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DETERMINATION OF 5-AMINOLEVULINIC ACID IN LIQUID CULTURES OF PURPLE NON-SULFUR PHOTOSYNTHESIZING BACTERIA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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A method of high-performance liquid chromatography (HPLC) was used for determination of producers of 5-aminolevulinic acid (ALA) in liquid cultures of purple non-sulfur photosynthesizing bacteria. ALA is derivatized with formaldehyde in which acetyl acetone in the presence of the amine group of ALA forms the fluorescent product 3,5-diacetyl-1,4-dihydro lutidine. The elaboration of the method was carried out on a liquid chromatograph "Waters Separations module 2695" (USA) with fluorescence detection. As a stationary phase, a chromatographic column Armsfer Si C_{18} was used. The chromatography of ALA was performed in an isocratic mode of elution.

Figs. 2, references 11.

Introduction

5-Aminolevulinic acid (δ-aminolevulinic acid or ALA), an endogenous non-proteinogenic amino acid, is the first compound in the porphyrin synthesis pathway, which leads to *heme* [1] in mammals and chlorophyll [2] in plants.

ALA has recently drawn increasing attention as a photodynamic chemical, which has been widely applied in medical and agricultural fields. This chemical not only has potential applications in tumor-localizing and photodynamic therapy in the case of various cancers, but it also can be used as a favorable biodegradable herbicide and insecticide which is harmless to crops, humans, and animals. Research efforts supporting the microbial production of ALA have received increasing interest due to its dominant

advantages over chemical synthesis, including higher yields, lesser pollutant emissions, and a lesser monetary cost.

ALA synthesis using photosynthetic bacteria (PSB) is a promising approach in various microbial synthesis methods [3-6]. In addition, ALA could increase plants' tolerance to low temperature and high salt concentration, and it might also be utilized as an efficient promoting factor for several crop yields even at low concentrations [7, 8]. ALA was mainly synthesized through chemical methods, and six potent synthetic methods have been proposed [7].

Nowadays, 5-ALA is mostly produced using microbial fermentation, namely by photosynthetic bacteria because chemical synthesis of 5-ALA has lower yields and is more complicated in comparison to microbial production [9]. Production of 5-ALA has been reported using both wild strains of bacteria and their mutants. Application of mutant strains is far more appropriate for 5-ALA production. So far many different strains of photosynthetic bacteria together with their mutants have been tested for their 5-ALA production capacities [10].

This paper is devoted to determination of 5-ALA in several wild strains of photosynthetic non-sulfur purple *Rhodobacter (Rba.) capsulatus*, *Rba. sphaeroides*, *Rhodopseudomonas (Rps.) palustris* using HPLC methodology.

Materials and methods

Chromatography

Waters 2695 Separations Module (USA) liquid chromatograph with a Waters 2475 fluorescence detector was used. As a stationary phase, a chromatographic column Armsfer Si C_{18} 5-8µ, 250×4 *mm* (PLIVA - Lachema a. s., Brno, Czech) was used. The determination of ALA was performed in an isocratic mode of elution. As a mobile phase CH_3CN (0.1% TFA) and water (0.1% TFA) (v/v 30/70) was used, flow rate was 1 ml/min, detection was carried out at a wavelength of λ_{ex}=350, λ_{em}=450, column temperature was 30^{o}C, injection volume – 10 μl. Chemical reagents and eluents from Sigma-Aldrich with a purity of > 99.9% (gradient grade, for HPLC) were used.

HPLC ALA assay

A fluorescent derivative of ALA was prepared based on the Hantzsch reaction of formaldehyde in which acetyl acetone in the presence of the amine group of ALA forms the fluorescent product 3,5-diacetyl-1,4-dihydrolutidine [11]. 50 µl of the culture supernatant was reacted with 3.5 ml of acetyl acetone reagent, acetyl acetone: ethanol: water (15:10:75 by vol.) containing 0.4% NaCl, and 0.45 *ml* formaldehyde solution (8.5% v/v). This mixture was heated for 30 *min* at 100^{o}C and then cooled in an ice bath. It

was left to stand in the dark until HPLC analysis with a fluorescence detector (Waters USA). 10 μl of the reaction mixture was injected into the Armsfer Si C_{18} column.

Results and discussion

In this research, we studied representatives of photosynthetic non-sulfur purple bacteria of species *Rba. capsulatus* MDC 6508, *Rba. sphaeroides* MDC 6509, *Rba. sphaeroides* MDC 6511 and *Rps. palustris* MDC 6506, previously isolated from various sources of mineral waters of Armenia and maintained in the Laboratory of Energy Alternative Sources.

HPLC analysis showed that the yield of 5-ALA on chromatograms averaged 310 seconds. (Fig. 1). The test substance is well detected, which makes it possible to use this method both for the purpose of identification and quantitative assessment of the content of 5-ALA in the metabolic products of the investigated cultures of purple bacteria.

