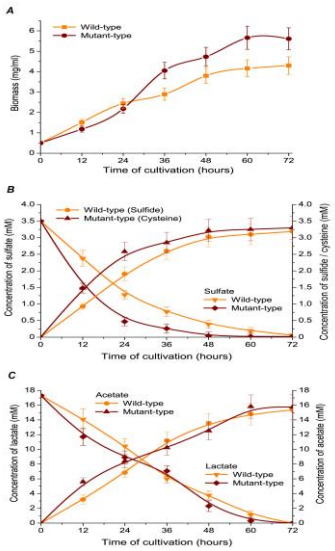


Fig. 1. Physiological properties of the *Desulfovibrio piger* Vib-7 wild- and mutant-type (M ± m, n = 5): *A* – bacterial growth in Kravtsov-Sorokin's liquid medium; *B* – consumption of sulfate as an electron acceptor accumulation of sulfide and cysteine; *C* – consumption of lactate as an electron donor and accumulation of acetate.

In literature, there are some data on the enzyme activity inhibition by product (Fimmel and Loughlin, 1977; Wiater and Hulanicka, 1979; Boronat *et al*., 1984). Therefore, the inhibition of the enzyme activity of *O*-acetylserine (thiol) lyase under the effect of cysteine during bacterial growth in the cultivation medium was studied (Fig. 2*C*). The level of the enzyme inhibition depended on time of bacterial growth and, accordingly, additional accumulation of cysteine in the cultivation medium. As result of the enzyme activity inhibition, sulfide accumulation in the medium was observed. The highest level of sulfide

(3.22±0.33, 4.35±0.41, 5.36±0.54 U × mg^{-1} protein) at the 84th hour of cultivation was measured in medium with cysteine+sulfate, cysteine+sulfite and cysteine+sulfide, respectively. Under these conditions, the activity of *O*-acetylserine(thiol)lyase was the lowest compared to the 36th hour of cultivation and the enzyme was almost completely inhibited.

Cysteine is an essential amino acid, unique in its ability to form disulfide linkages and also critical in the catalytic centers of many proteins (Droux, 2004; Rausch and Wachter, 2005). In bacteria, cysteine is synthesized from serine by incorporation of sulfide or thiosulfate. In the first step, *O*-acetylserine is formed by serine transacetylase, the *cysE* gene product (Boronat *et al*., 1984). Cysteine is then produced in a reaction catalyzed by the enzyme *O*-acetylserine (thiol)-lyase-A or *O*-acetylserine(thiol)-lyase-B, encoded by the *cysK* and *cysM* genes, respectively. Cysteine biosynthesis in plants is quite similar, although the respective genes have only recently been cloned and only one isozyme of *O*-acetylserine (thiol)-lyase has so far been identified. In animals, the transsulfuration pathway derives the sulfur group of cysteine from methionine and the carbon skeleton and amino group from serine (Kitabatake *et al*., 2000).

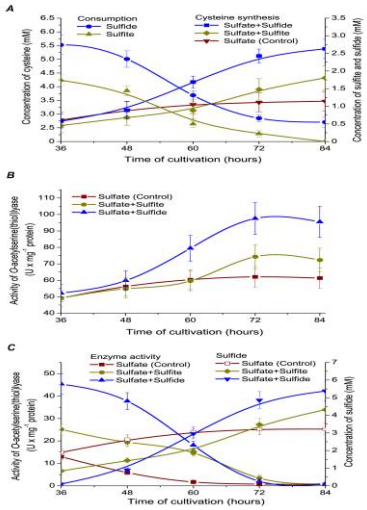


Fig. 2. Biochemical properties of the *Desulfovibrio piger* Vib-7 wild- and mutant-type (M ± m, n = 5): *A* – cysteine accumulation and consumption of sulfite and sulfide; *B* – enzyme activity of *O*-acetylserine(thiol)lyase under the effect of sulfate, sulfite and sulfide during bacterial growth in the cultivation medium; *C* – inhibition of the enzyme activity of *O*-acetylserine(thiol)lyase and accumulation of sulfide under the effect of cysteine (3 mM) during bacterial growth in the cultivation medium.

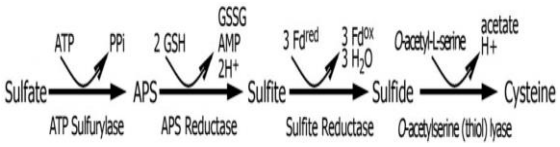
The pathway of cysteine biosynthesis in archaea *Methanosarcina barkeri* was demonstrated by Kitabatake *et al*. (2000). Complementation of a cysteine auxotrophic *Escherichia coli* strain NK3 led to the isolation of the *M. barkeri cysK* gene, which displays great similarity to bacterial *cysK* genes. Adjacent to *cysK* is anopen reading frame orthologous to bacterial *cysE* (serine transacetylase) genes. Analysis of recent genome data revealed the presence of bacteria-like *cysM* genes in *Pyrococcus* spp., *Sulfolobus solfataricus*, and *Thermoplasma acidophilum*. However, no orthologs for these genes can be found in *Methanococcus jannaschii*, *Methanobacterium thermoautotro-phicum*, and *Archaeoglobus fulgidus*, implying the existence of unrecognizable genes for the same function or a different cysteine biosynthesis pathway (Kitabatake *et al*., 2000).

