Chemistry of Natural Compounds, Vol. 44, No. 3, 2008

CAROTENE-FORMING ACTIVITY OF CERTAIN HALOPHILIC BACTERIA FROM BARSAKELMES SALINE SOIL

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UDC 579.222

Strains of halophilic bacteria from samples of Barsakelmes saline soil were screened for ability to synthesize carotinoid pigments. An active strain that accumulated β-carotene as the main pigment was selected. The β-carotene was shown to be identical to the standard pigment.

Key words: halophilic bacteria, carotinoid pigments, β-carotene.

Anaerobic photosynthetic bacteria, cyanobacteria, yeast, algae, higher plants, fungi, and extremely halophilic bacteria are known microbial producers of carotinoids [1]. Cells of halophiles contain many carotinoid pigments that color the colonies from pink to red. This is very important for halophilic microorganisms because it protects them from the intense radiation that is typical of their habitat [2]. Several halophilic microorganisms have been previously isolated and characterized from samples of saline soils of the Republic [3]. However, their pigment-forming activity has not be examined. Our goal was to identify carotene-forming halophiles and study the array of carotinoids synthesized by them.

We previously isolated 37 bacterial isolates growing in media with salt concentrations 15-25% from saline soils in various regions of the Republic of Uzbekistan. A characteristic signature of most isolated strains was the ability to accumulate various red shaded pigments [4]. Based on the literature [5], we assumed that the characteristic color of the isolated bacteria was due to the presence of carotinoid pigments. Fourteen cultures colored various shades of red and pink were selected for the investigation of their carotene-forming properties. We found that the selected isolates were able to grow in a medium containing 25% NaCl. This salt concentration was optimal for some of them. All selected cultures to one degree or another were able to accumulate carotinoid pigments. Figure 1 shows that the total carotinoid content in most cultures varied in the range 0.157-1.5 mg/L of culture liquid. Moreover, the carotinoid level in three cultures, designated K38, K91, and K91r, was significantly higher. Thus, isolate K91r accumulated up to 5 mg/L; K91, 4.6; K38, 4.3 mg/L. According to the literature, the accumulation level of carotinoid pigments in microorganism producers reaches 8 mg/L of culture liquid [6, 7]. For example, the single-celled alga *Dunaliella*, the most famous producer of β-carotene, could synthesize up to 30 mg of carotinoids per liter under the optimal growth conditions. Of these, 60% were the *cis*-isomers of β-carotene, which are much more active than chemically synthesized *trans*-isomers of the pigment [8].

β-Carotene is commonly regarded as the principal biotechnologically valuable carotinoid pigment. Therefore, we performed HPLC analysis of extracts from cultures K38, K91, and K91r using ethylacetate as the solvent and mobile phase in order to determine the β-carotene content in the total carotinoid preparations.

Isolate K91 contained a fraction corresponding with standard β-carotene according to the chromatographic separation.

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 0009-3130/08/4403-0306

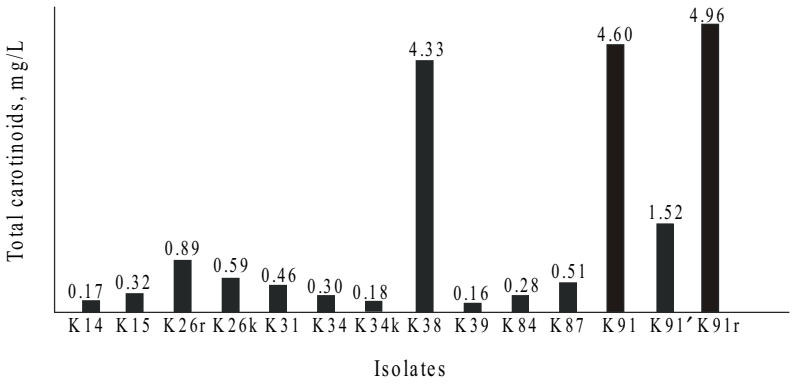

Fig. 1. Level of carotinoids in extracts of halophilic bacteria (average of three determinations).

EXPERIMENTAL

Cultivation Subject and Medium. We used 14 pigment-forming bacteria isolates isolated from saline soil of Barsakelmes (Ustyurt Plateau, Karakalpakiya). Medium No. 44 for *Halobacteria* of the following composition was used for cultivation and pigment-formation of the microorganisms (g/L of tapwater): NaCl, 250; $MgSO_4$, 10; KCl, 5: $CaCl_2 \cdot 6H_2O$, 0.2; tryptone, 2.5; yeast extract, 5. A medium of the same composition with added agar (20 g/L) was used to preserve the cultures.

Total Carotinoid Content. Fat-soluble carotinoids were isolated from biomass of pigmented bacteria by the literature method [9] using extraction of bacterial biomass in acetone:hexane until the sample was completely decolorized. All water-soluble pigments were transferred into the acetone; fat-soluble ones remained in the hexane.

The extinction of the resulting extract was measured at 465 nm. The amount of carotinoids was determined from a calibration curve for standard solutions of β-carotene.

HPLC Analysis of β-Carotene. The composition of the carotinoids in the resulting extracts were analyzed by HPLC (Agilent, USA) [10]. Because hexane cannot be used with the Zorbax Eclipse XDB C18 column (4.6 × 150 mm), extracts were dried beforehand in a rotary evaporator at 50°C. The dry solid was redissolved in ethylacetate. The standard was commercial β-carotene in ethylacetate for chromatography (Fluka). Samples were eluted over the Zorbax column by ethylacetate:water (98:2) mobile phase. The sample size was 30 μL; elution flow rate, 1 mL/min.

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