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Գ.00.04 – Կենսաքիմիա մասնագիտությամբ
կենսաբանական գիտությունների թեկնածուի
գիտական աստիճանի հայցման ատենախոսության

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MINISTRY OF SCIENCE AND EDUCATION OF RA

YEREVAN STATE UNIVERSITY

BLBULYAN SYUZANNA SUREN

THE CHARACTERIZATION OF ATP-ASE ACTIVITY OF SOME BACTERIA UPON GLYCEROL AND GLUCOSE OXIDATION CONDITIONS

SYNOPSIS

of dissertation for conferring of scientific degree of
Candidate of Biological Sciences
In the specialty of 03.00.04-Biochemistry

YEREVAN 2019

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական ղեկավար՝ ՀՀ ԳԱԱ թղթակից անդամ, կենս. գիտ. դոկտոր, պրոֆեսոր Ա. Հ. Թռչունյան

Պաշտոնական ընդդիմախոսներ՝ կենս. գիտ. դոկտոր, պրոֆեսոր Պ. Ա. Ղազարյան
կենս. գիտ. դոկտոր, պրոֆեսոր Ա. Զ. Փեփոյան

Առաջատար կազմակերպություն՝ ՀՀ ԳԱԱ Հր. Բունիաթյանի անվան կենսաքիմիայի ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2019թ. հուլիսի 16-ին ժամը 14^{00}-ին, Երևանի պետական համալսարանում գործող ՀՀ ԲՈԿ-ի Կենսաֆիզիկայի 051 մասնագիտական խորհրդի նիստում (0025, Երևան, Ալեք Մանուկյան փ. 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությանը կարելի է ծանոթանալ Երևանի պետական համալսարանի գրադարանում:
Ատենախոսության սեղմագիրն առաքված է 2019թ. հունիսի 5-ին:

051 մասնագիտական խորհրդի գիտական քարտուղար, կենս. գիտ. թեկնածու, դոցենտ՝ Մ.Ա. Փարսադանյան

The theme of dissertation has been approved at Yerevan State University

Academic advisor: Corresponding Member of NAS RA, Dr. of Biological Sciences, Professor A. Trchounian

Official opponents: Dr. of Biological Sciences, Professor P. Ghazaryan
Dr. Of Biological Sciences, Professor A. Pepoyan

Leading organization: H. Buniatian Institute of Biochemistry NAS RA

The defense of the dissertation will be held on 16th July, 2019, at 14:00, at the session of 051 Scientific Specialized Council on Biophysics of SCC of RA at Yerevan State University (0025, Yerevan, Alex Manoogian str. 1, YSU, Faculty of Biology).
The dissertation is available at the library of Yerevan State University.
The synopsis has been sent on 5th June, 2019.

Scientific Secretary of 051 Specialized Council, PhD., Associate Professor M. A. Parsadanyan

INDRODUCTION

Topic's significance. Metabolism of all living organisms, including bacteria, depends on the environment, energy sources and other factors. *Escherichia coli* (*E. coli*) can ferment different carbohydrates, such as glucose and as a result of which are formed various organic acids (succinic acid, acetic acid, formic acid, lactic acid and etc.), ethanol and gases (carbon dioxide (CO_2), hydrogen (H_2)). That's why these bacteria are widely used for industrial purposes for the production of bioethanol, bio-hydrogen. At the same time, the issue of finding inexpensive and easily accessible carbon sources is also topical. In 2006, it has been discovered that *E. coli* implements glycerol fermentation, resulting in different organic acids, gases, and alcohols are formed also (Dharmadi et al., 2006; Gonzalez et al., 2008). Glycerol is the most common waste of biodiesel production, which makes it quite affordable, and its' fermentation is very promising for bio-hydrogen and bio-ethanol production (Chaudhary, 2012). Formic acid, formed during fermentation in *E. coli*, oxidazes up to CO_2 and H_2 by the membrane-bound enzyme complex - formate hydrogen lyase (FHL). H_2 is ecologically clean, renewable source of fuel from which burning ~ 140 kJ/g energy is released (Chu and Majumdar, 2012). Different bacteria are producing H_2 during dark- or photo-fermentation (Trchounian, 2015; Hallenbeck and Liu, 2016). Special enzymes named hydrogenases (Hyd) are involved in this process. It has been shown that upon glucose fermentation for Hyds and FHL activities, their energetics requirements is necessary the H^+-translocating F_OF_1-ATPase, which is the essential membrane mechanism generating proton motive force (Δp) (Blbulyan et al., 2011, Trchounian et al., 2013). Studies in our laboratory have shown that production of H_2 and H^+ efflux are sensitive to *N,N'*-dicyclohexylcarbodiimide (DCCD) - inhibitor of F_oF_1 (Trchounian and Sawers, 2014, Blbulyan & Trchounian, 2015): On the other hand, it has been shown that ATPase activity of membrane vesicles F_OF_1 was significantly enhanced by formate when bacteria were grown during glucose fermentation (Bagramyan and Trchounian, 2003). How can explain the such importance of F_OF_1? Perhaps, during fermentation, ATPase combines hydrolysis of ATP with H^+ transfer across the membrane, as a result of which energy of ATP converts to Δp, required for the functioning of FHL and Hyds (Trchounian and Sawers, 2014) However, during glycerol fermentation, the data on the cooperation and interaction mechanisms of the above mentioned enzymes are limited.

The role of F_oF_1 in oxidation–reduction (redox) sensing by bacteria under glucose or glycerol fermentation has been proposed (Kirakosyan & Trhounian, 2007; Vassilian & Trchounian, 2009). However, the pathways and mechanisms involved in redox sensing by bacteria and the regulation of the bacterial metabolism are still not clear for many bacteria and are completely unknown for others, especially for thermophiles (Geobacilli) (Ghazaryan et al., 2015).

Research topics and tasks. The main purpose of this study was to investigate the ATPase activity of various bacteria depending on the carbon source oxidation nature.

Constituted tasks of the research were to:

1. investigate the membrane vesicles ATPase activity of *E. coli* BW25113 wild type and different Hyds lacking mutants during glycerol fermentation;
2. study of ATPase activity changes in *E. coli* wild type and various Hyd mutants membrane vesicles upon mixed carbon (glucose and glycerol) fermentation and compare with alone glycerol fermentation;
3. determine changes of H^+/K^+ fluxes in the cell extracts upon glycerol fermentation
4. investigate influence of different concentration of glucose on *E. coli* and Hyd mutant ATPase activity;
5. reveal the growth, kinetics of oxidation-reduction potential (ORP, E_h), pH changes and ATPase activity of thermophile *Geobacillus toebii* (*G. toebii*) ArzA-8 strain.

Scientific novelty and practical value of the study. Within the frames of the conducted work it has been revealed that pH of medium influence on *E. coli* F_oF_1 activity upon glycerol fermentation, in addition the highest activity, was observed at pH 7.5. Hyd-1 and Hyd-2 are required for the F_oF_1- ATPase activity. It has been discovered that F_0F_1 is involved in formation of H_2 cycle across the membrane and operates as an internal pH adjuster. It has been also shown that functional link between F_0F_1 and secondary transport systems like TrkA depends on glucose concentration and external pH. Obtained results point out the nature of interaction between TrkA and F_0F_1 and the importance of this during fermentation. For the first time in this investigation, it has been shown that regulation of ORP can increase the biotechnological applicability of thermophiles for obtaining both biomass and various valuable products. These findings are absolutely novel for these bacteria and provide an opportunity to clarify the functional relationship of different Hyds and H^+-ATPase in conditions of fermentation of different carbon sources as well as the role of ATPase in cell metabolism regulation. Various strains of *E. coli* have a great biotechnological potential and are widely used for biofuels production. Consequently, obtained data about different enzymes interaction mechanisms, functional link and regulation, can be used for both basic scientific research and biotechnological purposes.

Main points to present at the defense.

1. *E. coli* Hyd-1 and Hyd-2 enzymes directly interacts with F_oF_1-ATPase or transfer H^+ across the membrane upon glycerol fermentation, at slightly alkaline pH (pH 7.5).
2. ATPase activity of membrane vesicles of *E. coli* depends on the medium pH under glycerol fermentation. Nature of functional link between ATPase and Hyds depending on fermentation substrate is different.
3. *E. coli* ATPase activity and its interaction with Hyd-4, depend on the glucose concentration and the medium pH.
4. The growth and the ATPase activity of *G. toebii* ArzA-8 strain depend on presence of carbon source and environmental redox state.

Work approbation. Main results of the dissertation were discussed at seminars in Department of Biochemistry, Microbiology and Biotechnology, Biology Faculty of Yerevan State University, and at scientific conferences: 13th Int. School-Conference

for Young Scientists (Pushchino, Russia, 2009), Int. Symposium ''Solvation and ionic effects in Biomuleculs: Theory to experiment'' (Tsakhkadzor, Armenia, 2010), 17th and 18th European Bionergetics Conference (EBEC) (Freiburg, Germany, 2012, and Lisbon, Portugal, 2014), 13rd Young Scientists forum and 38th FEBS Congress (St. Petersburg, Russia, 2013), FEBS-EMBO Conference (Paris, France, 2014), Int. Scientific Workshop ''Trends in Microbiology and Microbial Biotechnology'' (Yerevan, Armenia, 2014), Int. Workshop on Expression, Structure and Function of Membrane Proteins (Florence, Italy, 2015), 7th Congress of Federation of European Microbiological Societies Congress (FEMS), (Valencia, Spain, 2017), 19th IUPAB and 11th EBSA Congress (Edinburgh, UK, 2017), 42nd FEBS Congress (Jerusalem, Israel, 2017).

