Науковий вісник Ужгородського університету
Серія Біологія, Випуск 34, 2013: 96-102
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UDC 571.1+577.1 C50

# CONTENT OF VITAMINS IN *HALOBACTERIUM HALOBIUM* 353П ВКПМ В - 1739 AND EFFECT OF ITS "PURPLE MEMBRANE"- FREE LYSATE ONTO COLONY GROWTH OF BACTERIA

Sharga B.M., Nikolaychuk V.I., Maga I.M., Bandrovska I.K., Chekulaeva L.N.

***Вміст вітамінів у Halobacterium halobium 353П ВКПМ В-1739 і ефект її лізату, звільненого від «пурпурних мембран», на ріст колоній бактерій — Б.М.Шарга[1], В.І.Ніколайчук[1], І.М.Мага[1], І.К.Бандровська[1], Л.М.Чекулаєва[2]*** *— Клітини штаму Halobacterium halobium 353П ВКПМ В-1739 перевірено на вміст вітамінів А, β-каротин, D, E, K, C, $B_1$, $B_2$, $B_3$, $B_5$, $B_6$, Bc, $B_{12}$, H та PP. З біомаси, відмитої центрифугуванням в ізотонічному розчині NaCl, готували суспензію $10^9$ кл/мл в цьому ж розчині за оптичним стандартом. Однакові об'єми цієї суспензії центрифугували, осаджені клітин піддавали лізису в дистильованій воді для одержання проб для аналізів. Вміст вітамінів був незначним, так як бактерія синтезує їх у межах своїх потреб. Було виявлено такі вітаміни, μг/л: ретинальдегід – 38,4 ± 0,3; β-каротин – 74,6 ± 0,5; α-токоферол – 16,8 ± 0,4; тіамін – 38,2 ± 0,1; рибофлавін - 53,5 ± 0,4; вітамін В5-49,7 ± 0,3; піридоксин – 10,0 ± 0,1; ціанкобаламін – 8,1 ± 0,2; фолієву кислоту -18,6 ± 0,3; біотин – 2,9 ± 0,2; нікотинову кислоту 172,1 ± 0,4. Отже, даний штам продукує вітаміни А, Е, $B_1$, $B_c$ і PP, комерційне мікробіологічне виробництво яких ще відсутнє. Тому він є перспективним для біотехнологічного вдосконалення як продуцент цих сполук або як донор генів для їхнього синтезу.*

*Коли очищений від «пурпурних мембран» лізат цієї культури додавали у пропорції 1:1 до води, на якій готували поживні середовища, то він стимулював ріст колоній Escherichia coli, Shigella sp., Klebsiella pneumoniae, Erwinia sp. і Pseudomonas sp. на 11-200, 50-60, 29-125, 60-130 та 95-106%, відповідно. Найбільше прискорення росту, близько 350%, спостерігали у колоній H. halobium 353 П ВКПМ В – 1739.*

*Звільнений від «пурпурних мембран» лізат ліофілізували. Це дозволило встановити 1,5% вміст сухої речовини у ньому. Він не втратив своєї активності після 6 місяців зберігання у такому стані при 4°С. Тому його можна використовувати як стимулятор росту у мікробіологічних середовищах.*

***Ключові слова**: вітаміни Halobacterium halobium, очищений від «пурпурних мембран» лізат, бактерії різних екологічних груп, стимуляція росту колоній.*

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***Content of vitamins in Halobacterium halobium 353П ВКПМ В - 1739 and effect of its "purple membrane"- free lysate onto colony growth of bacteria. — B.M.Sharga [1], V.I.Nikolaychuk [1], I.M.Maga [1], I.K.Bandrovska [1], L.N.Chekulaeva [2] —*** *Cells of Halobacterium halobium strain 353П ВКПМ В-1739 were tested for content of vitamins A, β-carotene, D, E, K, C, $B_1$, $B_2$, $B_3$, $B_5$, $B_6$, Bc, $B_{12}$, H and PP. Cell suspension of $10^9$ cells/ml was prepared in isotonic NaCl solution with use of an optical standard. To obtain probes for analyses, equal volumes of the suspension were centrifuged and cell pellets were subjected to lysis in distilled water. The vitamin content was not significant, as bacterium produced them according to its own requirements. Particularly, we determined the vitamins, μg/L: retinaldehyde – 38.4 ± 0.3; β-carotene – 74.6 ± 0.5; α-tocopherol – 16.8 ± 0.4; thiamine – 38.2 ± 0.1; riboflavin – 53.5 ± 0.4; vitamin B5 – 49.7 ± 0.3; pyridoxine – 10.0 ± 0.1; cyanocobalamin – 8.1 ± 0.2; folic acid – 18.6 ± 0.3; biotin – 2.9 ± 0.2; nicotinic acid – 172.1 ± 0.4. The strain is producing vitamins A, E, $B_1$, Bc and PP, the commercial microbiological production of which does not yet exist. The strain has prospects for biotechnological improvement as a producer of these compounds or as a donor of genes for their synthesis.*

