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# PRODUCTION OF BIOMASS OF PHOTOSYNTHETIC BACTERIA ON BASE OF STOCK-BREEDING AND POULTRY WASTES

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On base of stock-breeding and poultry wastes the cheap nutrient media for cultivation and production of biomass of phototrophic bacteria have been developed. Cultivation of purple nonsulfur bacteria *Rhodobacter sphaeroides* together with *Bacillus megaterium* in mixed culture has been succeeded in high biomass yield. The obtained biomass was distinguished with rich contents of biologically active substances.

#### Photosynthetic bacteria – biomass – mixed culture

Անասնապահական և թռչնաբուծական թափոնների հիման վրա մշակվել են էժան սննդամիջավայրեր ֆոտոտրոֆ բակտերիաների աձեցման և կենսազանգվածի ստացման համար։ Կիրառելով խառը կուլտուրաների՝ *Rhodobacter sphaeroides* ոչ ծծմբային ծիրանագույն բակտերիաների և *Bacillus megaterium*⊡ի համատեղ աձեցումը, հաջողվել է զգալի բարձրացնել կենսազանգվածի ելքը։ Ստացված կենսազանգվածը առանձնանում է կենսաբանական ակտիվ միացությունների հարուստ պարունակությամբ։

Ֆոտոսինթետիկ բակտերիաներ 🗆 կենսազանգված 🗆 խառը կուլտուրա

Разработаны дешевые питательные среды на основе животноводческих и птицеводческих отходов для выращивания и получения биомассы фототрофных бактерий. С использованием смешанного культивирования несерных пурпурных бактерий *Rhodobacter sphaeroides* и *Bacillus megaterium* удалось заметно увеличить выход биомассы. Полученная биомасса отличается богатым содержанием биологически активных соединений.

Фотосинтетические бактерии – биомасса – смешанная культура

The abilities of photosynthetic bacteria to assimilate carbon dioxide, to fix molecular nitrogen possessing with photosynthetic metabolism, as well as to grow on different wastes permit to use phototrophs in biotechnology [7,11]. The phototrophic bacteria are effective producers of biologically active substances, such as carotinoides, organic acids, moreover the biomass of phototrophs contains valuable substances and has practical importance as feed protein [2, 10].

Application of phototrophs in Armenia has high perspectives since this group of microorganisms are widely distributed, especially, in mineral springs, lakes, natural reservoirs, and can be grown from early spring till late autumn [6]. The literature data on cultivation and production of biomass of phototrophic bacteria on different agricultural wastes as nutrient media are less known.

The present work reports the results of investigation on production of biomass of phototrophic bacteria using as nutrient media the agricultural wastes such as stock-breeding manure and poultry dung.

*Materials and methods.* The strains of purple nonsulfur photosynthetic bacteria *Rhodobacter sphaeroides* sp.D-8 and *Rhodobacter sphaeroides* sp. A-10 isolated from mineral springs of Jermuk and Arzni as well as strain *Bacillus megaterium* INMIA B-1502 have been studied. All strains of bacteria were deposited in Culture Collection of the *Centre of Microbiology and Microbial Depository (CMMD)*, NAS of Armenia.

Strains of phototrophic bacteria were grown on Ormerud's medium [9]. The strain of *Bacillus megaterium* was grown on a medium containing fish hydrolysate [1].

The agricultural wastes such as stock-breeding manure and poultry dung were periodically taken from stock-breeding and poultry farms of Abovyan region. The wastes were dried at 60° for 15 days until containing 30 % of humidity in manure and 20% of humidity in dung. The 100 g dry matter samples after grinding were dissolved in one litre of tap water and were kept under room conditions periodically stirring for 4-6 hours. After filtration the extracts obtained were used as a base of nutrient media for cultivation of phototrophs. The initial pH was nearly 6.5-6.9. The chemical analysis of extracts showed that they contain ions of HCO<sub>3</sub> - 1321.0-1858. 5 mg/l, carbohydrates (glucose, sucrose) - 0.08-0.86 %, salts of organic acids which could satisfy the growth requirements of purple nonsulfur bacteria.