Publications. According to experimental data observed in dissertation, 16 papers, including 5 articles in peer-reviewed journals and 11 abstracts were published.

Volume and structure of dissertation. The dissertation contains following chapters: introduction, literature review (Chapter 1), experimental part (Chapter 2), results and discussion (Chapter 3), concluding remarks, conclusions and cited literature (total 185 papers and books). The document consists of 128 pages, 7 tables and 19 figures.

MATERIALS AND METHODS

Objects. *E. coli* BW25113 or MC4100 wild type (WT) strains and corresponding mutants (Table 1) were used in experiments.

Table 1. Charachteriistcs of *E.coli* strains used in this study

Strain	Genotype	Appropriate absent or defective proteins	Reference
BW25113	$lacI^q$ $rrnB_{T14}$ $\Delta lacZ_{W116}$ *hsdR514* $\Delta araBAD_{AH33}$ Δrha BAD_{LD78}	Wild type	Baba et al., 2006
JW 0955	BW25113 *ΔhyaB*	Large subunit of Hyd-1	Maeda et al., 2007
JW 2962*	BW25113 *ΔhybC*	Large subunit of Hyd-2	Maeda et al., 2007
JW 2701*	BW25113 *ΔfhlA*	FHL activator	Maeda et al., 2007
MW 1000	BW 25113 *ΔhyaB ΔhybC*	Large subunits of Hyd-1 and Hyd-2	Maeda et al., 2007
KT 2110	BW 25113 *ΔhyaB ΔhybC ΔselC*	Large subunits of Hyd-1 and Hyd-2 and $tRNA^{sec}$	Trchounian et al, 2012
FM 460*	MC 4100 Δ*selC*	$tRNA^{sec}$	Soboh et al., 2011
DHP-F2	MC 4100 *ΔhypF*	All four Hyd enzymes	Blbulyan & Trchounian, 2015
JRG3621**	MC4100 *Δ(hyfB-R)::spc*	Subunits of Hyd-4	Blbulyan et al., 2016

* Resistant to Kanamycin

** Resistant to Spectinomycin

The *G. toebii* strain ArzA-8 isolated from the Arzakan geothermal mineral spring in Armenia (temperature > 44 °C, pH 7.0–7.2) was also used (Panosyan et al., 2018).

Bacterial growth and preparation for assays. *E. coli* were grown at 37°C for 18-20 h in anaerobic conditions by direct transfer from nutrient agar surface in Petri dish into high buffered liquid peptone growth medium containing 20 g/l peptone, 15 g/l K_2HPO_4, 1.08 g/l KH_2PO_4, 5 g/l NaCl **(pH 7.5)**, 20 g/l peptone, 7.4 g/l K_2HPO_4, 8.6 g/l KH_2PO_4, 5 g/l NaCl **(pH 6.5)** or 20 g/l peptone, 15 g/l KH_2PO_4, 1.08 g/l K_2HPO_4, 5 g/l NaCl **(pH 5.5)**. 10 g/l glycerol and/or 2 g/l or 8 g/l glucose was added. Kanamycin (25 µg/ml final concentration) was added where appropriate (see Table 1). *G. toebii* were grown under aerobic conditions at 55 °C with shaking with 250 rpm in nutrient broth (NB) containing 5 g/l peptone, 1.5 g/l beef extract, 1.5 g/l yeast extract and 5 g/l NaCl, either without glucose or with glucose at different concentrations (5, 11 and 22 mM) at pH 7.5 or pH 6.5.

Isolation of membrane vesicles. Right–side-out (RSO) membrane vesicles were isolated from bacteria treated with 0.5 mg/ml lysozyme and 20 mM ethylenediaminetetra-acetic acid by osmotic lysis of spheroplasts (Konings and Kaback, 1973) and in-side out (ISO) vesicles - by disrupting the spheroplasts with a French press (Trchounian & Vassilian, 1994).

Determination of growth characteristics and measuring of E_h. The specific growth rate was determined by dividing 0.693 (lg2=0.693) by the doubling time of the optical density in the ranges where changes in the logarithm of optical density depended on time in a linear manner (Trchounian et al., 2012). E_h was measured by both platinum (Pt) and titanium-silicate (Ti-Si) electrodes.

ATPase assays. ATPase activity of membrane vesicles was determined by the amount of inorganic phosphate (Pi) liberated in the reaction of membrane vesicles with 5 mM ATP (pH 7.5, 6.5 and 5.5) in the assay mixture (50 mM Tris–HCl buffer (pH 7.5, 6.5 and 5.5) containing 1 mM $MgSO_4$). The ATPase activity was expressed in nMol P_i (min µg protein)$^{-1}$. P_i was determined spectrophotometrically (with UV–VIS Auto PS scanning spectrophotometer, LaboMed, USA) by the method Taussky and Shorr (1953). *N,N'*-dicyclohexcylcarbodiimide (DCCD) was used as an inhibitor of F_OF_1. For DCCD inhibition studies, whole cells or vesicles were incubated with 0.2 and/or 0.5 mM DCCD for 10 min. The DCCD-inhibited ATPase activity was calculated as difference between activities in the absence and in the presence of the inhibitor. Protein levels were measured by the method of Lowry using bovine serum albumin (BSA) as a standard. All assays were done at 37 °C.

Proton and potassium ions transport study. H^+ and K^+ fluxes by whole cells were determined by monitoring changes in H^+ and K^+ activities in the medium with the use of selective pH and K^+ electrodes. The electrode readings were calibrated by titration of the medium with small quantities of 0.01 N HCl and 0.01 M KCl. Ion fluxes were expressed in mMol/min per number of cells in a unit of volume (ml) (Trchounian et al., 2012).

Data processing. The average data obtained from 3 independent assays were represented, and standard deviation of values did not exceed 3 %. For the differences between different series of experiments, Student validity criteria (p) were determined using Microsoft Excel 2016; the difference was valid if $p<0.05$.

RESULTS AND DISCUSSION

The link of *E. coli* different hydrogenases and the F_OF_1-ATPase activity during glycerol fermentation

E. coli has ability to anaerobically ferment not only sugars (glucose) as well as glycerol at a pH-depended manner leading to different acids evolution (Gonzalez et al., 2008, Trchounian et al., 2015).

For the first time were investigated ATPase activity and H^+ efflux of the glycerol fermented *E. coli* WT and mutants of different Hyd enzymes in slightly alkaline and acidic medium. We have shown that ATPase activity of membrane vesicles of the glycerol-fermented *E. coli* BW25113 at pH 7.5 was strongly inhibited by 0.2 mM DCCD (Fig. 1A). ATPase activity was ~2-fold lowered at pH 7.5 compared with ATPase activity for the cells grown under glucose fermentation (not shown). Previous studies in our laboratory have shown that the addition of K^+ in the medium has stimulated the activity of H^+-ATP-ase during glucose fermentation (Trchounian & Vassilian, 1994). For the interpretation of H^+-ATPase and K^+ uptake TrkA system interactions under glycerol fermentation, F_oF_1 activity was also investigated in the presence of 100 mM K^+, and it should be noted that, in contrast to glucose, stimulation of ATPase activity was not observed (Blbulyan et al., 2011). Membrane vesicles ATPase activity at pH 7.5 compared with that in WT cells was lowered in ~2-fold with *hyaB* and ~20-fold with *hybC* mutants (see Fig. 1A). It was stimulated markedly (~1.5-time) in the *hyaB hybC* double mutant and suppressed significantly (~3-time) in the *fhlA* mutant (see Fig. 1A). The markedly decreased total and DCCD-inhibited ATPase activity at pH 7.5 in *hyaB* and *hybC* mutants leads to the suggestion that Hyd-2 more than Hyd-1 might have a closed relationship with F_oF_1.

We have examined the ATPase activity of WT and mentioned mutants at pH 5.5. *hyaB*, *hybC* mutants showed similar values which were higher compared to the WT. The ATPase activity was suppressed ~1.5-fold in *hyaB hybC* and ~2.5-times in *fhlA* mutants, respectively, compared with WT (Fig. 1B). Moreover, the low effect of DCCD on the ATPase activities at pH 5.5 observed is in confirmation with previous study pointed out that this reagent did not affect cells at a low pH (see Fig. 1B) (Trchounian & Kobayashi, 1999).

It was shown that FHL is required for H^+/K^+ exchange: most probably it serves to supply reducing equivalents for energy transfer to facilitate K^+ uptake via TrkA (Trchounian, 2004). However, the interaction between mentioned enzyme systems under glycerol fermentation was not known. The kinetics of H^+ and K^+ fluxes by *E. coli* wt whole cells grown under glycerol or glucose fermentation at pH 7.5 were investigated.

The H^+ and K^+ fluxes were lowered in the glycerol fermented cells than those for cells grown under glucose fermentation (Fig. 2). 0.2 mM DCCD inhibited H^+ efflux in WT ~1.3-fold and in such a way suggested the participation of F_oF_1 in H^+ secretion (Fig. 3A). The H^+ efflux in the *hyaB, hybC* and *hyaB hybC* mutants compared with that in WT was lowered (Fig. 3A). In opposite to the other mutants, the observably high H^+ efflux was observed in the *fhlA* mutant (see Fig. 3A).