*When "purple membrane"- free lysate of H. halobium strain 353П ВКПМ В-1739 was added in proportion 1:1 to the water used for the nutritive media preparation, it stimulated the colony growth of Escherichia coli, Shigella sp., Klebsiella pneumoniae, Erwinia sp. and Pseudomonas sp. by 11-200, 50-60, 29-125, 60-130 and 95-106%, respectively. The highest enhancement of colony growth, around 350%, was observed for H. halobium 353 П ВКПМ В -1739.*

*The "purple membrane"- free lysate was lyophilized. This allowed us to estimate the lysate dry matter content as 1.5%. In this state it was stored at 4°C for 6 months of observation without loss of its effect on bacterial growth. Thus, it can be used as a growth stimulator in microbiological media.*

***Key words:** Halobacterium halobium vitamins, "purple membrane"- free lysate, bacteria from different ecological groups, colony growth stimulation*

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## Introduction

Halophilic archaea, particularly *H. halobium* (syn. *H. salinarium*), remains in the focus of attention of scientists due to its unique biological properties.

This microbe is able to survive completely encased in salt crust for extended period and requires more than 15% NaCl content in the medium for growth. Exceptional resistance to solar radiation is another survival adaptation of this extreme halophile.

Bacteriorhodopsin, the plasma membrane protein of this bacterium, acts as a light driven proton pump for the ATP production. The protein forms so-called "purple membranes", randomly distributed as patches in the cell envelope. The bacteriorhodopsin in purple membranes is remarkably stable *in vitro*. For example, imbedded into thin gelatinous film and dried in our laboratory at Uzhgorod National University, it remains active in its photo-cycle conduction after more than 20 years of observation. Pumping of proton in response to photon absorption by bacteriorhodopsin *in vitro* coupled with color change and this could have technical applications.

Several patents were issued for use of either *H. halobium* cell mass or its constituents as biologically active preparations. For instance, bacteriorhodopsin is promising for use in medicine, particularly in ophthalmology. Protein-based artificial retinas, which comprise native bacteriorhodopsin and/or specialized bacteriorhodopsin mutants as the photoactive element were patented [35]. The lyophilized cell mass of *H. halobium* was patented as the feed additive "Баксин". This formulation has anti oxidative activity and modulative effects on physiological and immunological processes in mammals [15]. A method for treating hyperkeratotic diseases such as psoriasis which comprises applying a lysate of *H. halobium* to the affected skin area and irradiating with light was patented [24]. Cosmetic composition, characterized by the presence of at least one product comprising a glycoprotein fraction from *H. halobium* provided in the form of a gel, a milk, a lotion, or a cream was developed [22].

Biologically active substances of *H. halobium* 353П ВКПМ В-1739 had not been studied sufficiently.

The purpose of our study was to estimate the level of vitamins in the of *H. halobium* 353П ВКПМ В-1739 strain and to evaluate the effect of its "purple membrane"- free lysate onto growth of bacteria from different genera.

## Materials and Methods

Bacterial cultures and "purple membrane"- free lysate. All cultures were refridgerated at 4°C unless used. Enteric bacteria of *Escherichia*, *Shigella* and *Klebsiella* genera were preserved in tubes with 0.2% nutritive agar. Cultures of *Erwinia amylovora* were supported on 0.2% potato nutritive agar containing 5% sucrose, and other erwinias and bacteria of the genus *Pseudomonas* on 0.2% potato nutritive agar. For experiment, they were transferred to the same solid agar media with and without "purple membrane"- free lysate of *H. halobium* 353П ВКПМ В-1739.