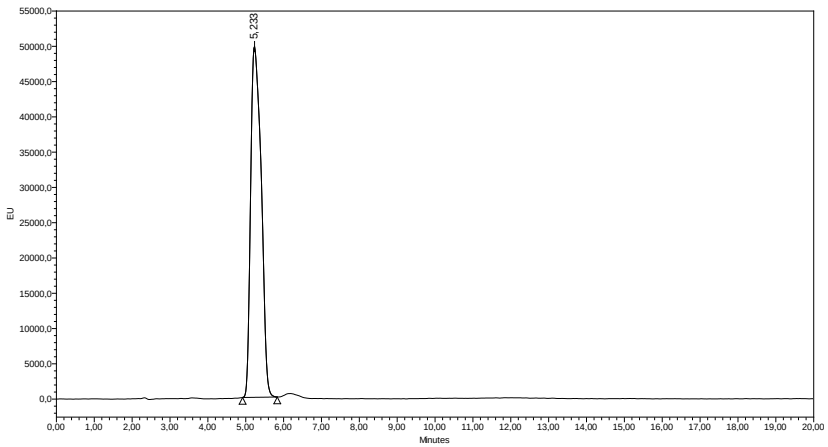

Fig.1. HPLC chromatogram of a standard sample of 5-ALA hydrochloride.

At the same time, after the corresponding pretreatment, metabolic products (culture liquid) of selected crops – potential producers of 5-ALA *Rba. capsulatus* MDC 6508, *Rba. sphaeroides* MDC 6509, *Rba. sphaeroides* MDC 6511 and *Rps. palustris* MDC 6506, were prepared for analysis. Figure 2 presents HPLC analyses of the metabolites of these strains, from which it can be seen that the objects of research contain 5-ALA similarly to the standard.

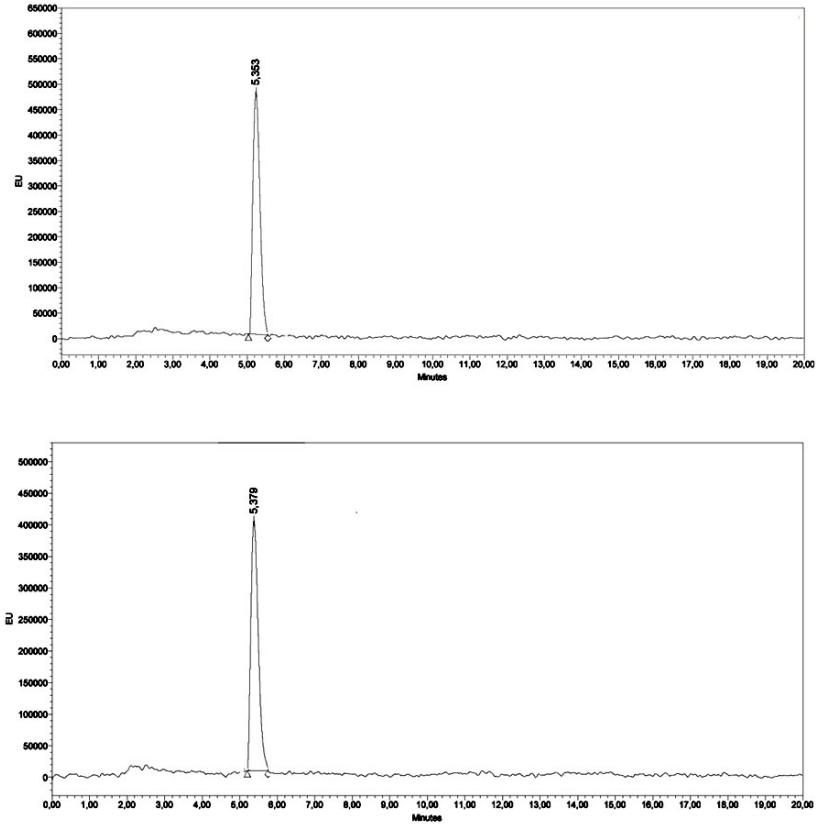

Fig. 2. HPLC chromatogram of culture fluids of photosynthetic non-sulfur purple bacteria: A - *Rba.sphaeroides* MDC 6509, B – *Rps. palustris* MDC 6506.

Using the data obtained (Fig. 2), the values of the concentrations of 5-aminolevulinic acid were calculated.

The concentration of 5-ALA in the culture fluids was calculated by an external standard method. The accuracy of this method is 2–3%. To perform this method, standard solutions of known concentrations of the compound of interest are prepared with one standard that is similar in concentration to the unknown. A fixed amount of sample is injected.

The concentration of the unknown is then determined according to the following formula:

$$Conc._{unkown} = \left(\frac{Area_{unknown}}{Area_{known}}\right) * Conc._{known}$$

Thus, it was established that the studied strains of photosynthetic non-sulfur purple bacteria, cultivated on Ormerod liquid medium, under standard growth conditions, synthesize ALA. The best producers according to the results of the analysis, were cultures of *Rba. sphaeroides* MDC 6509 and *Rps. palustris* MDC 6506, synthesizing 13 and 13.3 *mg* of 5-ALA per liter, respectively.