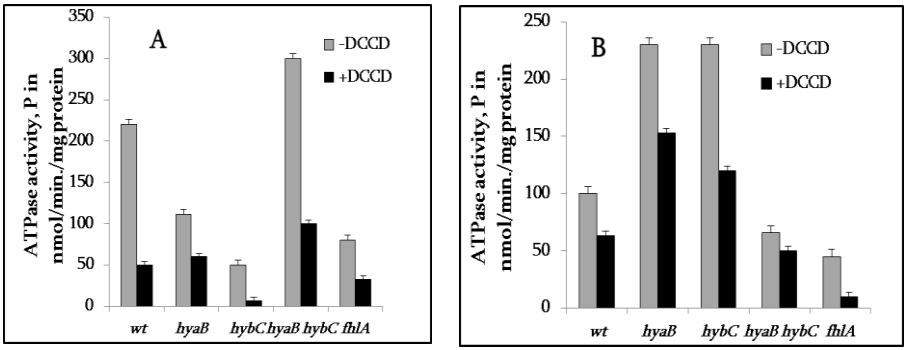
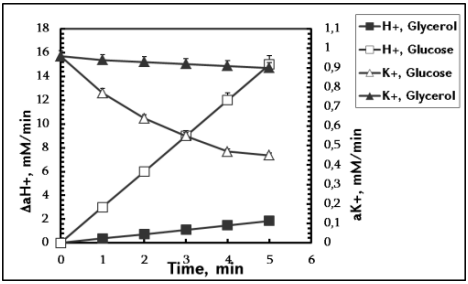


Fig.1. ATPase activity of membrane vesicles of *E. coli* wild type and *fhlA*, *hyaB*, *hybC*, *hyaB hybC* mutant strains at pH 7.5 **(A)** and pH 5.5 **(B).** Mebrane vesicles were treated in the presence of 0.2 mM DCCD. The assays pH was the same as growth pH. RSO membrane vesicles were used. For others see Materials and Methods.

Fig.2. Kinetics of H^+/K^+ exchange by *E. coli* wild type BW25113 during fermentation of glycerol and glucose at pH 7.5. For others see Materials and Methods.



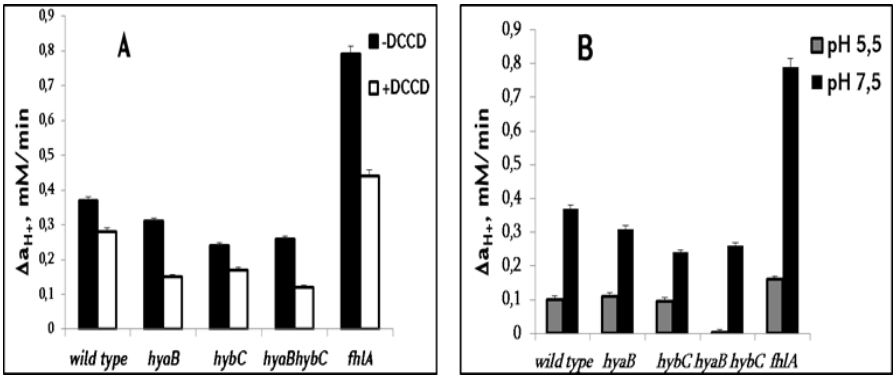


Fig.3. H^+ efflux by whole cells of *E. coli* WT and *hyaB, hybC, hyaB hybC, fhlA* mutant strains during glycerol fermentation at pH 7.5 in the presence of 0.2 mM DCCD **(A)** and pH 5.5 **(B).** For the strains see Table 1.

DCCD inhibited H^+ efflux ~2-fold in *hyaB*, ~1.2-fold in *hybC*, ~2.2-fold in *hyaB hybC* and ~1.8-fold in *fhlA* mutants (see Fig. 3A). It has also been shown that the accumulation of K^+ in these mutants was either low or absolutely absent (not shown). H^+ efflux was also investigated at pH 5.5: it was 3 times lower in with

compared with that at pH 7.5 (Fig. 3B). The H^+ efflux in *hyaB*, *hybC* mutants was similar each other (see Fig. 3B). Interestingly, H^+ efflux was again increased markedly in the *fhlA* mutant and absent in the *hyaB hybC* double mutant compared with that in wild type (see Fig. 3B).

The impact of membrane-bound hydrogenases of *E. coli* on the F_0F_1-ATPase activity upon glycerol and mixed carbon sources fermentation, at different pHs

The activities of FHL and F_0F_1 have been also found out to affect the fermentative metabolism of glycerol in *E. coli* (Trchounian et al., 2012). Some requirement of F_0F_1 and its relationship with Hyd enzymes in *E. coli* were suggested upon both glucose and glycerol fermentations (Trchounian et al., 2011; Blbulyan & Trchounian, 2015).

At this part of the work, we have studied the growth of *E. coli* mutants lacking Hyd enzymes at different pHs. *E. coli* WT and different Hyd mutant strains (see Table 1) could grow well in peptone medium fermenting glycerol only or glycerol added to glucose at pH 7.5, 6.5 and 5.5 (Fig. 4). The cells growth was more intensive during mixed carbon fermentation of glycerol added to glucose (Fig. 4B). This might be explained by different membrane mechanisms for glycerol and glucose transfer into the cells and some differences in their metabolic pathways (Poladyan et al., 2013; Trchounian & Trchounian, 2014). pH 6.5 was optimal for growth of *E. coli* on glycerol whereas specific growth rate was ~1.4-fold higher (Fig. 4A). Specific growth rate was lowered in *E. coli hypF* mutant strain lacking all Hyd enzymes at pH 7.5 and pH 5.5, but not pH 6.5 (see Fig. 4A). Furthermore, specific growth rate during mixed carbon fermentation on glycerol added to glucose was markedly low at pH 5.5 (see Fig. 4B); this rate of *selC* mutant was low at pH 6.5 too (see Fig. 4B). These data suggested the role of Hyd enzymes in bacterial growth on glycerol only or glycerol and glucose mixture depending on pH.

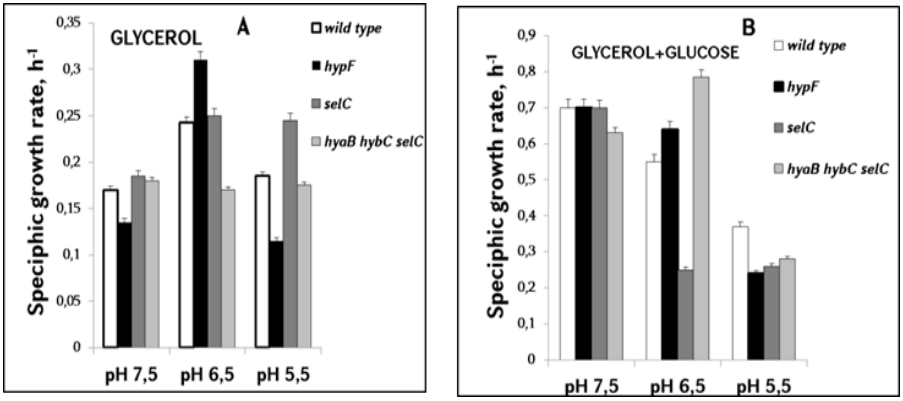


Fig.4. Growth of *E. coli* WT and *selC*, *hyaB hybC selC* and *hypF* mutant strains at different pHs. Bacteria were grown during glycerol **(A)** and glycerol with glucose fermentation **(B)**. For others, see Materials and methods.

The relationship of Hyd enzymes with the F_oF_1-ATPase was studied during the glycerol and mixed carbon sources fermentation. The overall ATPase activity of glycerol-fermented *E. coli* WT and mentioned mutants and its inhibition by DCCD were investigated at different pHs. ATPase activity of RSO vesicles was 223±8 nMol P_i (min μg protein)$^{-1}$. Interestingly, this ATPase activity was 74% of that in ISO membrane vesicles. Moreover, DCCD markedly inhibited ATPase activity of both types of membrane vesicles.

Indeed, ATPase activity of glycerol-fermented *E. coli* was ~3-fold higher in WT at pH 7.5 compared with that at pH 6.5 (Fig. 5). 0.5 mM DCCD inhibited markedly (10-fold) ATPase activity at pH 7.5; but the inhibition was less (1.3-fold) at pH 6.5 (see Fig. 5).