Strain of *H. halobium* 353П ВКПМ В-1739 was supported onto slants of medium consisting of the following ingredients in grams per liter of distilled water: NaCl – 25, Mg $SO_4$ – 20, KCl – 2, $NH_4NO_3$ – 2, Na citrate – 3, peptone – 5, yeast extract – 5, glycerol – 2, agar – 20. The required volume of medium was reached by dilution of all the ingredients in distilled water. The pH of the medium was leveled to 7.2 by adding 1M KOH or HCl solutions. When needed, culture was seeded onto the same fresh medium. Colonies of *H. halobium* 353П ВКПМ В-1739 were grown aerobically at 37°C for one week in Petri dishes with illumination from fluorescent tubes with light intensity of $0.2\text{-}0.3\times10^6$ erg/cm$^2$c. The same medium without agar was used for *H. halobium* 353П ВКПМ В-1739 cultivation in liquid culture. Cell mass was separated from the medium by centrifugation and washed twice in 25% NaCl solution. Then cell masses were transferred to 16 mL tubes and suspended in sterile 25% NaCl solution to produce suspension of $10^9$ cells/mL by use of an optical standard. The same cell density was reached at the finish of cultivation in liquid medium. Equal volumes of the suspensions were centrifuged at 5000 g and pellets of *H. halobium* 353П ВКПМ В-1739 cells were suspended in distilled water resulting in their lysis and to liberation of cell constituents. The lysate was analyzed for vitamins.

Bacteriorhodopsin was separated from the lysate in form of "purple membranes" by the method of Oesterhelt, Stoeckenius [34]. The "purple membrane"- free lysate was added in proportion (1:1, v/v) to the distilled water used for preparation of media: solid medium for *Halobacterium*; nutritive agar for *Escherichia*, *Shigella*, *Klebsiella*; potato-sucrose agar for *Erwinia amylovora*; potato agar for other species of *Erwinia* and *Pseudomonas*.

To evaluate "purple membrane"- free lysate effect on colonial growth of these bacteria, each strain was inoculated with a microbiological needle 3 times onto 5 dishes of solid medium both containing and not containing lysate. Petri dishes inoculated with *H. halobium* 353П ВКПМ В-1739 were incubated at 37°C for 1 week, those seeded with *Escherichia*, *Shigella* and *Klebsiella* were cultured at 37°C for 2 days, and media inoculated by *Erwinia* or *Pseudomonas* were kept at 28°C for 3 days. The mean diameter of grown colonies was measured in both variants of inoculations and compared.

The percentage of dry matter of the "purple membrane"- free lysate was determined after its lyophilization. Lyophilized lysate was sterilized by

filtration and refridgerated. Its activity on bacterial growth was verified after 6 months of storage.

Determination of vitamins. Vitamin A and β-carotene. Photoelectrocolorimetric method of vitamin A determination with $SbCl_3$ was used for estimation of this vitamin content. The measurements were done at $\lambda = 620$ nm. The vitamin A content was determined with use of calibration curve optical density *versus* concentration of the standard dilutions of retinol acetate.

The β-carotene liberated by osmotic disruption of the cells in distilled water was identified and quantitatively determined with the aid of a spectrophotometer after separation from other carotenoids by chromatography in $Al_2O_3$ column. The α- and β-carotene were eluted from the column by hexane, followed by 1% solution of acetate in hexane. The quantity of β-carotene was estimated by taking into calculations the data of optical density measured at 450-475 nm (β-carotene has maximums in hexane at 450 - 451, 475 нм) [8].

Tocopherol. Reaction of Emmerie A., Engel C. (1938) and Furter, Meyer (1939) in modification of Кибардин [10] was used for estimation of vitamin E content. After the heating of bacterial cell mass in 30% NaOH at 90°C water bath, the tocopherol was extracted together with other vitamins and sterols in petroleum ether. Vitamin A was removed from the cell mass by reaction with $H_2SO_4$, carotene was separated from vitamin E by chromatography in $Al_2O_3$ column and sterols were precipitated by aeskulin prepared as described by Гласова и Сабов [3]. Other vitamins did not intervene in reaction of $FeCl_3$ with tocopherol. Petroleum ether was evaporated completely and tocopherol was determined in specific reaction with $FeCl_3$ carried out at the presence of 2,4,6-threepiridil-8-triazine, which developed pink complex with Fe(II) produced in the reaction. Optical density of the resulting colored solution correlated with quantity of tocopherol reacted with $FeCl_3$.

