

Bacteria *Cellulosimicrobium* sp. as a food substrate for free-living amoebae

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Free-living amoebae are ubiquitous microorganisms that can be isolated from environmental objects as well as from patients with amoebic keratitis, sinusitis, encephalitis etc. There are two possible variants of cultivation these protozoa: monoxenic (on culture of bacteria) and axenic (without using microorganisms). Cultures of *E. coli*, *Enterobacter* sp. and non-capsule strains *Klebsiella pneumoniae* are used for monoxenic cultivation most often. In this study, bacteria of genus *Cellulosimicrobium* was used as a food substrate for amoebae. As these bacteria were isolated from bentonite clays in Ukraine, as amoebae, it was suggested that amoebae can feed by them, both in laboratory and in natural conditions.

THE AIM OF THE STUDY. The determination of the features of monoxenic cultivation representatives of the genus *Acanthamoeba*, using bacteria *Cellulosimicrobium* sp.

RESULTS. The specific "bentonite" amoebae were isolated during the study of microbial consortia of bentonite clays. They were classified as genus *Acanthamoeba* after the detailed microbiological study and sequencing of the gene 18S RNA. The bacteriological method – co-cultivation with *Escherichia coli* was used to isolate amoeba cultures on the initial stages. However, the results of such cultivation were poorly reproduced. During further experimental studies, bacteria that were used as a food substrate for "bentonite" amoebae were isolated. The growth of amoebae was accompanied by a creeping vitreous lysis of the lawn of these bacteria, starting from the second day of cultivation. Using different methods of microscopy and staining allowed us to establish that the amoeba can actively absorb bacteria that were isolated by us. They were visualized both outside the protozoan cells and in the amoeba vacuoles. This microorganism was named *Cellulosimicrobium* sp. strain *bent-1*. These bacteria are managed to isolate from the Kurtsivske deposit of bentonite clays in Ukraine. A series of morphological, cultural, biochemical signs and sequencing of the gene 16S RNA was used to characterize these microorganisms. Obtained result made it possible to refer this strain of bacteria to the genus *Cellulosimicrobium*. Strain *bent-1* has 99% genetic similarity to the bacteria: *Cellulosimicrobium cellulans* and *Cellulosimicrobium funkei*.

The phenomenon of plaque formation – the creation of round spots with crateroid depression in agar was observed in case of co-cultivation bacteria of the genus *Cellulosimicrobium* with amoebae in the agar plates. This phenomenon makes it possible to calculate viable amoebae.

Nowadays, studies are being conducted to determine the possibility of using these bacteria for cultivation free-living amoebae that are isolated from other environmental objects.

CONCLUSION. Thus, bacteria of the genus *Cellulosimicrobium* strain *bent-1* were used for the cultivation of acanthamoebae in these experiments for the first time. The results allow us to conclude that the possibility using bacteria of this genus for monoxenic cultivation of the "bentonite" amoebae in laboratory conditions.