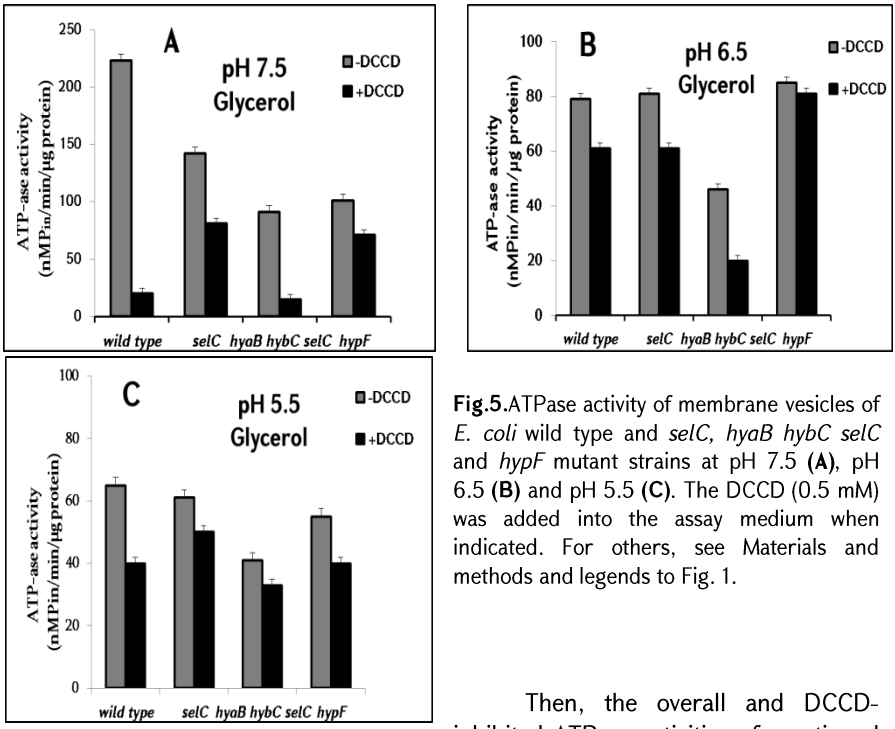


Fig.5.ATPase activity of membrane vesicles of *E. coli* wild type and *selC, hyaB hybC selC* and *hypF* mutant strains at pH 7.5 **(A)**, pH 6.5 **(B)** and pH 5.5 **(C)**. The DCCD (0.5 mM) was added into the assay medium when indicated. For others, see Materials and methods and legends to Fig. 1.

Then, the overall and DCCD-inhibited ATPase activities of mentioned mutants grown under glycerol fermentation at pH 7.5 were decreased compared with WT (see Fig.5A). The overall and DCCD-inhibited ATPase activity of *hyaB hybC selC* triple mutant grown under glycerol fermentation was the lowest at pH 7.5.

At pH 6.5 wt, *selC* and *hypF* mutants had similar ATPase activity but *hyaB hybC selC* mutant had ~1.7-fold lower ATPase activity than WT; the ATPase activity was suppressed ~2.3-fold in this mutant by DCCD (see Fig. 5B). These results indicated that there is no direct relationship between Hyd-3 and the F_0F_1-ATPase upon the fermentation of glycerol at slightly acidic pH.

The ATPase activities were lower at pH 5.5 (Fig. 5C). than at pH 7.5 and pH 6.5 for the mutants used; in the case of *hyaB hybC selC* mutant ATPase activity at pH 5.5 was similar with that at pH 6.5 (see Fig. 5C). At acidic pH a stronger effect of DCCD was observed in *hyaB hybC selC* mutant (see Fig. 5C).These results indicated that acidic pH is not optimal for ATPase activity upon glycerol fermentation. Low overall and DCCD-inhibited ATPase activity of *hyaB hybC selC* mutant again suggests the requirement of Hyd-1 and Hyd-2 for the F_oF_1-activity.

We have examined the ATPase activities of mixed carbon (glucose and glycerol) fermented *E. coli* WT and Hyd mutants (see Table 1). It is worth mentioning that the all strains during mixed carbon sources fermentation showed higher ATPase activity at pH 7.5, 6.5 and 5.5 compared with ATPase activity of cells grown under glycerol only fermentation at appropriate pHs.

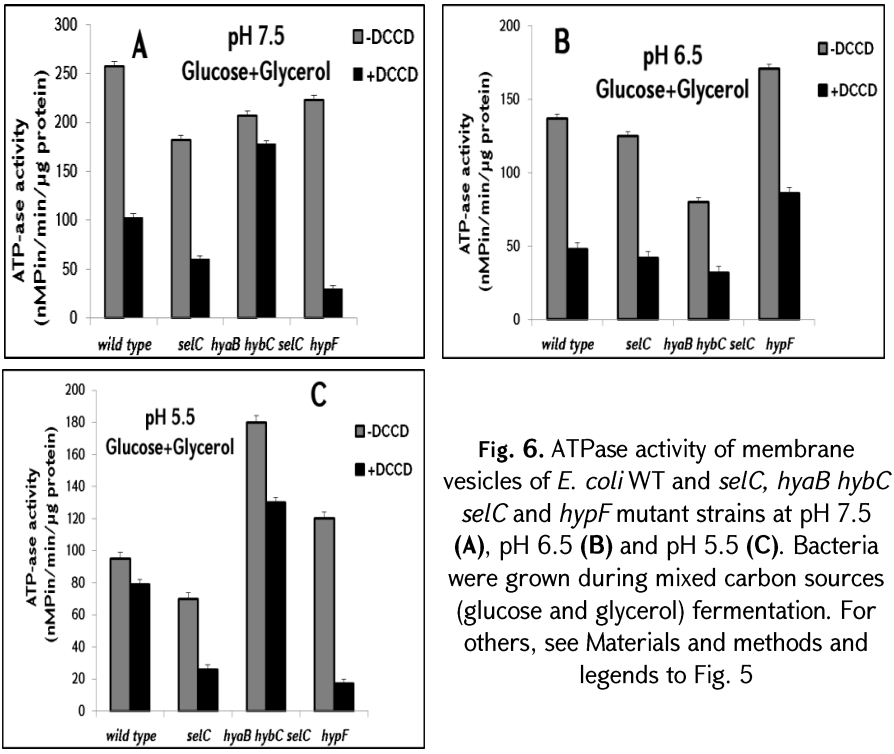


Fig. 6. ATPase activity of membrane vesicles of *E. coli* WT and *selC, hyaB hybC selC* and *hypF* mutant strains at pH 7.5 **(A)**, pH 6.5 **(B)** and pH 5.5 **(C)**. Bacteria were grown during mixed carbon sources (glucose and glycerol) fermentation. For others, see Materials and methods and legends to Fig. 5

Mixed carbon fermented *E. coli* WT cells demonstrated significant overall ATPase activity of 255±2 nMol P_i (min µg protein)$^{-1}$. Interestingly, ATPase activity upon mixed carbon fermentation compared with glycerol fermentation was stimulated (~2.2-fold) in *hypF* mutant at pH 7.5; this activity was closed to WT (Fig. 6A). DCCD markedly inhibited (~7-fold) ATPase activity of this mutant (see Fig. 6A).These results indicated the major input of F_OF_1 in overall ATPase activity upon mixed carbon fermentation at pH 7.5.

In spite of a higher ATPase activity in *hyaB hybC selC* mutant, DCCD inhibition was less whereas DCCD inhibited ATPase activity ~3-fold in *selC* mutant (see Fig. 6A). These results suggested that, during mixed carbon fermentation at slightly alkaline pH, FHL and probably Hyd-3 are more necessary for F_oF_1 activity than Hyd-1 and Hyd-2.

The *hypF* mutant had the highest total ATPase activity at pH 6.5 during the fermentation of glucose and glycerol (Fig. 6B). The WT had ~2-fold lower ATPase activity at pH 6.5 than at pH 7.5. Thus, correlation between total ATPase activity and growth medium pH was also observed upon mixed carbon fermentation as for glycerol only fermentation. The obtained data allowed suggesting that, during mixed carbon fermentation at slightly acidic pH, Hyd-1 and Hyd-2 but not Hyd-3 are required for the F_oF_1-ATPase activity.

The situation upon mixed carbon fermentation was different at pH 5.5. The WT had ~3-fold lower overall ATPase activity than at pH 7.5, while ATPase activities of *hypF* and *hyaB hybC selC* mutants were higher compared with WT (Fig. 6C). Probably, in these conditions (glucose and glycerol fermentation) when end products of fermentation, such as formate and other acids, might be accumulated within the cell and, therefore, decrease intracellular pH, the F_0F_1-ATPase is playing an important role in regulation of intracellular pH by detoxification of different acids formed during fermentation, specifically of formic acid.

Glucose concentration dependent ATP-ase activity in *E. coli* during fermentation and the role of Hyd 4

The previous studies in our laboratory have shown under fermentative conditions F_0F_1 can directly interact with K^+ uptake TrkA system, responsible for K^+ accumulation in the cells and form F_oF_1-TrkA protein-protein supercomplex (Trchounian & Kobayashi, 2000; Trchounian, 2004). Hyd-4 activity have been shown to depend on glucose concentration and seen during glucose low concentration (Trchounian & Trchounian, 2014). But the dependence of Hyd activity on K^+ was not clear.

We have investigated dependence of the F_oF_1 activity of *E. coli* on glucose concentration under different conditions determining Hyd-4 input in such dependence if any. The DCCD-sensitive ATPase activity of membrane vesicles from glucose-fermented (0.2% and 0.8%) *E. coli* WT and *hyfB-R* mutant was investigated at different pHs. These activities were increased in both cases ~1.4-fold and ~1.2-fold, respectively, by 100 mM K^+ compared to that at the absence of K^+ (Fig.7A). 0.2 mM DCCD strongly inhibited ATP-ase activity of both strains at the presence of K^+ (0.2% glucose) (Table 2). These data confirmed a role of Hyd-4 during glucose fermentation at slightly alkaline pH that is in maintaining of proton-motive force as suggested before (Trchounian & Sawers, 2014; Trchounian & Trchounian, 2014). In contrast to 0.2% glucose fermentation at pH 7.5, during growth on 0.8% glucose in K^+-free medium, the overall and DCCD-sensitive ATPase activities of WT were approximately 18-22 % higher (Fig.7, B). These results indicated that F_oF_1-ATPase

activity is glucose-concentration dependent: F_0F_1 is active mainly at low concentration of glucose (0.2%) at pH 7.5.

