Biolog. Journal of Armenia, 1 (69), 2017

BIO-HYDROGEN PRODUCTION BY *RHODOBACTERSPHAEROIDES*DURING MIXEDCARBON FERMENTATION

L.Y. HAKOBYAN[1], L.S. GABRIELYAN[1,2], A.H. TRCHOUNIAN[1,2]

Yerevan State University,1Research Institute of Biology
;2Department of Biochemistry, Microbiology and Biotechnology,
lilit_hy@ysu.am

Bio-hydrogen (H_2) production is considered as one of the promising ways to generate ecologically clean and renewable energy from various cheap and effective carbon sources like glycerol or industrial wastes. Experiments were carried out to reveal the H_2 production by Rhodo bacter sphaeroides MDC6521 during mixed carbon (succinate, glucose and glycerol) fermentation and the input of the F_oF_1-ATPase in this process. The results show that bacteria grown in a medium containing glucose and glycerol (15 mM) in addition to succinate (15 mM), exhibit an increase not only in H_2 production, but alsoin F_oF_1-ATPase activity of membrane vesicles obtained from them. Significant ~1.7 fold increase of DCCD sensitive ATPase activity was obtained with glycerol addition compared with control (30 mM succinate), and since it had a positive effect also on H_2 yield, further experiments will be performed to obtain the optimal succinate/glycerol ratio to get the maximum H_2 yield.

Bio-hydrogen production–Rhodo bacter sphaeroides - mixed carbon fermentation

Կենսաջրածնի (H_2) արտադրությունը համարվում է էկոլոգիապես մաքուր և վերարտադրվող էներգիայի ստացման խոստումնալից ուղի՝ բազմաթիվ էժանև արդյունավետ ածխածնի աղբյուրների (գլիցերին, արդյունաբերական թափոններ) կիրառման հնարավորությամբ: Փորձերի նպատակն է եղել բացահայտել *Rhodobacter sphaeroides MDC6521*-ի կողմից H_2-ի արտադրության առանձնահատկությունները խառը ածխածնային (սուկցինատ, գլյուկոզ և գլիցերին) խմորման պայմաններում և F_oF_1-ԱԵՖազի ներդրումը այդ գործընթացում: Արդյունքները ցույց են տվել, որ սուկցինատից (15 մՄ) բացի նա և գլյուկոզ (15 մՄ) և գլիցերին (15 մՄ) պարունակող միջավայրում աճած բակտերիաները դրսևորում են ոչ միայն H_2-իարտադրության, այլ նաև թաղանթային բշտիկների F_oF_1-ԱԵՖազի ակտիվության խթանում: ԴՑԿԴ-զգայուն F_oF_1-ԱԵՖազի ակտիվության զգալի՝ ~1.7 անգամ աճ է գրանցվել սուկցինատի և գլիցերինի (15-ականմՄ) խառնուրդում ստուգիչի (30 մՄ սուկցինատ) համեմատությամբ, և քանի որ այն ուներ դրական ազդեցություն նաև H_2-ի ելքի վրա, հետագա ուսումնասիրություններ կիրականացվեն՝ ստանալու առավելագույն H_2-ի ելք ապահովող սուկցինատ/գլիցերին հարաբերությունը:

Կենսաջրածնի արտադրություն–R. sphaeroides –խառը ածխածնային խմորում

Производство биоводорода (H_2) считается перспективным способом получения чистой и возобновляемой энергии из различных дешевых и эффективных источников углерода, таких как глицерин или промышленные отходы. Были проведены исследования для выявления особенностей выделения H_2 бактерией *Rhodobacter sphaeroides* MDC6521 при сбраживании смешанных источников углеродов (сукцинат, глюкоза и глицерин) и роли F_oF_1-АТФазы в этом процессе. Результаты показывают, что бактерии, выращенные в среде, содержащей глюкозу и глицерин (15 мМ) в дополнение к сукцинату (15 мМ), демонстрируют увеличение не только выхода H_2, но также АТФазной активности мембранных везикул. При добавлении глицерина ДЦКД-чувствительная АТФазная активность увеличивалась в ~1.7 раз по сравнению с контролем (30 мМ сукцинат). Так как добавление глицерина положительно сказывалось и на выделении H_2 бактерией, будут проведены дальнейшие исследования для получения оптимального соотношения сукцинат/глицерин, обеспечивающего максимальный выход H_2.

