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Comparative Characterization of Extracellular and Intracellular Hydrocarbons of *Clostridium pasteurianum*

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Abstract—Extracellular and intracellular hydrocarbons produced by *Clostridium pasteurianum* VKM 1774 during cultivation on glucose-containing media in an argon atmosphere or in the presence of carbon dioxide and molecular hydrogen were analyzed by gas-liquid chromatography. Intracellular hydrocarbons were 50-55% (C_{25}-C_{35}) *n*-alkanes. Carbon dioxide and molecular hydrogen stimulated synthesis of extracellular hydrocarbons, which comprised 90-95% (C_{11}-C_{24}) *n*-alkanes.

Key words: hydrocarbons, intracellular, extracellular, *Clostridium pasteurianum*

Study of the ability of anaerobic bacteria to synthesize hydrocarbons is of great importance because of the fuel crisis and the possibility of biological production of hydrocarbon supplies. In addition, there is an opinion about the protective role of the produced hydrocarbons, [1] and their capability for autoregulation of adhesion [2, 3]. The protective role of hydrocarbons is connected with their making the cell wall of microorganisms hydrophobic, and this increases their adaptation under natural conditions. This is especially important for microorganisms that produce acids as end products of metabolism. Also, some authors think that extracellular hydrocarbons decrease adhesion properties on glass. The mechanism of this process is not studied well, but it has been suggested that because the basis of adhesion is connected with hydrophobic links of the cell with glass and ionic interactions, the hydrocarbons become a competitive inhibitor of adhesion blocking hydrophobic microlocuses on the surfaces of cells. It was also shown that hydrocarbons connect with cells and cause the sorption of other cells, forming heavy aggregates, this also assisting in desorption of the microorganisms from glass.

Different microorganisms have been noted to synthesize intracellular hydrocarbons [4-7]. And extracellular hydrocarbons can be produced not only by methanogens, but also by *Desulfovibrio desulfuricans* and *Pseudomonas fluorescens* [3, 8, 9]. In the case of *D. desulfuricans* both intracellular and extracellular hydrocarbons have been studied [10, 11].

This work was devoted to the investigation of intracellular and extracellular hydrocarbons of *Clostridium pasteurianum* growing on glucose-containing medium in the presence of Ar or CO_2 and H_2.

MATERIALS AND METHODS

Clostridium pasteurianum VKM 1774 was obtained from the Collection of Microorganisms, Russian Academy of Sciences and cultivated in vials on nutrient medium of containing (g/liter): K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.005; $FeSO_4 \cdot 7H_2O$, 0.005; $MnSO_4 \cdot 7H_2O$, 0.005; glucose, 10.0; yeast extract, 0.1, in an atmosphere of Ar or a mixture of 10% CO_2 and 90% H_2, pH 7.2-7.4. After inoculation, the vials were flushed with gas passed through a bacterial filter and closed hermetically. The volume ratio of the gas phase and the nutrient medium was 2 : 1. The vials were incubated at 30°C for 60 h, this corresponding with the beginning of stationary phase of bacterial growth. Biomass content was determined using a Photoelectrocolorimeter-56.

Hydrocarbons were determined using a GLD chromatograph (France) on a 25-m capillary column of Apiezon L. Chromatograms were recorded and analyzed by area using a LKB integrator. To determine extracellular hydrocarbons, bacterial cells were separated by centrifugation at 7000*g* for 15 min, and then the supernatant was tested for the absence of cellular protein and treated with chloroform (1 : 10 v/v). The mixture was kept at 25°C for 24 h and then analyzed. Bacterial cells were washed with

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Intracellular and extracellular hydrocarbons from cells and culture fluid of *Cl. pasteurianum* VKM 1774