Boronat *et al*., (1984) have reported that the plasmid pAB65, derived from a specialized transducing phage carrying DNA from about 52 min on the *E. coli* genome, coded for two polypeptides of M_r approx. 34000. The expression of one was regulated by cystine and the *cysB* gene product and the other by the *cysB* gene product only. One of these polypeptides was a subunit of *O*-acetylserine(thiol)-lyase. The pattern of peptide synthesis directed by plasmids carrying smaller DNA fragments indicated that the gene for *O*-acetylserine(thiol)lyase was transcribed clockwise. The spectrum, amino acid composition and subunit number of the enzyme were determined. The enzyme appears homologous with the *Salmonella typhimurium cysK* gene product (Boronat *et al*., 1984).

Yamamoto *et al*. (2011) have showed that the expression of cysK in *E. coli* was under the control of *CysB*, a *LysR* family transcription facto rand theexpression of *cysK* was regulated by several genetic and environmental factors in addition to *CysB*: two genetic factors, *OmpR* and *CysE*, and lithium. Based on the findings, authors constructed the high-level expression system of cysK (Yamamoto *et al*., 2011). Mutants of *CysK*, deficient in *O*-acetylserine sulphydrylase A [O-acetyl-L-serine acetate-lyase (adding hydrogen-sulphide)], were isolated as strains resistant to selenite or giving a black colour reaction on bismuth citrate indicator medium by Fimmel and Loughlin (1977). Authors have demonstrated that all strains were resistant to the inhibitor 1,2,4-triazole. Strains containing both a *cysE* mutation and a *cysK* mutation lacked the constitutive levels of NADPH-sulfite reductase showing that these levels were due to the *in vivo* concentration of the inducer, *O*-acetylserine. The *cysK* locus was found to be 81 % cotransducible with the *ptsI* gene (Fimmel and Loughlin, 1977). Triazole and azaserine resistant *CysK* mutants of *E. coli* K12 were also studied by Wiater and Hulanicka in 1979.

The cysteine role as an amino acid in proteins, cysteine functions as a precursor for a huge number of essential biomolecules, such as vitamins and cofactors (Droux, 2004; Wirtz *et al*., 2004), antioxidants like glutathione, which is regarded as the major determinant of cellular redox homeostasis, and many defense compounds is very important (Rausch and Wachter, 2005). All of these biomolecules contain sulfur moieties that act as functional groups and are derived from cysteine. The biosynthesis of cysteine is accomplished through two sequential reactions catalyzed by the enzymes serine acetyltransferase, which synthesizes the intermediary product *O*-acetylserine, and O-acetylserine(thiol)lyase, which combines sulfide with *O*-acetylserine to produce cysteine. Together, these two enzymes form the heterooligomeric cysteine synthase complex, which was first described in bacteria (Droux *et al*., 1998).

In summarizing all results of our studies, we can conclude that the *cysK* gene from *E. coli* genome was cloned and transferred in the intestinal sulfate-reducing bacterium *D. piger* Vib-7 wild-type. The expression of the *cysK* gene by the formation of the final reaction product (cysteine) and the activity of O-acetylserine(thiol)lyase enzyme was studied. Schematic of sulfate reduction to cysteine in *D. piger* Vib-7 mutant-type was demonstrated:



ATP sufurylase produces adenosine-5′-phosphosulfate (APS); sulfite is then formed from APS by glutathione-dependent APS reductase; sulfide is catalyzed from the generated sulfite by ferredoxin-dependent sulfite reductase; *O*-acetylserine(thiol)-lyase catalyzes the reaction of sulfide and O-acetyl-L-serine to synthesize cysteine (Brychkova *et al*., 2012).

Conclusion

The *cysK* gene mutant-type of sulfate-reducing bacterium *D. piger* Vib-7 was genetically constructed. This mutant-type accumulated biomass by 36% higher than the wild-type. That was consistent to the studies of dynamics of sulfate and lactate consumption as well as

accumulation of sulfide/cysteine and acetate. The *D. piger* Vib-7 mutant-type also consumed these substrates much faster than the wild-type. The expression of the *cysK* gene in the mutant-type was studied by the formation of the final reaction product (cysteine) and the activity of *O*-acetylserine(thiol)lyase enzyme.