Производство биоводорода –Rhodo bacter sphaeroides–сбраживание смешанных источников углеродов

H_2 is an energy carrier with high energy content (–122 kJg^{-1}). It is recognized as the most promising alternative to fossil fuels as it is clean, renewable, and efficient [1,10]. Biological H_2 production with microorganisms is considered as more perspective way of H_2 production at an industrial scale, not only compared to chemical production, but also compared to other biotechnological methods, due to a variety of substrates and organic wastes the microorganisms can use [7,11]. Photosynthetic purple non-sulfur bacteria such as *Rhodobacter* species are promising candidates for the H_2 production due to their high substrates conversion rate [3,6]. Under anaerobic conditions *Rhodobacter sphaeroides,* isolated from Armenian mineral springs, has been shown to perform a photo-fermentation of various carbon- and nitrogen-containing organic substrates with H_2 production [2,4,5]. The selection of the source for H_2 is a serious problem, because it strongly affects the H_2 yield by photosynthetic bacteria. The source should be relatively inexpensive and be effectively utilized by bacteria resulting in high H_2 yield. Various organic substrates, generally used in laboratory for research on H_2 production have been used in our research previously, providing a fast and efficientH_2 production [3,6]. But one of the key problems was always high cost of various organic carbon and nitrogen sources. A key to the solution of this problem can be the use of different organic wastes, which are cheaper and more effective for H_2 production and can provide inexpensive energy generation and simultaneous waste utilization [9]. The promising results obtained by our group for H_2 production during substrate utilization led to number of questions regarding the mechanisms of this process. The analysis of pathways of H_2 production during substrate utilization is difficult due to a complex composition of industrial wastes. One way to address this issue can be analysis of H_2 production process during a mixed carbon fermentation using a limited number of carbon sources [8].

Materials and method

Phototrophic bacterium *R. sphaeroides* strain MDC6521 (Microbial Depository Center, National Academy of Sciences of Armenia, Yerevan, Armenia, WDCM803), isolated from Arzni mineral spring in Armenian mountains, was cultivated in glass vessels of 150 ml capacities with plastic press caps in anaerobic conditions on Ormerod medium with different carbon sources upon illumination (~36 W m^{-2}) as described previously [2-5,9]. The growth of batch culture was monitored by changes in optical density (OD) by Spectro UV-Vis Auto spectrophotometer (Labomed, USA). Specific growth rate was calculated as ln2/doubling time of OD within a logarithmic growth phase, and it was expressed as h^{-1} [2-5].The medium E_h was determined during *R. sphaeroides*anaerobic growth by potentiometric method using a pair of redox electrodes: platinum (Pt) and titanium-silicate (Ti-Si) electrodes [2-5]. The H_2 yield was calculated by the decrease of E_h to low negative values during bacterial growth and expressed in mmol/L[2-5].ATPase activity of *R. sphaeroides* membrane vesicles was estimated by the liberation of inorganic phosphate (P_{in}) in the reaction with ATP by themethod of Taussky and Shorr [2-5]. Various reagents of analytical grade were used in this study. Each experiment was repeated three times to determine deviation, which is presented as error bars on the figures.

Results and discussion

The growth was monitored during *R. sphaeroides* cultivation in media containing different carbon source combinations. The results were compared with the control, which contained 30 mM succinate as a carbon source. Bacteria grown under mixed carbon fermentation conditions exhibited slight increase of specific growth rate, compared with control (Fig. 1).

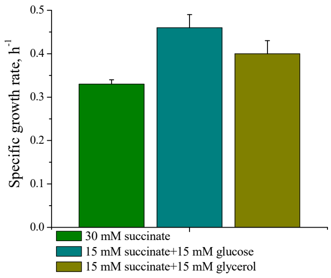

Fig. 1. Specific growth rate of *R. sphaeroides* MDC6521 during mixed carbon fermentation.

As for the H_2 production, during mixed carbon fermentation conditions, the change was significant in case of glycerol, but not the glucose addition (Table 1). There was a ~2.3 fold increase of H_2yield after 72 hours of growth, compared with control. Since, such an increase in H_2 yield couldn't be explained by bacterial growth enhancement only, membrane associated systems should be examined to find out the possible causes.

Table 1. The effect of mixed carbon fermentation on H_2yield of*R. sphaeroides*MDC6521.

H_2 yield, mmol/L (72 hours of growth)	
Control (30 mM succinate)	2.2 ±0.1
15 mM succinate + 15 mM glucose	2.12 ±0.1
15 mM succinate + 15 mM glycerol	5.06 ± 0.2

Consequently, we have analyzed effect of mixed carbon fermentation on DCCD-inhibited F_oF_1-ATPase activity of *R. sphaeroides* MDC6521membrane vesicles. The results show that bacteria grown in a medium containing glucose and glycerol (15 mM) in addition to succinate (15 mM), exhibit an increased F_oF_1-ATPase activity of membrane vesicles obtained from them (Fig. 2).