The phototrophs were grown at 30-32° with 2500-3000 lux illuminating and under different conditions of aeration. The inoculum was added in amount of 5 % (v/v) in the nutrient media. The growth of cultures was monitored spectrophotometrically determining the optical density of cell suspension at wave length 660 nm and by determining the dry weight of bacterial biomass. Microscopic control was performed using microscope MBB-1A and a KF-4 phase contrast device. Absorption spectra of whole cells were measured on "Specord" UV-Vis spectrophotometer. The phototrophs were grown for 5-8 days until the considerable de-crease of growth. The content of wet protein was determined by nitrogen method. The amino acids composition was determined on amino acid analyzer AAA-339. The vitamins were determined by Odincova's method [8], vitamin  $B_{12}$  - by Kuceva's method [5]. Three-day-old cultures of phototrophic bacteria grown ana-erobically under 2500-3000 lux illumination and one-day-old culture of B. mega-terium grown on a flask-shaker were used as an inoculum for mixed cultivation with optimal combination 2:1 (v/v) of phototrophic and heterotrophic cultures.

Statistical analysis was based on date from experiments of ten replicates.

**Results and discussion.** The yield of biomass is few on clean extracts. It is explained by the high contents of calcium salts in extracts which affects on penetration of light increasing the optical density of the media. Good results of the growth were mentioned when the extracts were diluted with tap water in ratio 1:1(v/v). The accumulation of biomass in considerably increased when small amount of yeast extract (0.02%) is added in different media as a growth stimulator. The results of composition and content of amino acids (Table 1), total nitrogen and protein (Table 2), valuable vitamins (Table 3) and carotenoids (Fig. 1) of obtained biomass are represented respectively on tables.

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Amino acids	Ormerud's	Medium with	Medium with
	medium	stock-breeding	poultry
	(control)	manure	dung
DL-Alanine	4.57	4.55	4.3
L-Arginine	3.2	3.2	3.12
DL-Aspartic acid	4.34	4.35	4.05
Glycine	2.38	2.18	2.0
L-Glutamic acid	5.43	5.43	5.33
L-Histidine	1.1	1.1	1.05
L-Isoleucine	2.42	2.25	2.2
DL-Leucine	3.15	2.88	2.72
DL-Lysine	2.72	2.68	2.68
DL-Methionine	1.08	0.85	0.64
DL-Phenylalanine	1.99	1.9	1.69
L-Proline	2.6	2.5	2.16
DL-Serine	1.58	1.58	1.58
DL-Threonine	2.83	2.8	2.68
DL-Tryptophane	+	+	+
Dl-Tyrosine	1.48	1.3	1.07
DL-Valine	3.43	3.3	3.11

**Table 1.** Amino acid composition of biomass of *Rh. sphaeroides sp.D-8* grown on different media (g/100g dry weight of biomass)

**Table 2.** Contents of total nitrogen and protein in biomass of phototrophic bacteria grown on different media

Media	<i>1)Total nitrogen,</i> %	1)Protein, %	2)Total nitrogen, %	2)Protein, %
Ormerud's medium (control)	9.4	59	8.67	54.1
Medium with stock-breeding manure	9.25	58.6	8.45	53.7
Medium with poultry dung	9.2	58.2	8.22	53.3

Legend: 1)- Rh. sphaeroides sp. D-8, 2) – Rh. sphaeroides sp. A-10.

Amino acids	Ormerud's medium	Medium with stock-	Medium with
	meatum	breeding manure	poundy duing
Biotin	0.42	0.39	0.37
Cyanocobalamin	513	503	487
Niacin	415	398	389
Pantothenic acid	2.5	2.3	2.3
Pyredoxin	2.6	2.6	2.17
Thiamin	6.03	5.8	5.35

*Legend: Vitamins content,*  $\mu g/g dry weight$ .

The results of composition and content of amino acids, total nitrogen and protein, valuable vitamins in biomass obtained on media with waste extracts didn't give up and in some cases exceeded the same results on the control Ormerud's medium (Tables 1, 2, 3).