Vitamin K. The method based on color reaction of Dam [19], Irreverre, Sullivan [27] with substitution of sodium ethylate by NaOH was used to determine the vitamin K.

To estimate the level of vitamin $B_1$ production by *H. halobium* 353П ВКПМ В-1739, the method based on Jansen reaction was used [29]. Cell mass was extracted by HCl. The protein-bonded vitamin B was released by enzymatic cleavage. Then, thiamine was oxidized to thiochrome by potassium ferrocyanide at basic pH and content of vitamin B1 was determined by blue luminescence in serial dilutions [11].

The level of vitamin $B_2$ was determined by luminescence titration according to the methods of Najjar (1941) and Scott et al. (1946) in modification of Елисеева [7]. The vitamin was extracted by HCl, protein-bounded vitamin $B_2$ was released by enzymatic cleavage. Other luminescent compounds were removed by $KMnO_4$ treatment. The thiochrome luminescence partially produced in reaction was removed by special filter.

Vitamin $B_5$ was quantified by the colorimetric method of Schmull, Wollish (1957) and Strohecker, Henning (1965) in modification of Мойсеенок [12]. The principle of the method consists of hydrolytic cleavage of amide bound between carboxylic group of pantoic acid and β-alanine and colorimetric measuring of β-alanine released.

The determination of vitamin $B_6$ was done by the method of Lepkovsky (1943) in modification of Горяченкова Е.В. [4]. The method is based on extraction of vitamin $B_6$ by HCl followed by neutralization of acid and colorimetric reaction with $FeCl_3$. The intensity of greenish color was measured in colorimeter.

Vitamin $B_{12}$ was determined by turbidimetric procedure described by Букин В.Н. (1954) with use of *E.coli* strain unable to grow without cyanocobalamin in medium [1].

Vitamin Bc was extracted from cell mass by HCl, absorbed by activated charcoal, eluted by ammonium solution and treated by potassium permanganate. Pteridin-6-carbonic acid produced blue luminescence. Intensity of it was determined by titration in UV-light. The luminescence of vitamin B1 and B2 was filtered by special optical filters [18].

Biotin content was estimated by microbiological method of Воронкова и др. (1980) using *Saccharomices cerevisiae* ATCC 7754, the biotin auxotroph strain [2].

Vitamin PP was determined by the method of Степанова [16]. Cell mass was filtered after hydrolysis in 2% HCl in warm water bath for 30 min. Hydrobromic thiocyanate reagent with ethanol-aniline was added to the filtrate in a ventilated fume cabin. Light-pink color developed. The intensity of color reaction was measured in colorimeter with red light filter. (Nicotinic acid standard solution with concentration 0.2 mg per ml of reaction mixture has an extinction of 0.049, and this was a factor in our calculations).

Vitamins C and D. Standard methods [5, 6] were used to check *Halobacterium halobium* 353П ВКПМ В-1739 for production of vitamins C and D.

**Results and Discussion**

Of the four fat-soluble vitamins, *i.e.* vitamins A, D, E and K, only vitamins A and E were determined in *Halobacterium halobium* 353 П ВКПМ В – 1739. The vitamins D and K were not found in this strain by the methods used in our study.

The content of vitamin A was determined in the cell lysate at 38.4 ± 0.3 μg/L. This level is approximately half the content of its precursor, β-carotene, 74.6 ± 0.5 μg/L (Table 1). This can be explained by the fact that retinal is bound to apoprotein, forming Schiff bases in

bacteriorhodopsin, and thus cannot be fully extracted from cells by organic solvents.

No success has (yet) been obtained in direct biotechnological production of vitamins A or D yet by any bacterial cultures. They are produced mainly chemically now [44].

Gochnauer et al. [23] observed that addition of glucose into the medium increased the formation both of β-carotene, a precursor of vitamin A, and bacterioruberins. However, glycerol decreased the formation of bacterioruberins and increased the formation of carotenes in *H. halobium*. El-Sayed et al. [20] revealed that light enhances the biosynthesis of bacterioruberin and the conversion of β-carotene to retinal, but does not affect β-carotene biosynthesis in *H. salinarum* Oyon Moussa-16. Low oxygen tension given in the light brought a slight increase in retinal accumulation, although its biosynthesis from β-carotene is an oxygenation reaction. This paradox was explained by the authors as a consequence of the increase in β-carotene biosynthesis.

