ATLANTIS PRESS

Advances in Biological Sciences Research, volume 7

1st International Symposium Innovations in Life Sciences (ISILS 2019)

Isolation of Local Shewanella sp. Strain from Vezjolka River at Belgorod District in Belgorod Region, Russia

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***Abstract*—The work describes the procedures used to isolate microorganisms and the initial determination of *Shewanella sp*. from the Vezjolka River, Belgorod Region of Russia. The isolates obtained during isolation are characterized by signs corresponding to those declared by the authors of the recipe for selective nutrient medium for isolation and determination of *Shewanella sp*, namely, the green color of the colonies, the zones of turbid precipitate around them, arising due to the activity of bacterial lipase, and a black center indicating production hydrogen sulfide.**

Keywords*—*Shewanella; Belgorod region; algicidal activity; bioremediation

I. INTRODUCTION

Shewanella is the only genus in the *Shewanellaceae* family, some species inside which were previously included by researchers in the genuses *Alteromonas* [1] and *Achromobacter*. All known species of the genus *Shewanella* are facultatively anaerobic gram-negative rod-shaped bacteria, most of which are inhabitants of extreme marine environments with low temperature and high pressure, but freshwater species are also found.

One of the key physiological characteristics of these microorganisms that attract the attention of researchers is their ability to use a wide range of insoluble metal oxides and other compounds as electron acceptors for electron acceptors under anaerobic conditions. Due to this, the species *Shewanella oneidensis* MR-1 is currently widely used in research related to bioremediation of wastewater and the creation of microbial fuel cells.

There is evidence of the ability of *S. oneidensis* to purify water from heavy metals like uranium (U), and chromium (Cr) [2], .iron (Fe), manganese (Mn) and many different metal oxides [3]. The bacterium reduces the vanadate ion (V5) to the vanadyl ion (V4), which precipitates as a solid [4].

S. oneidensis MR-1 also showed resistance to relatively high concentrations of lead (Pb) in the culture medium (about 700 mg per 1) [5]. As a result of natural emissions, an average of 27 thousand tons of lead annually enters the atmosphere, including volcanic eruptions, etc. However, most of it enters the environment due to anthropogenic factors. It was noted that the level of lead in plants growing near highways is higher than in areas remote from the road [6]. In addition, environmental pollution by lead is characteristic of industrial regions of the former USSR. For example, due to the presence of metallurgical production on the territory of the Stary Oskol district and the city of Stary Oskol, a technological increase in pollution with heavy metals is observed. In general, the industrial revolution and the subsequent development of industry left a significant imprint on the biosphere.

In recent years scientists have also reported about alternative uses for *Shewanella's* species. In an article published by researchers at Shanghai University, the problem of toxic cyanobacterial blooming of freshwater Taihu Lake is described [7]. Authors isolated the *Shewanella sp*. Lzh-2 strain from a natural algal consortium of the lake and showed its capability of effectively inhibit the growth of harmful cyanobacteria.

The problem of harmful cyanobacterial blooming exists in many countries including Russia. There have been reports of rapid development of macroalgae from 2010-2011, and appearance of cyanobacterial colonies since 2015 [8].

Rumyantsev V.A. with co-authors points out the significant negative effects of cyanobacteria on Russian reservoirs, noting that the safety of recreational activities, the consumption of fish and drinking water; increasing the service life of pipelines, dams, locks and equipment of power plants are closely related to cyanobacterial flowering of water [9]. Cyanobacteria are capable of adhesion on solid

surfaces, such as pipelines, dams and sluices, which leads to their corrosion failure.

Thus, screening of members of natural algobacterial communities seems to be a promising way to search for strains of natural bacterial antagonists of cyanobacteria that can be also used in bioremediation.

II. EXPERIMENTAL

In our primary experiment the local strain was isolated at the end of August and beginning of September of 2019 from the surface of the coastal section of the Vezjolka river, which is located in a zone of dense urban development, and exhibiting presumptive signs of the beginning of eutrophication, including turbid green color of water, and the extensive distribution of duckweed on the water surface (Fig. 1).

III. RESULTS AND DISCUSSION

According to the data of a chemical analysis of the water composition of the study area in 2017, the content of nitrite ions in it significantly exceeds the norms of fishery MPC [10]. Sowing was done by serial dilution of samples on a selective solid medium to isolate bacteria of the genus Shewanella sp. without the addition of irgazan and using GMF agar as the basis. Modifying components are presented in Table I. [11].

TABLE I. COMPONENTS OF THE SELECTIVE MEDIUM FOR ISOLATING MICROORGANISMS OF THE GENUS SHEWANELLA SP. ACCORDING TO THE PATENT OF THE RUSSIAN FEDERATION NO. 2435845

Ingredients	**g / l**
pancreatic hydrolyzate of fish meal	12.0
meat peptone	12.0
NaCl	6.0
twin-80	5.0
$CaCl_2$	0.2
$Na_2S_2O_3 \cdot 5H_2O$	0.35
$(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$	0.25
sorbitol	13.0
bromothymol blue	0.08
Irgazan (DP-300)	0.14–0.2
rifampicin	0.0005–0.001
NaOH	0.8
agar	12.0
distilled water	1 *l*

After incubation for 2 days in a thermostat at a temperature of 27 ° C, the grown colonies were identified as Shewanella sp. by three specific signs for growth on the indicated medium signs such as the green color of the colonies, the zones of turbid precipitate around them, arising from the activity of bacterial lipase, and the black colony center, indicating the production of hydrogen sulfide.

During the sowing, isolates were identified that were similar in character to those declared in RF patent No. 2435845 — the green color of the colonies, the zones of turbid precipitate around them and the black center. Based on this, we can conclude that some physiological properties of the isolated microorganisms, namely, the ability to form hydrogen sulfide, have an active lipase. These characteristics when grown on selective media indicate that the isolate belongs to the genus *Shewanella sp*. (Fig. 2)

Gram staining followed by microscopy showed that the selected microorganisms are gram-negative, motile and have a rod-shaped shape with bent ends (Fig. 3).

It was revealed that this isolate is represented by facultative anaerobes, since it can exist under aerobic and anaerobic conditions, which was verified in the course of the experiment carried out by the Fortner method.

Fig. 1. Water collection point.

Fig. 2. Isolated *Shewanella sp* strain.

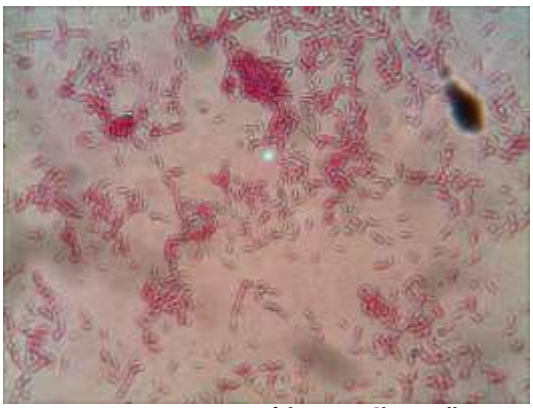

Fig. 3. Gram-negative microorganisms of the genus *Shewanella sp.*

IV. CONCLUSION

In the course of the work, a culture was identified that exhibits the claims stated in the patent, namely, green colonies with a black center and a cloudy precipitate, optionally anaerobic, gram-negative rods with rounded ends, characterized by active lipase and producing hydrogen sulfide. However, further studies of morphology, biochemical properties, and genetic features are needed to specifically position the isolated pure culture isolate as a strain.

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