Table 2. DCCD-inhibited ATPase activities of membrane vesicles of *E. coli* wild type and mutant grown under different concentrations of glucose fermentation

Strain	DCCD-inibited ATPase activity, %*							
	pH 7.5				pH 6.5			
	0.2% Glucose		0.8% Glucose		0.2% Glucose		0.8% Glucose	
	$-K^+$	$+K^+$	$-K^+$	$+K^+$	$-K^+$	$+K^+$	$-K^+$	$+K^+$
wt	79±1	85±1	86±1	73±2	84±1	92±1	84±1	63±2
hyfB-R	82±1	87±1	92±1	82±2	85±2	84±1	67±1	87±2

*DCCD-inhibited ATPase activity was calculated as percentage of overall ATPase activity which was 100 % for *wt* and *hyfB-R* at appropriate pH during glucose fermentation.

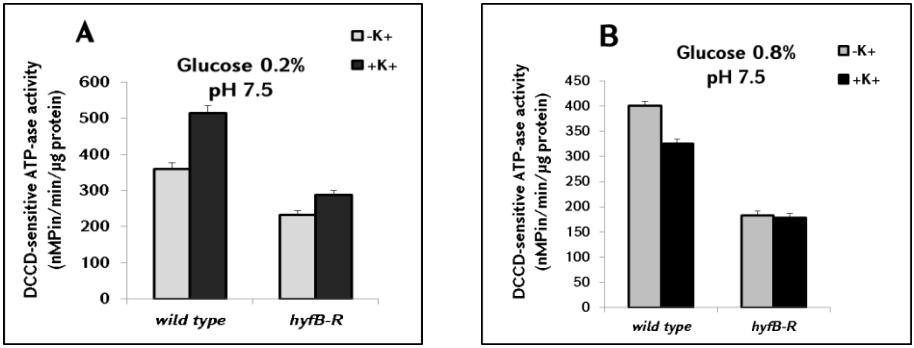


Fig. 7. ATPase activity of membrane vesicles of *E. coli* wild type and *hyfB-R* mutant grown on peptone medium supplemented with 0.2% glucose **(A)** and 0.8% glucose **(B)** at pH 7.5. The DCCD (0.2 mM) was added into the assay medium when indicated. For others, see Materials and methods

Upon 0.8% glucose *hyfB-R* demonstrated ~2.2-fold lower ATPase activity in K^+-free medium, compared to wild type, and K^+ had no effect on ATPase activity (see Fig.7B). Under 0.2% glucose fermentation WT demonstrated lower ATPase activity in both cases, in K^+-free and in K^+-contented media at pH 6.5, compared with those at pH 7.5 (comp. Figs.8A, with 8 B). K^+ had slight effect (~1.2-fold) on the DCCD-sensitive ATPase activity of WT, whereas DCCD markedly inhibited ATPase activity ~7-fold and ~12-fold in K^+-free and in K^+-contented assay buffers, respectively (see Table 2).

HyfB-R mutant also showed similar DCCD-sensitive ATPase activity, as WT, in K^+-free medium (see Fig. 8A). Note, that K^+ had no effect on ATPase activity of *hyfB-R*; moreover, it was decreased in K^+-containing medium (see Fig. 8A). The obtained results allowed suggestion of a mediated role of Hyd-4 for F_0F_1-TrkA association. Then, in 0.8% glucose fermented cells, compared with 0.2% glucose fermented cells, DCCD-sensitive ATPase activity at pH 6.5 in K^+-free medium, was lower ~1.2-fold in wt and ~4-fold in *hyfB-R* mutant (Fig. 8). In K^+-containing medium wt showed ~1.6-fold lower ATPase activity compared with that in K^+-free medium, whereas in mutant

K^+ increased ATPase activity ~1.7-fold (see Fig.8B). These data pointed out a relationship between F_oF_1 and K^+-uptake upon 0.8% glucose fermentation at pH 6.5 but Hyd-4 had no any role.

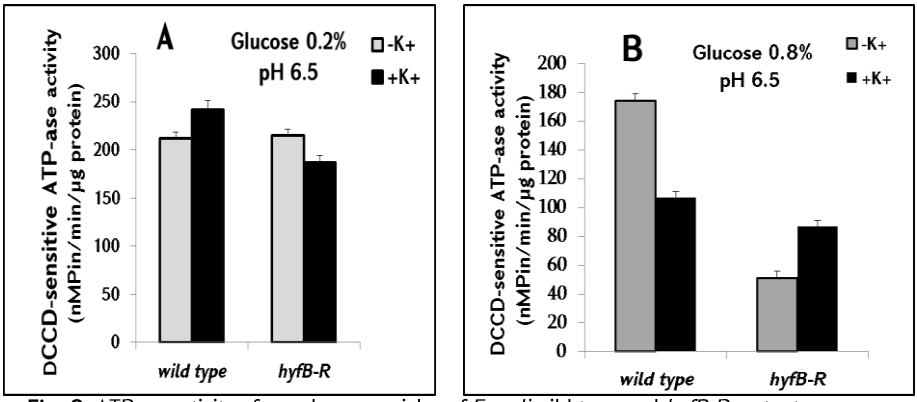


Fig. 8. ATPase activity of membrane vesicles of *E. coli* wild type and *hyfB-R* mutant grown on peptone medium supplemented with 0.2% glucose **(A)** and 0.8% glucose **(B)** at pH 7.5. The DCCD (0.2 mM) was added into the assay medium when indicated. For others, see Materials and methods

G. toebii growth, ORP kinetics and ATPase activity

Geobacilli have a potential worldwide distribution, as they are able to grow in various environments where redox processes are performed. However, nothing is known about redox processes and ATPase activity or redox stress in *Geobacillus*. The maximal growth yield of *G. toebii* ArzA-8 (OD ~0.9) was observed in NB medium rich in organic substrates (see Materials and methods) without glucose supplementation; some differences were detected between added glucose concentrations of 5, 11 and 22 mM (Fig. 9).

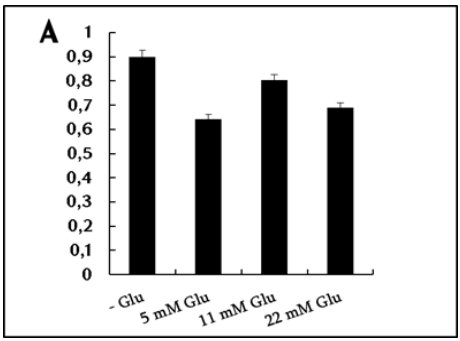


Fig.9. The changes in absorbance (A) during *G. toebii* ArzA-8 growth in the absence of glucose (–Gl) and presence of glucose (Gl) at different concentrations (5, 11 and 22mM), pH 7.5. A_{600} was presented after 5 h of bacterial growth.

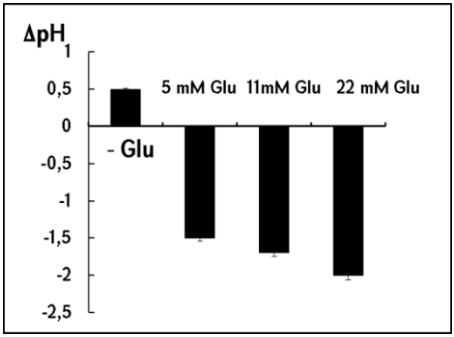


Fig. 10. The changes in the medium pH during *G. toebii* ArzA-8 growth in the absence and presence of glucose at different concentrations. ΔpH is a difference between initial pH of growth medium and pH after 8 h of growth.

Simultaneously, alkalization of the growth medium was also observed in the absence of glucose: after 24 h of growth, the pH was 8.4. However, an acidification effect was detected in the presence of 5, 11 or 22 mM glucose at the end of the log growth phase: the growth medium pH decreased to 5.9, 5.7 or 5.4, respectively (Fig.10).

We have examined the evaluation of ORP kinetics by the bacteria during their growth in NB medium in the absence and presence of glucose and the effects of various oxidizers and reducers at different pHs. A decrease in the ORP was observed during the bacterial log growth phase, as measured by the redox electrodes (see Materials and methods) at both pH 6.5 and pH 7.5 (Fig. 11).