Pisal, Lele [26] and Rad *et al*. [38] observed increased production of β-carotene in unicellular halotolerant green alga *Dunaliella salina* under stressed conditions of growth, particularly, with increasing NaCl in the medium.

Whether or not the level of NaCl in growth medium had effect on vitamin production by *H. halobium* 353 П ВКПМ В – 1739 remains to be studied.

Commercial β-carotene is produced chemically or by extraction from natural sources such as cultivated plants and algae [21, 25] and some fungi [9, 14]. However, it is possible to improve *H. halobium* 353 П ВКПМ В – 1739 by genetic engineering for higher production of vitamin A and β-carotene or use the halobacterial genes for synthesis of these compounds in cells of other species cells. Hitherto, among bacteria, only in *Halobacterium* has the ability to produce vitamin A been estimated.

We determined the amount of vitamin E in *H. halobium* 353 П ВКПМ В – 1739 at the level of 16.8 ± 0.4 μg/L. Microalgae *Euglena gracilis* Z. [46] and *Dunaliella tertiolecta* [17] produce vitamin E in concentrations much higher than we opened in the strain we used. Optimized fermentative production of *a*-tocopherol from glucose by *E. gracilis* reached a 1.21 mg/g dry cell mass [46].

Lactic acid bacteria and *Flavabacterium* mutant are able to synthesise vitamin K in the form of menaquinones with the yield of 29–123 mg/L [45] and 182 mg/L [47], respectively. The highest vitamin K production by *B. subtilis* from soybean extract, 36.6 mg/g of okara-natto wet mass, was in mutant *Unnan* SL-001 [43].

Thus, *H. halobium* 353 П ВКПМ В – 1739 cells are able to produce the fat-soluble vitamins A, E and β-carotene, however in low quantities.

Water-soluble vitamins were determined in this strain also. Compared to other vitamins, the highest cell content we discovered was for vitamin PP. Its yield comprised 172.1 ± 0.4 μg /L. This finding is promising as no biotechnological production of niacin from bacteria has been developed yet. It is produced only chemically in industry now [42].

The lowest production in *H. halobium* 353 П ВКПМ В – 1739 cells was observed for vitamin $B_{12}$, 8.1 ± 0.2 μg/L. This is too low, compared with several species of microbial producers of vitamin $B_{12}$ including *Propionibacterium freudenreichii, Pr. shermanii, Rhodopseudomonas protamicus, Pseudomonas denitrificans, Nocardia rugosa, Rhizobium cobalaminogenum, Micromonospora* sp., *Streptomyces olivaceus, Nocardia gardner, Butyribacterium methylotrophicum, Pseudomonas* sp. and *Arthrobacter hyalinus.* From these, the highest yield, 206.0 mg/L of cyanocobalamin, was produced in *P. freudenreichii* at anaerobiosis on glucose as the main component with addition of 5,6-dimethyl benzimidazole to the medium. The lowest yield of vitamin $B_{12}$, 1.1 mg/L, was produced by *A. hyalinus* on isopropanol as the main component with the same amendment to fermentation broth [32]. This vitamin is produced in nature and in industry exclusively by microorganisms.

The level of thiamine production by *H. halobium* 353 П ВКПМ В – 1739 was 38.2 ± 0.1 μg/L. The effective biotechnological method of vitamin B1 production has not been developed yet. Usually, thiamine is produced by microbes in extremely small amounts which do not exceed their needs. All commercial thiamine is produced by chemical method [42].

Riboflavin content in *H.halobium* 353 П ВКПМ В – 1739 was determined at 53.5 ± 0.4 μg/L. A single step fermentative riboflavin production has been developed using a recombinant *B. subtilis* strain [28]. In the highest producer, recombinant strain *Corynebacterium ammoniagenes*, riboflavin was produced at the level of 15.3 g/L [30]. *Clostridium* sp. and *Candida* sp. are good riboflavin producers too, however ascomycetes *Eremothecium ashbyii* and *Ashbya gossypii* are considered the best [41]. Vitamin B2 production in a culture of *A. gossypii* was enhanced after 4 days to 2.5 g/L by adding 1% mineral support with adsorbed soybean oil to the medium [31].