Cysteine level was directly proportional to consumption of sulfate in the mutant-type and accumulation of sulfide in the wild-type. The *D. piger* Vib-7 mutant-type completely used sulfate the 48th hour of cultivation, thereafter additional sulfite and sulfide doses from the medium were also consumed and converted to cysteine. However, additional cysteine dose (3 mM) caused the inhibition of *O*-acetylserine(thiol)lyase activity and accumulation of sulfide that was typical for wild-type of the sulfate-reducing bacteria.

According to all obtained results, the studies of the dissimilatory sulfate reduction in the *D. piger* Vib-7 mutant-type, its consumption of substrates, utilization of toxic sulfide and production of cysteine in detail can be perspective for clarification of their etiological role in the development of the humans and animals bowel diseases. The obtained genetically constructed mutant strain bacterium *D. piger* Vib-7 for therapeutic strategy could be applied as a probiotic substance for subjects with inflammatory bowel disease and ulcerative colitis. This strain could compete with other intestinal sulfate-reducing bacteria, actively growth consuming sulfate and lactate much faster, and converting the toxic sulfide to untoxic cysteine in the gut. At present, the only possibility to hand is to try to manipulate the intestinal microflora by adding potential protective bacteria such as probiotics. Preliminary clinical studies have confirmed that this approach might be extremely useful. However, much more work is necessary to understand why probiotics are able to compete with aggressive bacteria and how the communication between microflora and the immune system in healthy and ulcerative colitis patients works. The *D. piger* Vib-7 mutant-type is valuable and indispensable tools that provide a wide range of options for investigating involvement of various factors into the pathogenesis of inflammatory bowel disease and ulcerative colitis to evaluate different therapeutic options.

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References

Bailey, N.T.J. Statistical Methods in Biology. 1995. *Cambridge University Press. 3rd edition*, 252 p. http://dx.doi.org/ 10.1017/CBO9781139170840

Barton, L.L., Hamilton, W.A. 2010. Sulphate-Reducing Bacteria. Environmental and Engineered Systems. *Cambridge: Cambridge University Pres*. 553 p. http://dx.doi.org/10.1017/ CBO9780511541490

Becker, M.A., Kredich, N.M., Tomkins, G.M. 1969. The purification and characterization of O-acetylserine sulfhydrylase-A from *Salmonella typhimurium*. *J. Biol Chem*., 244: 2418-2427.

Boronat, A., Britton, P., Jones-Mortimer, M.C., Kornberg, H.L., Lee, L.G., Murfitt, D., Parra, F. 1984. Location on the *Escherichia coli* genome of a gene specifying O-acetylserine(thiol)lyase. *J. Gen. Microbiol.*, 130: 673-685.

Brychkova, G., Yarmolinsky, D., Sagi, M. 2012. Kinetic assays for determining in vitro APS reductase activity in plants without the use of radioactive substances. *Plant Cell Physiol.*, 53(9): 1648-1658.

Chapman, M.A.S., Grahn, M.F., Boyle, M.A. 1994. Butyrate oxidation is impaired in the colonic mucosa of sufferers of quiescent ulcerative colitis. *Gut*, 35: 73-76.

Cline, J.D. 1969. Spectrophotometric determination of hydrogen sulfide in natural water. *Limnol Oceangr*, 14: 454-458.

Cummings, J.H., Macfarlane, G.T., Macfarlane, S. 2003. Intestinal Bacteria and Ulcerative Colitis. Curr. Issues Intest. Microbiol, 4: 9-20.

Droux, M., Ruffet, M.-L., Douce, R., Job, D. 1998. Interactions between serine acetyltransferase and O-acetylserine(thiol) lyase in higher plants. Structural and kinetic properties of the free and bound enzymes. *European Journal of Biochemistry*, 255: 235-245.

Droux, M. 2004. Sulfur assimilation and the role of sulfur in plant metabolism: a survey. *Photosynthesis Research*, 79: 331-348.

Fimmel, A.L., Loughlin, R.E. 1977. Isolation and characterization of cysK mutants of *Escherichia coli* K12. *J. Gen Microbiol*., 103(1): 37-43.

Gaitonde, M.K. 1967. A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochem J.*, 104: 627-633.

Gibson, G.R., Cummings, J.H., Macfarlane, G.T. 1991.Growth and activities of sulphate-reducing bacteria in gut contents of health subjects and patients with ulcerative colitis. *FEMS Microbiol. Ecol*, 86: 103-112. http://dx.doi.org/ 10.1111/j.1574-6968.1991.tb04799.x

Jones-Mortimer, M.C. 1968): Positive control of sulphate reduction in *Escherichia coli*. Isolation, characterization and mapping of cysteineless mutants of *E. coli* K12. *Biochem. J.*, 110(3): 589-595.