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ԾԻՐԱՆԱԳՈՒՅՆ ՈՉ ԾԾՄԲԱՅԻՆ ՖՈՏՈՍԻՆԹԵԶՈՂ ԲԱԿՏԵՐԻԱՆԵՐԻ ԿՈՒԼՏՈՒՐԱԼ ՀԵՂՈՒԿՈՒՄ 5-ԱՄԻՆԱԼԵՎՈՒԼԻՆԱԹԹՎԻ ՆՈՒՅՆԱԿԱՆԱՑՈՒՄԸ ԲԱՐՁ ԵՂԱՆԱԿՈՎ

Է. Վ. ՄԻՆԱՍՅԱՆ, Ա. Հ. ԾԱՏՈՒՐՅԱՆ,
Ն. Ք. ՔԱԼԱՆԹԱՐՅԱՆ և Բ. Ա. ՀԱՐՈՒԹՅՈՒՆՅԱՆ

Բարձր արդյունավետության հեղուկային քրոմատոգրաֆիայի (ԲԱՀՔ) մեթոդով նույնականացվել են ծիրանագույն ոչ ծծմբային ֆոտոսինթեզող բակտերիաների կուլտուրալ հեղուկներում 5-ամինալևուլինաթթուն: Մեթոդը իրականացվել է «Waters Separations module 2695» (ԱՄՆ) հեղուկային քրոմատոգրաֆի վրա՝ ֆլյուրոսցենտային դետեկցմամբ: Որպես անշարժ փազ օգտագործվել է Armsfer Si C_{18} 5-8 μ, 250×4 մմ (PLIVA - Lachema a. s., Բռնո, Չեխիա) աշտարակը: 5-ամինալևուլինաթթվի քրոմատոգրաֆիան իրականացվել է իզոկրատիկ եղանակով: Որպես շարժական փազ օգտագործվել է CH_3CN (0, 1% TFA) և ջուր (0, 1% TFA) 30/70 ծավալային հարաբերությամբ: Հոսքի արագությունը՝ 1 մլ/րոպե, ֆլյուրոսցենտային ալիքի երկարությունը՝ λex=350, λem=450:

ОПРЕДЕЛЕНИЕ 5-АМИНОЛЕВУЛИНОВОЙ КИСЛОТЫ В КУЛЬТУРАЛЬНЫХ ЖИДКОСТЯХ НЕСЕРНЫХ ФОТОСИНТЕЗИРУЮЩИХ БАКТЕРИЙ МЕТОДОМ ВЫСОКОЭФФЕКТИВНОЙ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ

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Методом высокоэффективной жидкостной хроматографии определено содержание 5-аминолевулиновой кислоты (АЛК) в культуральных жидкостях фотосинтезирующих несерных пурпурных бактерий. Разработка метода осуществлялась на жидкостном хроматографе “Waters Separations module 2695” (США) с помощью флуоресцентного детектирования. В качестве хиральной фазы использовали хроматографическую колонку Armsfer Si C_{18} 5-8 *мкм*, 250×4 *мм* (PLIVA – Lachema a. S., Брно, Чехия). Хроматографию АЛК выполняли в изократическом режиме элюирования. В качестве подвижной фазы использовали CH_3CN (0,1%

TFA) и воду (0,1% TFA) (об./об. 30/70), при скорости потока – 1 *мл / мин*. Детектирование проводили при длине волны λ_{ex} = 350 и λ_{em} = 450.

REFERENCES

[1] *Gardener L.C., Cox T.M*. // The Journal of Biological Chemistry, 1988, v. 263, №14, p. 6676.

[2] *Wettstein D., Gough S., Kannangara C.G.* // The Plant Cell, 1995, v. 7, p. 1039.

[3] *Beck T.J., Kreth F.W., Beyer W., Mehrkens J.H., Obermeier A., Stepp H., Stummer W., Baumgartner R.* // Lasers in Surgery and Medicine, 2007, v. 39, p. 386.

[4] *Bhowmick R., Girotti A.W.* // Free Radical Biology and Medicine, 2010, v. 48, p. 1296.

[5] *Johansson A., Palte G., Schnell O., Tonn JC., Herms J., Stepp H*. // Photochemistry and Photobiology, 2010, v. 86, p. 1373.

[6] *Kang Z., Zhang J., Zhou J., Qi Q., Du G., Chen J.* // Biotechnol Adv., 2012, v. 30, p. 1533.

[7] *Sasaki K., Watanabe M., Tanaka T*. // Applied Microbiology and Biotechnology, 2002, v. 58, p. 23.

[8] *Liu S., Zhang G., Li X., Zhang J*. // Applied Microbiology and Biotechnology, 2014, v. 98, p. 7349.

[9] *Zhang Z., Li H., Zhou W., Takeuchi Y., Yoneyama K*. // Plant Growth Regulation, 2007, v. 49, p. 27.

[10] *Sasaki K., Tanaka T., Nishizawa Y., Hayashi M*. // Journal of Fermentation and Bioengineering, 1991, v. 71, p. 403.

[11] *Li Q., Sritharathikhum P., Oshima M., Motomizu S.* // Analytica chimica acta, 2008, v. 612, p. 165.