It is known that the organic compounds of carbon serve as a source of carbon and electron donor for purple nonsulfur bacteria. The less of car-bon source media affects not only on growth of the cultures but also on biosyntheses of carotinoides.

The absorption spectra showed that the synthesized carotinoides on medium with stock-breeding manure by their quantitative and qualitative characteristics are similar to same results obtained on control Ormerud's medium (Fig. 1).



Fig. 1. Absorption spectra of whole cells of *Rh. sphaeroides sp. D-8* grown in different media: 1. Ormerud's medium, 2. Medium with stock-breeding manure

The results showed that the extracts of poultry dung were poor of so-me elements in comparison with the stock-breeding manure. The addition of 0.3% glucose to poultry dung stimulates the growth of bacteria up to 3.3-3.8g/l. It is more effective to use molasses in concentration of 0.25% in media with the extracts of poultry dung.

It is known that during the mixed cultivation of different microor-ganisms the constant balance or stimulation of the growth of single or mixed cultures of microorganisms is observed. Physiological properties of microorganisms, the composition of media and the conditions of cultivation have great role in this process [3]. The data on cultivation statistics of mixed cultures under different conditions confirm the great influence on the growth of monocultures and mixed cultures [4].

In our experiments we have used the method of mixed cultivation for production of high level of biomass of phototrophic bacteria on base of wastes. As heterotrophic microorganism *B. megaterium* was used.

The purple nonsulfur bacteria are facultative anaerobes though they grow also well under conditions of weak aeration in dark. In association of facultative anaerobic phototrophic bacteria and aerobic *B. megaterium* es-sentially the partners are differed with their requirements to oxygen.

The results on mixed cultivation of phototrophic bacteria and *B. me-gaterium* are represented in Table 4.

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In our experiments the active growth of *B. megaterium* was observed under aerobic conditions using both monoculture and mixed culture. Microairation in mixed culture increased the growth of phototrophs and decreased the growth of heterotrophic culture but the heterotrophic culture in this case had rather stimulating influence on growth of phototrophs. In case of anaerobic conditions the growth of phototrophic bacteria was high in monoculture but the single cells of *B. megaterium* were observed in monoculture. In mixed culture the growth of phototrophic bacteria under anaerobic conditions was high in comparison with monoculture. In this case the stimulation effect of *B. megaterium* even with weak growth was available (Table 4).

Cultures	Aerobic	Microaerophilic	Anaerobic
	(dark)	(light)	(light)
Rh. sphaeroides, sp. D-8	0.43	3.36	3.06
Rh. sphaeroides, sp. A-10	0.54	3.85	3.7
B. megaterium	2.2	1.9	0.048
Rh. sphaeroides, sp. D-8 +			
B. megaterium	2.41	4.8	4.24
Rh. sphaeroides, sp. A-10 +			
B. megaterium	2.66	5.03	4.94

 Table 4. Influence of aeration on the biomass yield during mixed cultivation of phototrophic bacteria and B. megaterium on medium with stock-breeding manure extracts (g/l dry weight of biomass)

The analysis of the growth of single culture-components showed that the mixed growth of cultures is based on symbiotic interaction as commensalism. The commensal relations among phototrophic and heterotrophic bacteria have their best effect under microaerophilic conditions. The stimulating influence of heterotrophic bacterial culture was the highest when the correlation of phototrophic and heterotrophic bacteria was in ratio 2:1(v/v) in inoculum. In association of phototrophic and heterotrophic bacteria the yield of biomass could be increased about 33-42% in comparison with monoculture. Microscopic examination conformed that the biomass obtained was mainly consisted of phototrophic bacteria.

Thus, the cheap nutrient media on the base of agricultural wastes such as stock-breeding manure and poultry dung have been developed for production of biomass of phototrophic bacteria. The highest yield of biomass was observed by mixed cultivation under microaerophilic conditions of growth. Besides being rich feed product the biomass can be also used as a source of valuable biologically active substances and can be applied as fertilizer in agriculture.

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