Content of vitamin $B_5$ (pantothenic acid + pantothenol) in *H. halobium* 353 П ВКПМ В – 1739 was determined at the level of 49.7 ± 0.3 μg/L. Cells of many microorganisms (*Bifidobacterium bifidum*, *Lactobacillus arabinosus, L. butiricus, Escherichia coli*, *etc.*) are able to synthesise pantothenate. Commercial pantothenic acid is produced chemically or by fermentation [42]. Recombinant strain of *E. coli*, overexpressing pantothenic acid biosynthesis enzymes, produced 65 g/L of D-pantothenic acid from glucose when β-alanine was added as a precursor [37].

Biotechnological production of pyridoxine has not been developed yet. Vitamin B6 is produced chemically. We found that *H. halobium* 353 П ВКПМ В – 1739 cells at lisis released around 10.0 ± 0.1 μg/L of pyridoxine. Of course, this not enough for economically viable production. Screening for vitamin $B_6$ producers among other bacteria found potential strains, such as *Klebsiella* sp., *Flavobacterium* sp., *Pichia guilliermondii*, *Bacillus subtilis*, *Rhizobium meliloti* and so on [40]. However, not one was used for commercial production of pyridoxine.

Content of vitamin $B_c$ in *H. halobium* 353 П ВКПМ В – 1739 was 18.6 ± 0.3 μg/L of suspension. Some enteric bacteria are able to produce folic acid also. Pteroilglutamic acid is present in high amounts in plant leaves. All commercial folic acid is produced by chemical method today [40, 42].

Table 1. Vitamins in *Halobacterium halobium* 353 П ВКПМ В - 1739 cell suspension

| Vitamins | Content, μg/L , $\bar{x} \pm SE$ |
|---|---|
| Fat-soluble vitamins | |
| A (retinaldehyde) | 38.4 ± 0.3 |
| β-carotene | 74.6 ± 0.5 |
| E (α-tocopherol) | 16.8 ± 0.4 |
| Water-Soluble Vitamins | |
| $B_1$ (thiamine) | 38.2 ± 0.1 |
| $B_2$ (riboflavin) | 53.5 ± 0.4 |
| $B_5$ (pantothenic acid + pantothenol) | 49.7 ± 0.3 |
| $B_6$ (pyridoxine) | 10.0 ± 0.1 |
| $B_{12}$ (cyanocobalamin) | 8.1 ± 0.2 |
| $B_c$ (pteroylglutamic acid or folic acid) | 18.6 ± 0.3 |
| H (biotin) | 2.9 ± 0.2 |
| PP (nicotinic acid) | 172.1 ± 0.4 |

*Note: The levels of different vitamins determined in *H. halobium* 353 П ВКПМ В – 1739 were expressed in μg per L of the bacterial suspension $10^9$cells/mL.

Strain of *H. halobium* 353 П ВКПМ В – 1739 produces 2.9 ± 0.2 μg /L of vitamin H. This amount we regard as low, in comparison to the biotin production by recombinant *Serratia marcescense* resistant to vitamin H antimetabolites. This microbe yields 600 mg/L of D-biotin in the presence of high levels of sulfur and ferrous iron [33]. *Agrobacterium/Rhizobium* HK4 was transformed by plasmid containing modified *E. coli bio* operon. This microbe then produced 110 mg/L of biotin, growing on a defined medium with diaminononanoic acid as the starting material [39].

Vitamins are required by bacterial cells in minute quantities. Considering the low levels of this and other vitamin production in *H. halobium* 353 П ВКПМ В – 1739, we must take into account that this strain has not been modified genetically yet for their higher production. However, the use of microbes from species *H. halobium* is promising as they are non-pathogenic and require less strict precaution for cultivation. The production of vitamins in *H. halobium* 353 П ВКПМ В – 1739 may be enhanced by developing better cultivation methods and by using modern recombinant DNA techniques also.

We discovered strong effect of growth stimulation in different groups of bacteria caused by “purple membrane”- free lysate (Table 2). When this was added in proportion 1:1 to the water used for the media preparation, it stimulated the colony growth of *Escherichia coli*, *Shigella* sp., *Klebsiella pneumoniae*, *Erwinia* sp. and *Pseudomonas* sp. by 11-200, 50-60, 29-125, 60-130 and 95-106%, respectively. The highest stimulation of around 350% was observed for *H. halobium* 353 П ВКПМ В – 1739, the microorganism from which the “purple membrane”- free lysate was obtained.