Kitabatake, M., So, M.W., Tumbula, D.L., Soll, D. 2000. Cysteine biosynthesis pathway in the archaeon *Methanosarcina barkeri* encoded by acquired bacterial genes? *J. Bacteriol*., 182(1): 143-145.

Kolmert, A., Wikstrom, P., Hallberg, K.B. 2000. A fast and simple turbidimetric method for the determination of sulfate in sulfate-reducing bacterial cultures. *J. Microbiol. Methods*, 41: 179-184.

Kushkevych, I.V. 2012a. Sulfate-reducing bacteria of the human intestine. I. Dissimilatory sulfate reduction. *Studia Biologica*, 2012; 6(1): 149-180. http://bioweb.lnu.edu.ua /studia/?p=article&year=2012&tom=6&nomer=1&id=175

Kushkevych, I.V. 2012b. Sulfate-reducing bacteria of the human intestine. II. The role in the diseases development. *Studia Biologica*, 6(2): 221-250. http://bioweb.lnu.edu.ua/ studia/?p=article&year=2012&tom=6&nomer=2&id=199

Kushkevych, I.V. 2013. Identification of sulfate-reducing bacteria strains of human large intestine. *Studia Biologica*, 7(3): 115-124. http://bioweb.lnu.edu.ua/studia/?p=article &year=2013&tom=7&nomer=3&id=277

Kushkevych, I.V., Bartoš, M., Bartošová, L. 2014. Sequence analysis of the 16S rRNA gene of sulfate-reducing bacteria isolated from human intestine. *Int. J. Curr. Microbiol. Appl. Sci*., 3(2): 239–248. http://www.ijcmas.com/vol-3-2/Ivan%20Kushkevych,%20et%20al.pdf

Levine, J., Ellis, C.J., Furne, J.K., Springfield, J., Levitt, M.D. 1998. Fecal Hydrogen Sulfide Production in Ulcerative Colitis. *The American J. Gastroenterol*., 93(1): 83-87.

Loubinoux, J., Bronowicji, J.-P., Pereira, I.A. *et al*. 2002. Sulphate-reducing bacteria in human feces and their association with inflammatory diseases. *FEMS Microbiol Ecol*., 40: 107-112.

Loubinoux, J., Mory, F., Pereira, I.A. *et al*. 2000. Bacteremia caused by a strain of *Desulfovibrio* related to the provisionally named *Desulfovibrio fairfieldensis*. *J Clin Microbiol*., 38: 931-934.

Low, D., Nguyen, D.D., Mizoguchi, E. 2013. Animal models of ulcerative colitis and their application in drug research. *Drug Design, Development and Therapy*, 7: 1341-1357.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein determination with the Folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Ohge, H., Furne, J.K., Springfield, J., Sueda, T., Madoff, R.D., Levitt, M.D. 2003. The effect of antibiotics and bismuth on fecal hydrogen sulfide and sulfate-reducing bacteria in the rat. *FEMS Microbiol Lett*, 228: 137-142.

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

Rausch, T., Wachter, A. 2005. Sulfur metabolism: a versatile platform for launching defence operations. *Trends Plant Sci*, 10(10): 503-509.

Roediger, W.E.W., Duncan A., Kapaniris, O. 1993a. Sulphide impairment of substrate oxidation in rat colonocytes: A biochemical basis for ulcerative colitis? *Clin Sci*., 85: 623-627.

Roediger, W.E.W., Duncan, A., Kapaniris, O., Millard, S. 1993b. Reducing sulfur compounds of colon impair colonocyte nutrition: implications for ulcerative colitis. *Gastroenterology*. 104, 802–809.

Roediger, W.E.W., Nance, S. 1986. Metabolic induction of experimental ulcerative colitis by inhibition of fatty acid oxidation. *Br J Exp Pathol*., 67: 773-782.

Rowan, F.E., Docherty, N.G., Coffey, J.C. *et al*. 2009. Sulphate-reducing bacteria and hydrogen sulphide in the aetiology of ulcerative colitis. *British J Surgery*, 96: 151-158.

Sutton, S. 2011. Measurement of Microbial Cells by Optical Density. *J. Valid Tech*., 17(1): 46-49.

Wiater, A., Hulanicka, D. 1979. Properties of cysK mutants of *Escherichia coli* K12. *Acta Biochim Pol*., 26: 21-28.

Wirtz, M., Droux, M., Hell, R. 2004. O-acetylserine(thiol)lyase: an enigmatic enzyme of plant cysteine biosynthesis revisited in *Arabidopsis thaliana*. *J. Exp. Bot*., 55(404): 1785-1798.

Yamamoto, K., Oshima, T., Nonaka, G., Ito, H., Ishihama, A. 2011. Induction of the *Escherichia coli* cysK gene by genetic and environmental factors. *FEMS Microbiology Letters*, 323(1): 88-95.