Alkanes	Gaseous phase	Hydrocarbons, % of biomass	Content of hydrocarbons, %						
			ΣC_{11}-C_{18}	ΣC_{19}-C_{24}	ΣC_{25}-C_{35}	$(\Sigma Cco_2+H_2)/\Sigma C_{Ar}$	Σn-C	Σi-C	$\Sigma C_{odd}/\Sigma C_{even}$
Intracellular	Ar	2.5 ± 0.4	10.2 ± 1.2	35.6 ± 2.5	54.2 ± 4.2	1.2 ± 0.5	89.3 ± 3.5	10.5 ± 3.2	1.00 ± 0.02
	$CO_2 + H_2$	3.7 ± 0.2	14.0 ± 2.8	36.2 ± 2.0	49.8 ± 4.5		81.6 ± 2.5	18.4 ± 2.8	1.02 ± 0.02
Extracellular	Ar	3.8 ± 0.2	59.2 ± 3.8	34.6 ± 4.8	6.2 ± 0.5	2.0 ± 0.5	68.4 ± 3.5	31.6 ± 2.5	1.02 ± 0.02
	$CO_2 + H_2$	6.9 ± 0.4	63.2 ± 2.8	30.8 ± 4.2	6.0 ± 1.2		62.4 ± 3.0	37.6 ± 2.5	1.04 ± 0.04

Note: Σn-C and Σi-C are relative contents of *n*-alkanes and iso-alkanes + isoprenoids.

0.01 M Tris-phosphate buffer (pH 7.0), disintegrated using an UZDN-2T ultrasonic disintegrator at 22 kHz for 6 min, and centrifuged at 18,000*g* for 20 min to remove unbroken cells and cell fragments. The supernatant was treated with chloroform (1 : 10 v/v), kept for 24 h as described above, and intracellular hydrocarbons were determined.

Commercial Ar and CO_2 were used without further purification; H_2 was obtained using an SGS-2 generator. The purity of the bacterial culture was monitored by microscopy.

RESULTS AND DISCUSSION

Intracellular and extracellular hydrocarbons of *Cl. pasteurianum* VKM 1774 at the beginning of stationary phase of bacterial growth are compared in the table. Intracellular hydrocarbons produced during cultivation in the atmosphere of Ar or CO_2 and H_2 comprise 50-55% *n*-C_{25}-C_{35} alkanes. Amounts of isomers and isoprenoids (i-C) are insignificant. The ratio of alkanes with even and odd numbers of carbons is close to unity. The high molecular weight composition of intracellular hydrocarbons of *Desulfovibrio desulfuricans* was shown earlier [11].

The spectra of *n*-alkanes of the extracellular and intracellular hydrocarbons are similar. However, the extracellular hydrocarbons are of lower molecular weight, the content of *n*-C_{11}-C_{24} alkanes being 90-95%. The quantity of isoprenoids and isomers were a little higher (32-38%). The production of extracellular hydrocarbons in the presence of CO_2 and H_2 is twofold higher than that in the atmosphere of Ar. The hydrocarbon composition changed with increasing quantity of hydrocarbons of lower molecular weight. Increase in the hydrocarbon production in the atmosphere $CO_2 + H_2$ was connected with the following. First, adding H_2 into the gas mixture of the cultivation atmosphere leads to the shifting the equilibrium of biochemical reactions to reduction processes. Then enzymatic involvement of CO_2 and H_2 occurs with formaldehyde and formate production, which can increase hydrocarbon synthesis. Also, hydrocarbons synthesis might be connected with stress for clostridium, and adding of the $CO_2 + H_2$ gas mixture is a stress condition.

The extracellular hydrocarbons produced by *Cl. pasteurianum* have carbon chain lengths similar to those of *Desulfovibrio desulfuricans* and somewhat smaller than those of *Pseudomonas fluorescens* [3, 11]. In contrast to sulfate-reducing bacteria and pseudomonas, branched forms of alkanes were found.

The data show that the intracellular hydrocarbons are mainly higher molecular weight alkanes. The extracellular hydrocarbons are mainly lower molecular weight alkanes. The differences in the molecular composition of intracellular and extracellular hydrocarbons are connected with the functioning of the cytoplasmic membrane: the hydrocarbons of low molecular weight pass through the membrane to the cultural fluid, and the retained hydrocarbons are involved in biochemical processes that lengthen the hydrocarbon chains.

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