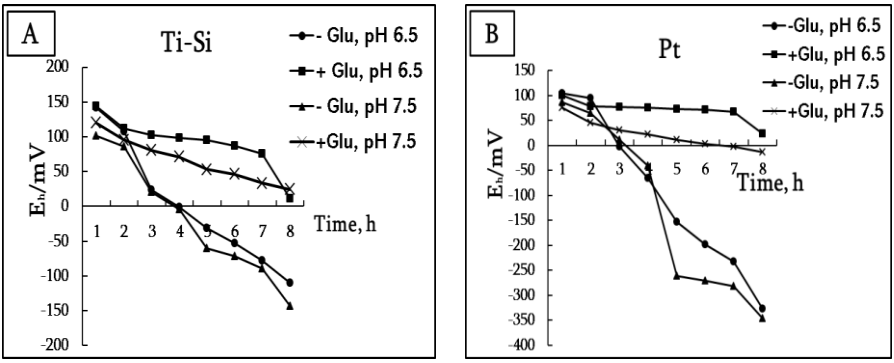


Fig. 11. The oxidation–reduction potential (ORP) kinetics during *G. toebii* ArzA-8 growth in the absence and presence of glucose (5mM) at pH 6.5 and pH 7.5. The ORP was measured by **(A)** E_h (Ti–Si electrode) and **(B)** E_h' (Pt electrode).

The investigation of the ORP kinetics revealed remarkable differences: E_h decreased to a negative value of –150±5 mV and E_h' to –350±4 mV with an increase of pH without glucose but increased to positive values of +111±3 mV and +80±3 mV with a decrease in pH when glucose was added at pH 7.5.

A determinant role of the ORP for bacterial growth means that various oxidizers and reducers affecting the ORP can mediate the growth of bacteria. The oxidizer potassium ferricyanide ($K_3[Fe(CN)_6]$) and the reducer dithiothreitol (DTT) were used. Indeed, both reagents inhibited the growth of *G. toebii*: in medium without glucose supplementation, 1 or 2 mM $K_3[Fe(CN)_6]$ stimulated growth at the beginning of the log growth phase but inhibited (~1.3-fold) subsequent bacterial growth (not shown). In conditions without glucose, the inhibitory effect of DTT (3 mM) on *G. toebii* growth was stronger (~4.4-fold).

We have also studied ATPase activity of *G. toebii*. The overall ATPase activity of *G. toebii* membrane vesicles was ~532±0.05 nMol Pi/min·μg protein for cells grown in the absence of glucose, which was ~1.3-fold higher compared with cells grown on glucose (Fig.12, A). 0.5 mM DCCD markedly inhibited total ATPase activity (~3-fold) (see Fig.12, A). 3 mM DTT repressed total ATPase activity by ~1.23-fold and the

DCCD-inhibited activity by ~5-fold in cells grown in the absence of glucose compared with the control, without reagent supplementation (see Fig.12, A).

It is significant that DTT stimulated the F_oF_1-ATPase activity ~1.24-fold in the cells grown on glucose compared with the control, without reagent supplementation (Fig. 12,B). Thus, the DTT stimulation effect could be attributed to the F_oF_1-ATPase.

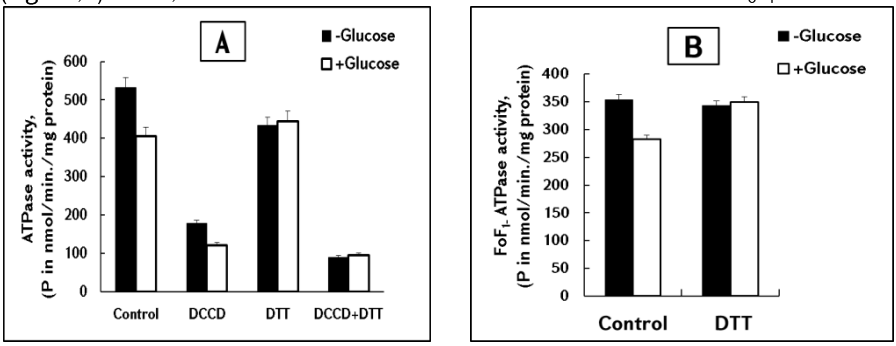


Fig. 12. Membrane vesicle ATPase activity of *G. toebii* ArzA-8. (**A**) Total ATPase activity; (**B**) the F_oF_1-ATPase activity. Bacteria were grown at pH 7.5. F_OF_1-ATPase activity was the DCCD-inhibited one, which was calculated as the difference between total ATPase activity and ATPase activity in the presence of DCCD. Control was without inhibitor or reducer supplementation

CONCLUDING REMARKS

Within the frame of this work was revealed that upon glycerol fermentation by *E. coli* at slightly alkaline pH ATPase activity of membrane vesicles as well as H^+ efflux and K^+ accumulation rates of whole cells are lowered than those for cells fermenting glucose: F_OF_1 participates in H^+ efflux and it is probably operates independently of TrkA. At pH 7.5 Hyd-1 and Hyd-2 could have close relationship to F_OF_1 or participate in H^+ translocation whereas Hyd-3 and Hyd-4 might operate in H^+ uptake mode. At pH 5.5 Hyd-1 and Hyd-2 more than F_oF_1 might be involved in H^+ efflux.

The results obtained indicate a correlation between membrane ATPase activity in *E. coli* and pH: alkaline pH is more optimal for the DCCD-inhibited F_oF_1-ATPase activity during glycerol fermentation. This is probably related with bacterial optimal growth at pH 7.5. Moreover, the F_oF_1-ATPase has a different role in maintaining proton motive force depending on Hyd enzymes as suggested (Trchounian et al., 2012; Trchounian & Sawers, 2014). The results with the *hyaB hybC selC* triple mutant suggest the requirement of Hyd-1 and Hyd-2 for the activity of the F_oF_1-ATPase during glycerol fermentation, and cooperation or interaction between these enzymes is more obvious at slightly alkaline pH. The higher ATPase activity of triple and *selC* mutants upon the mixed carbon (glucose and glycerol) fermentation at pH 7.5 presumably can be explained by that, during mixed carbon fermentation, the F_oF_1-ATPase is involved in the formation of H_2 cycling through the membrane as suggested (Trchounian & Sawers, 2014). It is possible that, when Hyd-3 is not active as a part of FHL, F_oF_1 operates for regulation of intracellular pH. Thus, the present results are important in understanding the role of F_oF_1 and Hyd enzymes in cells and especially the functional link between Hyd-1, Hyd-2 and F_oF_1 during anaerobic fermentation of glycerol and mixed carbon sources. It is also worth mentioning that, depending on fermentation substrate and pH, the form of relationship between these enzymes could be different.

These findings support F_oF_1–TrkA association formed during fermentative conditions at low concentration of glucose (0.2 %) and at alkaline pH in *E. coli*. It was suggested that Hyd-4 could be linked to F_oF_1 supplying reducing equivalents for energy transfer (Trchounian et al., 2014; Blbulyan, 2016).

The obtained results shown that during *G. toebii* growth, ORP kinetics doesn't depend on the initial pH variation in the medium (Ghazaryan et al., 2015). ATPase activity was quite high in *G. toebii*. It is of interest that different levels of ATPase activity were observed with cells in relation to glucose availability: reducing conditions suppressed total ATPase activity, but not F_0F_1 activity without glucose supplementation. These results indicate a role of F_oF_1 in bacterial physiology and ecology, especially in redox sensing by this bacterium. The ORP can clearly modify metabolic fluxes and might be a further environmental physicochemical parameter to be taken into account for the optimization of metabolic processes and the application of thermophiles in biotechnology.

These findings are novel for investigated bacteria and allow clarifying the interaction between F_oF_1 and different Hyds, as well as their functional link with secondary transport systems. Moreover, the received data will broaden the understanding the role and interaction nature mentioned above systems.

CONCLUSIONS

The following conclusions were made based on experimentally obtained results:

1. *E. coli* ATPase activity during glycerol fermentation, at pH 7.5, compared glucose is lower: Hyd-1 and Hyd-2 have close relationship to F_oF_1 and participate in H^+ translocation.
2. There is a direct correlation between *E. coli* ATPase activity and growth medium pH under glycerol fermentation: the highest activity was observed at pH 7.5, but lowest- at pH 5.5. In addition, 0.5 mM DCCD inhibition impact is more significant at pH 7.5. Moreover, Hyd-1 and Hyd-2 are required for the F_oF_1-ATPase activity under glycerol fermentation.
3. During mixed carbon (glucose and glycerol) sources fermentation, at pH 7.5, in total ATPase activity is essential the role of F_oF_1-ATPase. The absence of all Hyds have does no effect on total ATPase activity but increases F_oF_1-ATPase activity which indicates that upon mixed carbon sources fermentaion Hyds effect on F_oF_1-ATPase. At the same time, again, this activity is dependent on medium pH.
4. The high concentration of glucose (0.8%) impacts on *E. coli* F_oF_1-ATPase activity and its association with Hyd-4, as well as with secondary transporters, such as TrkA.
5. 100mM K^+ increased ATPase activity of wild type only at glucose limited conditions (0.2%) under fermentation at pH 7.5 and pH 6.5. Functional link between F_oF_1-ATPase and TrkA is depended on glucose concentration and external pH.
6. The maximal growth yield of *G. toebii* ArzA-8 (OD ~ 0.9) was observed in NB medium without glucose supplementation. At the same time alkalization of the growth medium was also observed. The decrease of ORP was observed parallel to the pH growth, whereas at low pH and with glucose supplementation ORP increased to the positive values.
7. In medium without glucose 1 or 2 mM potassium ferricyanide and 3 mM DTT inhibited growth of *G. toebii* ArzA-8. The decrease of ORP to the negative values was observed, compared to the control. DTT (reducing conditions) repressed total ATPase activity but not activity of F_oF_1-ATPase. While DTT stimulated F_oF_1-ATPase activity in bacteria upon glucose supplementation.
8. The bacterial F_oF_1-ATPase activity is characterized by interaction with Hyds, dependence on glycerol and glucose oxidation nature, and by regulation with oxidizer and reducer.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