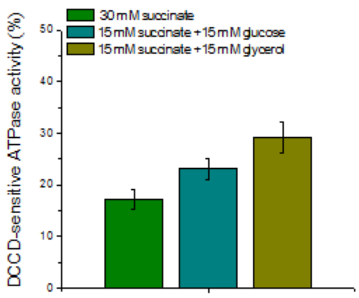

Fig.2. DCCD-sensitive ATPase activity of membrane vesicles of *R. sphaeroides* MDC6521 during mixed carbon fermentation.

Significant ~1.7 fold increase of DCCD sensitive ATPase activity was obtained with glycerol addition compared with control (30 mM succinate).Since it had a positive effect both on bacterial growth and H_2 production, further experiments will be performed to obtain the optimal succinate/glycerol ratio to get the maximum H_2 yield.The role of F_oF_1-ATPase in bioenergetics of *R. sphaeroides* during mixed carbon fermentation can also be considered in application in biotechnology as a tool to interfere the hydrogen metabolism in these bacteria.

References

1. *Basak N, Jana AK, Das D, Saiki D.* Photofermentative molecular biohydrogen production by purple-non-sulfur (PNS) bacteria in various modes: The present progress and future perspective. Int J Hydrogen Energy 2014;39:6853–71.
2. *Gabrielyan L, Sargsyan H, Hakobyan L, Trchounian A.* Regulation of hydrogen photoproduction in *Rhodobactersphaeroides*batch culture by external oxidizers and reducers. Appl Energy 2014; 131: 20–25.
3. *Gabrielyan L, Trchounian A.* Purple bacteria and cyanobacteria as potential producers of molecular hydrogen: an electrochemical and bioenergetic approach. In: Trchounian A, editor. Bacterial Membranes. Ultrastructure, bioelectrochemsitry, bioenergetics and biophysics, Kerala, India: Research Signpost; 2009, p. 233–273.
4. *Hakobyan L, Gabrielyan L, Trchounian A.* Bio-hydrogen production and the F_oF_1-ATPase activity of *Rhodo bacter sphaeroides*: effects of various heavy metal ions. Int J Hydrogen Energy 2012; 37: 17794–17800.
5. *Hakobyan L, Gabrielyan L, Trchounian A*. Yeast extract as an effective nitrogen source stimulating cell growth and enhancing hydrogen photoproduction by *Rhodo bacter sphaeroides*strains from mineral springs. Int J Hydrogen Energy 2012; 37: 6519–6526.
6. *Han H, Liu B, Yang H, Shen J.* Effect of carbon sources on the photobiological production of hydrogen using *Rhodobactersphaeroides* RV. Int J Hydrogen Energy 2012; 37: 12167–74.
7. *Marone A, Izzo G, Mentuccia L, Massini G, Paganin P, Rosa S, Varrone C, Signorini A.* Vegetable waste as substrate and source of suitable microflora for bio-hydrogen production. Renewable Energy 2014; 68: 6–13.
8. *Rollina JA, del Campoa JM, Myunga S et al*. High-yield hydrogen production from biomass by in vitro metabolic engineering: Mixed sugars co-utilization and kinetic modeling. *Proc Nat. Acad.Sci. USA* 2015; 112: 4964-4969.
9. *Sargsyan H., Gabrielyan L., Trchounian A*. The distillers grains with solubles as a perspective substrate for obtaining biomass and producing bio-hydrogen by *Rhodo bacter sphaeroides.* Biomass & Bioenergy 2016; 90, 90-94.
10. *Trchounian A.* Mechanisms for hydrogen production by different bacteria during mixed-acid and photo-fermentation and perspectives of hydrogen production biotechnology. Crit Rev Biotechnol 2015; 35: 103-13.
11. *Wang LJ.* Production of bioenergy and bioproducts from food processing wastes: a review. Transactions of the ASABE 2013; 56: 217–30.

Biolog. Journal of Armenia, 1 (69), 2017

THE CONTENT OF SULFUR DIOXIDE IN DRIED VINE FRUIT REALISED IN ARMENIA

L.L. HAKOBYAN[1], K.M. GRIGORYAN[2], A.H. TRCHOUNIAN[1,2]

[1] Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University;
[2] Research Institute of Biology, Yerevan State University
hakobyanl@ysu.am

In dried vine fruit production much attention is paid to the development of technologies and methods for inhibition the growth and evolution of potentially toxigenic fungi. The most effective method to limit the fungi growth is the treatment of raw grape with sulfur dioxide. However, the