Table 2. Growth stimulation of “purple membrane”- free cell lysate onto bacteria of different ecological groups

| Strains | Colony diameter on media, mm, $\bar{x} \pm SE$ | | Stimulatory effect, % |
|---|---|---|---|
| | without lysate | with lysate | |
| *Escherichia coli* Ca 62 | 4 ± 0.1 | 10 ± 0.2 | 150 |
| *Escherichia coli* Ca 42 | 4 ±0.2 | 7 ±0.1 | 75 |
| *Escherichia coli* Ca 7 | 8 ± 0.3 | 9 ± 0.2 | 11 |
| *Escherichia coli* Ca 18 | 6 ± 0.1 | 8 ± 0.3 | 33 |

| Strains | Colony diameter on media, mm, $\bar{X} \pm SE$ | | Stimulatory effect, % |
|---|---|---|---|
| | without lysate | with lysate | |
| *Escherichia coli* Ca 23 | 6 ± 0.1 | 9 ± 0.1 | 50 |
| *Escherichia coli* Ca 38 | 7 ± 0.2 | 8 ± 0.2 | 14 |
| *Escherichia coli* Ca 46 | 4 ± 0.3 | 12 ± 0.4 | 200 |
| *Escherichia coli* Ca 53 | 7 ± 0.2 | 12 ± 0.3 | 71 |
| *Escherichia coli* Ca 57 | 4 ±0.3 | 9 ±0.4 | 125 |
| *Escherichia coli* 235 | 4 ± 0.1 | 8 ± 0.2 | 100 |
| *Escherichia coli* $E_1$ | 5 ±0.2 | 7 ± 0.3 | 40 |
| *Shigella dispar* P14 | 5 ± 0.1 | 8 ± 0.2 | 60 |
| *Shigella paradysenteriae boydii* P1 | 4 ± 0.1 | 5 ± 0.1 | 50 |
| *Klebsiella pneumoniae* ATCC 13883 | 7 ± 0.2 | 10 ± 0.3 | 43 |
| *Klebsiella pneumoniae* 1032 | 5 ± 0.2 | 10 ± 0.1 | 100 |
| *Klebsiella pneumoniae* 7730t4435 | 6 ± 0.3 | 8 ± 0.2 | 33 |
| *Klebsiella pneumoniae* 1112 | 7 ± 0.4 | 9 ± 0.3 | 29 |
| *Klebsiella rhinoscleromatis* 2594 | 4 ± 0.2 | 9 ± 0.3 | 125 |
| *Klebsiella rhinoscleromatis* 808tz | 4 ± 0.1 | 6 ± 0.2 | 50 |
| *Klebsiella rhinoscleromatis* 2707 | 5 ± 0.1 | 9 ±0.2 | 80 |
| *Klebsiella rhinoscleromatis* 234 | 5 ± 0.2 | 10 ±0.4 | 100 |
| *Klebsiella rhinoscleromatis* 7734 | 6 ± 0.3 | 11 ± 0.4 | 83 |
| *Klebsiella ozaenae* 552t6 | 4 ± 0.2 | 8 ± 0.3 | 100 |
| *Erwinia amylovora* 595 a | 2 ± 0.3 | 4 ± 0.1 | 100 |
| *Erwinia chryzanthemi* NCPPB 898 | 1.5 ± 0.1 | 3.2 ± 0.1 | 113 |
| *Erwinia chryzanthemi* NCPPB 402 | 2.5 ± 0.2 | 4 ± 0.2 | 60 |
| *Erwinia herbicola* LMG 2565 | 2 ± 0.1 | 4.6 ± 0.3 | 130 |
| *Erwinia rhapontici* NCPPB 564 | 2.4 ± 0.1 | 5 ± 0.2 | 108 |
| *Pseudomonas syringae* 8511 | 2.3 ± 0.2 | 4.5 ± 0.3 | 95 |
| *Pseudomonas syringae* NCPPB 281 | 3 ± 0.2 | 6.2 ± 0.2 | 106 |
| *Pseudomonas cerasi* 8654 | 2.6 ± 0.1 | 5.3 ± 0.2 | 104 |
| *Halobacterium halobium* 353 П ВКПМ В – 1739 | 4 ± 0.2 | 16.2 ± 0.3 | 350 |

The “purple membrane”- free lysate was prepared as a dry powder by lyophilization. This allowed us to estimate the lysate dry matter content as 1,5%. In lyophilized state it was stored for 6 months of observation without loss of its effect on bacterial growth.


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Отримано: 11 жовтня 2013 р.
Прийнято до друку: 12 листопада 2013 р.