1. **Blbulyan S.**, Trchounian A. Influence of glucose concentration on *Escherichia coli* F_oF_1-ATPase and hydrogenase 4 (hyf) enzymes activities // *FEBS Congress,* Jerusalem, Israel, **2017**, p. 156. doi:10.1111/febs.14174
2. **Blbulyan S.**, Trchounian A. *Escherichia coli* F_oF_1-ATPase activity and its cooperation with hydrogenase 4 (hyf) depend on glucose concentration// 7th *FEMS Congress,* Valencia, Spain, **2017**, p. 054.
3. Poladyan A., Sahakyan M., **Blbulyan S.,** Trchounian K., Trchounian A. Glucose concentrations influence on activities of F_oF_1-ATPase and hydrogenase 4 in *Escherichia coli // 19th IUPAB and 11th EBSA Congress,* Edinburgh, UK, **2017**, p. S289.
4. **Blbulyan S.** Glucose concentration dependent ATP-ase activity in *Escherichia coli* during fermentation and the role of Hydrogenase 4 //*Biological Journal of Armenia*, **2016,** 68, p.85-91.
5. **Blbulyan S.,** Trchounian A. *Escherichia coli* membrane-associated ATPase activity and its interaction with hydogenase 4 (*hyf*) depend on glucose concentration // *4th Int. Workshop on Expression, Structure and Function of Membrane Proteins*, Florence, Italy, **2015**, p. 60.
6. Ghazaryan A., **Blbulyan S.,** Poladyan A., Trchounian A. Redox stress in geobacilli from geothermal springs: phenomenon and membrane-associated response mechanisms. // *Bioelectrochemistry*, **2015**, 105, p. 1-6.
7. **Blbulyan S.,** Trchounian A. Impact of membrane-associated hydrogenases on the F_oF_1-ATPase in *Escherichia coli* during glycerol and mixed carbon fermentation: ATPase activity and its inhibition by *N,N'*-dicyclohexylcarbodiimide in the mutants lacking hydrogenases // *Arch. Biochem. Biophys.*, **2015**, 579, p. 67-72.
8. **Blbulyan S.**, Trchounian A. Role of *Escherichia coli* hydrogenases in the F_oF_1-ATPase activity under mixed carbon fermentation at different pHs // *BBA-Bioenergetics, Suppl. to volume* 1837, **2014**,p. e107-e108, doi:10.1016/j.bbabio.2014.05.257
9. **Blbulyan S.,** Trchounian A. Proton translocating ATPase activity of *Escherichia coli* membrane vesicles under mixed carbon fermentation at alkaline and acidic pHs // *FEBS Journal*, 281, suppl. s1, **2014**, p.594, doi: 10.1111/febs.12919
10. **Blbulyan S.,** Poladyan A., Trchounian A. Role of the proton translocating ATPase in redox sensing of thermophilic *Geobacilli* isolated from Armenian mineral springs // Abstracts book of conference: *Trends in microbiology and microbial biotechnology*, **2014**, p. 48, Yerevan, Armenia**.**
11. **Blbulyan S**. and Trchounian A. *Escherichia coli* F_0F_1-ATPase activity under glycerol fermentation at different pH and role of hydrogenases // *FEBS Journal*, 280, suppl. 1, **2013**, p.599-600. doi: *10.1111/febs. 12339*.
12. Trchounian K., **Blbulyan S.,** Trchounian A. Hydrogenase activity and proton-motive force generation by *Escherichia coli* during glycerol fermentation // *J Bioenerg Biomembr,* **2013**, 45, p. 253-60. doi: 10.1007/s10863-012-9498-0.
13. **Blbulyan S.**, Trchounian A. *Escherichia coli* membrane vesicles F_oF_1-ATPase activity under glycerol fermentation at alkaline and acidic pH // *BBA-Bioenergetics,* **2012**, *Suppl. to volume* 1817, S131-S132.*doi: 10.1016/j.bbabio.2012.06.349*.
14. **Blbulyan S.**, Avagyan A., Poladyan A., Trchounian A. Role of *Escherichia coli* different hydrogenases in H^+ efflux and F_oF_1-ATPase activity during glycerol fermentation at different pH // *Biosci Rep*, **2011**, 31,p. 179-184.doi: http://dx.doi.org/10.1042/BSR20100053
15. **Blbulyan S.**, Poladyan A., Trchounian A. The *Escherichia coli* membrane vesiclesF_0F_1-ATPase activity under glycerol fermentation at alkaline and acidic pH. *Proceedings in Int. Symposium: "Solvation and Ionic Effects in Biomolecules: Theory to Experiment"*, **2010**, Tsakhkadzor, Armenia, p. 59-60.
16. **Blbulyan S.**, Poladyan A., Trchounian A. Converting energy by anaerobically grown *Escherichia coli* during fermentation of glucose and glycerol at acidic and alkaline pH. *Materials of the 13th Int. Youth Scientific Conf.: "Biology - the science of the XXI century"*, Pushino, **2009**, p.133-134 (in Russian).

ԲԼԲՈՒԼՅԱՆ ՍՅՈՒԶԱՆՆԱ ՍՈՒՐԵՆԻ

ՈՐՈՇ ԲԱԿՏԵՐԻԱՆԵՐԻ ԱԵՖ-ԱԶԱՅԻՆ ԱԿՏԻՎՈՒԹՅԱՆ ԲՆՈՒԹԱԳՐՈՒՄԸ ԳԼԻՑԵՐՈԼԻ և ԳԼՅՈՒԿՈԶԻ ՕՔՍԻԴԱՑՄԱՆ ՊԱՅՄԱՆՆԵՐՈՒՄ

Ամփոփագիր

Հանգույցային բառեր՝ *Escherichia coli*, *Geobacillus toebii*, ԱԵՖ-ազային ակտիվություն, գլիցերոլի և գլյուկոզի խմորում, հիդրոգենազներ (Հիդ), H^+-K^+-ական փոխանակություն, օքսիդավերականգնողական պոտենցիալ:

Աղիքային ցուպիկում (*E. coli*) խմորման ընթացքում առաջացած մրջնաթթուն՝ թաղանթակապ մրջնաթթու-ջրածին-լիազ (ՄՋԼ) ֆերմենտային համալիրի միջոցով օքսիդանում է մինչև ածխաթթու գազի (CO_2) և մոլեկուլային ջրածնի (H_2): Այս գործընթացում ընդգրկված հատուկ ֆերմենտների՝ հիդրոգենազների էներգետիկ պահանջների համար անհրաժեշտ է էներգիաապահովման տեսանկյունից կարևորագույն ֆերմենտի՝ պրոտոնային F_oF_1-ԱԵՖ-ազի գործունեությունը (Bagramyan and Trchounian, 2003):

Ներկայացված աշխատանքում առաջին անգամ ուսումնասիրվել է գլիցերոլի խմորման պայմաններում *E. coli*-ի ԱԵՖ-ազային ակտիվության առանձնահատկությունները, ինչպես նաև փորձ է արվել բացատրել պրոտոնային ԱԵՖ-ազի և հիդրոգենազների գործառական կապը խմորման և pH-ի տարբեր արժեքների պայմաններում: Ուսումնասիրվել է նաև ջերմասեր *G. toebii* ArzA-8 տեսակի աճը, օքսիդավերականգնողական պոտենցիալի (ՕՎՊ) կինետիկան և pH-ի փոփոխությունները, ինչպես նաև պրոտոնի տեղափոխությունն ամբողջական բջիջներում ՝ օքսիդիչի և վերականգնիչի առկայության պայմաններում: Այդ համատեքստում ջերմասեր *G. Toebii*-ի վերոհիշյալ ֆիզիոլոգիական գործընթացները համեմատվել են մեզոֆիլ *E.coli*-ի հետ:

Ցույց է տրվել, որ գլիցերոլի խմորման պայմաններում միջավայրի pH-ն ազդում է *E. coli* բակտերիաների F_oF_1-ԱԵՖ-ազի ակտիվության վրա, ընդ որում ամենաբարձր ակտիվություն դիտվել է pH 7.5-ում: Գլիցերոլի խմորում իրականացրած *E. coli*-ի BW25113 նախնու թաղանթային բշտիկների ԱԵՖ-ազային ակտիվությունը՝ համեմատած գլյուկոզի խմորման հետ, ցածր է գրեթե երկու անգամ: Այս ակտիվությունը զգալիորեն ճնշվում է F_oF_1-ի արգելակիչ *N,N'*-դիցիկլոհեքսիլկարբոդիիմիդի (0.2 մՄ ԴՑԿԴ)-ի ազդեցությամբ pH 7.5-ում: *hyaB* և *hybC* մուտանտների (բացակայում են, համապատասխանաբար, Հիդ-1-ի և Հիդ-2-ի մեծ ենթամիավորները) ընդհանուր և ԴՑԿԴ-զգայուն ցածր ԱԵՖ-ազային ակտիվության տվյալները թույլ են տվել ենթադրել, որ վերոհիշյալ պայմաններում՝ Հիդ-2-ը ավելին, քան Հիդ-1-ը, ունի ամուր փոխազդեցություն F_oF_1-ԱԵՖ-ազի հետ: Ստացված տվյալները վկայում են, թույլ թթվային pH-ի (5.5) և գլիցերոլի խմորման պայմաններում, F_oF_1-ԱԵՖ-ազի և ՄՋԼ համալիրի գործառական կապի մասին:

Ուսումնասիրվել է նաև *E. coli*-ի վայրի տիպի և նրա Հիդ-ային մուտանտների ԱԵՖ-ազային ակտիվությունը ածխածնի խառն աղբյուրների՝ գլյուկոզի և գլիցերոլի խմորման պայմաններում, pH-ի 7.5, 6.5 և 5.5 արժեքների դեպքում: Բոլոր շտամները խմորման այս պայմաններում և pH-ի վերոհիշյալ արժեքների դեպքում, ցուցաբերել են ավելի բարձր ԱԵՖ-ազային ակտիվություն՝ քան միայն գլիցերոլի խմորման դեպքում:

Ընդհանրացնելով ստացված արդյունքները՝ կարելի է եզրակացնել, որ *E. coli*-ի ԱԵՖ-ազային ակտիության և աճման միջավայրի pH-ի միջև կա ուղիղ կապ: Գլիցերոլի խմորման պայմաններում հիմնային pH-ը առավել օպտիմալ է ԴՑԿԴ-զգայուն ԱԵՖ-ազային ակտիվության համար:

Ուսումնասիրվել է F_oF_1-ԱԵՖ-ազի և երկրորդային տեղափոխիչ համակարգի՝ TrkA-ի, գործառական կապը գլյուկոզի տարբեր կոնցենտրացիաների և արտաքին pH-ի տարբեր արժեքների պայմաններում: Ցույց է տրվել, որ F_oF_1- TrkA գերհամալիրը ձևավորվում է գլյուկոզի ցածր կոնցենտրացիայի խմորման պայմաններում, հիմնային pH-ում:

Հայտնի է, որ բնության մեջ, որտեղ սահմանափակ են մանրէների աճի պայմանները, ջերմասերները՝ իրենց մենահատուկ ֆերմենտներով ցուցաբերում են տարբեր նյութափոխանակային ուղիներ:

Ածխածնի աղբյուրի (գլյուկոզ) առկայությունից կախված, *G. toebii* բակտերիայի ՕՎՊ-ի և pH-ի կինետիկայում ստացվել են զգալի տարբերություններ: Գլյուկոզի ներկայությամբ աճի ընթացքում դիտվում է միջավայրի թթվեցում և ՕՎՊ-ի դրական արժեք, մինչդեռ գլյուկոզի բացակայությամբ, երբ միջավայրը հիմնայնացվում է, գրանցվում են ՕՎՊ-ի ցածր բացասական արժեքներ

Հետաքրքրական է, որ *G. toebii*-ի ԱԵՖ-ազային ակտիվությունը բավականին բարձր էր: Ստացված արդյունքները ցույց են տալիս F_oF_1-ԱԵՖ-սինթազի դերը այս բակտերիայի ֆիզիոլոգիական և էկոլոգիական գործընթացներում, մասնավորապես ռեդօքս զգայնության մեջ: Հնարավոր է, որ բակտերիայի աճի ընթացքում ռեդօքս ռեագենտների ցուցաբերած ազդեցությունները պայմանավորված են հենց F_oF_1-ԱԵՖ-սինթազով: Հարկ է նշել, որ *G.toebii*-ի համար ստացված տվյալները լիովին տարբերվում էին *E. coli*-ի համար ստացված տվյալներից:

Կատարված հետազոտությունները թույլ են տալիս պարզաբանել F_oF_1-ի և Հիդ-ների, ինչպես նաև երկրորդային տեղափոխիչ համակարգերի գործառական կապը, փոխազդեցությունը, վերջինիս բնույթը տարբեր սուբստրատների խմորման պայմաններում և pH-ի տարբեր արժեքներում: Ստացված տվյալները կխորացնեն վերոհիշյալ համակարգերի մասին ունեցած պատկերացումները: Հաշվի առնելով *G.toebii*-ի ցուցաբերած արդյունքները, կարելի է ասել, որ ՕՎՊ-ի կարգավորումը, կարող է մեծացնել ջերմասեր բատերիաների կենսատեխնոլոգիական կիրառելիությունը, ինչպես կենսազանգվածի, այնպես էլ տարբեր արժեքավոր նյութերի ստացման նպատակով:

БЛБУЛЯН СЮЗАННА СУРЕНОВНА

ХАРАКТЕРИСТИКА АТФАЗНОЙ АКТИВНОСТИ НЕКОТОРЫХ БАКТЕРИЙ В УСЛОВИЯХ ОКИСЛЕНИЯ ГЛИЦЕРИНА И ГЛЮКОЗЫ

РЕЗЮМЕ

Ключевые слова: *Escherichia coli*, *Geobacillus toebii*, АТФазная активность, сбраживание глюкозы и глицерина, гидрогеназы (Гид), H^+-K^+-обмен, окислительно-восстановительный потенциал

Муравьиная кислота, образующаяся в кишечной палочке (*E. coli*) в процессе брожения, окисляется посредством формиат-водород-лиазного комплекса (ФВЛ) с образованием углекислого газа (CO_2) и молекулярного водорода (H_2). Для энергетических затрат, вовлеченных в данный процесс ферментов – гидрогеназ, необходимо участие другого важнейшего фермента – протонной F_oF_1-АТФазы (Bagramyan and Trchounian, 2003).

В представленной работе впервые исследованы особенности АТФазной активности *E. coli* в условиях сбраживания глицерина, а также сделана попытка выявления функциональной связи между протонной АТФазой и гидрогеназами при различных значениях pH среды.

Исследованы также рост, кинетика окислительно-восстановительного потенциала (ОВП) и изменения pH среды, перенос протона в присутствии окислителя и восстановителя в термофильной бактерии *G. toebii* ArzA-8. При этом перечисленные физиологические процессы в термофильной *G. toebii* сравнивались с мезофильной *E. coli*.

Показано, что при сбраживании глицерина, pH среды влияет на активность протонной F_oF_1-АТФазы *E.coli,* при этом наиболее высокая активность проявляется при pH 7.5. АТФазная активность мембранных везикул *E. coli* BW25113 при сбраживании глицерина была ниже почти в 2 раза, по сравнению с активностью при сбраживании глюкозы. Активность значительно подавлялась в присутствии ингибитора F_0F_1-АТФазы – *N,N'*-дициклогексилкарбодиимида (0.2 мМ ДЦКД) при pH 7.5. В *hyaB* и *hybC* мутантах (отсутствуют большие субъединицы Гид-1 и Гид-2, соответственно) общая и низкая ДЦКД-чувствительная АТФазные активности позволяют предположить, что в данных условиях Гид-2 сильнее взаимодействует с F_oF_1-АТФазой, чем Гид-1. Полученные данные свидетельствуют о наличии функциональной связи между F_0F_1-АТФазой и ФВЛ комплексом при слабокислом pH (5.5) и сбраживании глицерина.

Исследованы также АТФазные активности дикого вида *E. coli* и Гид-ых мутантов при сбраживании смешанных источников углерода – глюкозы и глицерина, при pH 7.5, 6.5 и 5.5. При этом все штаммы при данных значениях

pH обладали более высокой АТФазной активностью, по сравнению с глицерином.

Обобщая полученные результаты, можно предположить существование прямой связи между АТФазной активностью *E. coli* и pH среды роста. При сбраживании глицерина щелочной pH является наиболее оптимальным для ДЦКД-чувствительной АТФазной активности.

Исследована функциональная связь между F_oF_1-АТФазой и системой вторичного транспорта - TrkA-системой при различных концентрациях глюкозы и разных значениях pH внешней среды. Показано, что комплекс F_oF_1-TrkA формируется при низких концентрациях глюкозы и щелочном pH.

Как известно, в природе, где условия роста микроорганизмов ограничены, термофилы с присущими им специфическими ферментами обладают разными метаболическими путями.

В зависимости от источника углерода (глюкоза), кинетики ОВП и pH в бактерии *G. toebii* резко отличаются. При росте в присутствии глюкозы наблюдается закисление среды и положительные значения ОВП, тогда как в отсутствие глюкозы, при защелачивании среды регистрируются низкие отрицательные значения ОВП. Интересным является тот факт, что *G. toebii* обладает довольно высокой АТФазной активностью. Полученные результаты показывают роль F_oF_1-АТФазы в физиологических и экологических процессах данной бактерии, в частности, в редокс-чувствительности. Возможно, что эффекты редокс реагентов при росте бактерии связаны именно с F_oF_1-АТФсинтазой. Нужно отметить, что полученные для *G. toebii* данные полностью отличаются от результатов, полученных для *E. coli*.

Проведенные исследования позволяют выявить функциональную связь между F_oF_1-АТФазой и гидрогеназами, а также с системами вторичного транспорта, взаимодействия между ними, их характеристики при сбраживании различных субстратов и при различных значениях pH. Полученные данные расширят имеющиеся представления о данных системах. Учитывая результаты, полученные при исследовании *G. toebii,* можно утверждать, что регулирование ОВП может увеличить биотехнологическое применение термофильных бактерий с целью получения как биомассы, так и различных ценных продуктов.