ASSESSMENT OF TECHNOLOGICAL POTENTIAL OF BACTERIAL STRAINS ISOLATED FROM AQUATIC SEDIMENTS OF ROȘU, ROȘULEȚ AND PUIU DANUBIAN LAKES

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Abstract. The assessment of the technological potential of some bacterial strains from aquatic sediments has led to the detection of enzymes such as tributyrinase, amylase, citrate permeate, and some biopolymer-like products. Eight of the tested strains have the ability to synthesize polyhydroxyalkanoates, six bacterial strains secreting citrate esterases. Approximately 80% of the strains under study have the ability to metabolize starch and 60% metabolize tributyrin.

Keywords: bacterial strains, enzymes, polyhydroxyalkanoates.

Abstract. Evaluarea potenţialului tehnologic al tulpinilor bacteriene izolate din sedimentele acvatice ale lacurilor dunărene Roşu, Roşulet şi Puiu. Evaluarea potenţialului tehnologic al unor tulpini bacteriene provenite din sedimente acvatice a condus la semnalarea prezenţei unor enzime ca tributirinaza, amilaza, citrat permeaza, dar şi a unor produşi de tipul biopolimerilor. Opt dintre tulpinile testate au capacitate de a sintetiza polyhydroxyalkanoaţi, şase tulpini bacteriene secretă citrat esteraze. Aproximativ 80% dintre tulpinile luate în studiu au capacitatea de a metaboliza amidonul, iar 60% metabolizează tributirina.

Cuvinte cheie: tulpini bacteriene, enzime, polyhydroxyalkanoaţi.

INTRODUCTION

Natural environment microbiota is an inexhaustible source of new microorganisms that have the ability to synthesize products with technological applicative potential. Thus, the present study aimed to evaluate the capacity of some isolated strains from the aquatic sediment to synthesize polyhydroxyalkanoates (PHA) and/or enzymes involved in the metabolism of starch, citrate, tributyrin, etc.

Polyhydroxyalkanoates are natural polymers, biodegradable with physical characteristics similar to those of petrochemical polyesters, a reason that they are considered as an environmentally sustainable alternative to row materials of plastic (ANJUM A et al., 2016).

Amylases are enzymes with high applicability in food, textile, paper, fermentation, pharmaceutical and sugar industries (KUNAMNENI et al., 2005; WINDISH & MHATRE, 2012).

Urease enzyme is a virulence factor present in various pathogenic bacteria and has the role of hydrolyzing urea (KONIECZNA et al., 2012). Urea is a widely distributed organic compound: it is found in the natural environment (water and soil) and in the human body, where its appearance is related to protein degradation (SIRKO & BRODZIK, 2000).

Citrate permease intervenes in citrate metabolisation, playing an important role in the fermentation of many foods (HUGENHOLTZ, 1993).

MATERIAL AND METHODS

The biological material consisted of 15 bacterial strains isolated from the aquatic sediment of Roşu, Roşuleţ and Puiu Danube lakes.

The used media: agarose with 1% starch, Tween 80 agarose, Christensen medium, Simmons medium, Lugol solution, tributyrin supplemented agar medium, Luria-Bertani medium (LB), agarified mineral medium (SPIEKERMANN et al., 1999) supplemented with 1% Nile Blue with UV light (312 nm) viewing. Cultivation was carried out at 28 °C for 24-48 hours.

RESULTS AND DISCUTIONS

The bacterial strains used in the present study were isolated from sludge samples from Rosu, Rosulet and Puiu lakes. Isolation, selection and characterization were performed on agarized LB medium.

To highlight the ability to synthesize different bacterial products, the strains of interest were grown on specific media (Table 1).

To render evident the presence of tributyrase enzyme in some of the tested strains, they were cultivated on agar medium supplemented with 1% tributyrin; if the bacterial strain possesses this enzyme, the medium clarifies around the given bacterial culture (Fig. 1). Out of the fifteen strains under study, the presence of the tributyrinase enzyme was reported in nine. Approximately 60% of the tested strains have the ability to synthesize this bioproduct.

Table 1. Capacity of the bacterial strains under study to synthesize bioproducts of technological interest.

Bacterial strains	Bioproducts					
	Tributyrinase enzyme	**Amylases enzyme**	**Urease enzyme**	**Tween esterase enzyme**	**Citrart permease**	**PHA**
S20.1	-	+	-	-	+	-
S20.2	+	+	-	-	+	+
S17.1	+	+	-	-	+	-
S16.1	+	+	-	-	+	-
S34.2	-	+	-	-	-	+
S34.1	-	+	-	-	-	+
S36.1	-	+	-	-	+	-
S36.2	+	+	-	-	+	+
S30.2	+	+	-	-	0	+
S30.4	+	+	-	-	0	+
S30.1	+	+	-	0	0	+
S18.2	0	0	0	0	0	-
S12.1	+	+	0	-	+	+
S1.1	+	-	0	0	-	-
S18	0	+	-	-	+	-

Legend: **+** presence of the test product**, -** absence of the test product**, 0** no development of the bacterial culture.

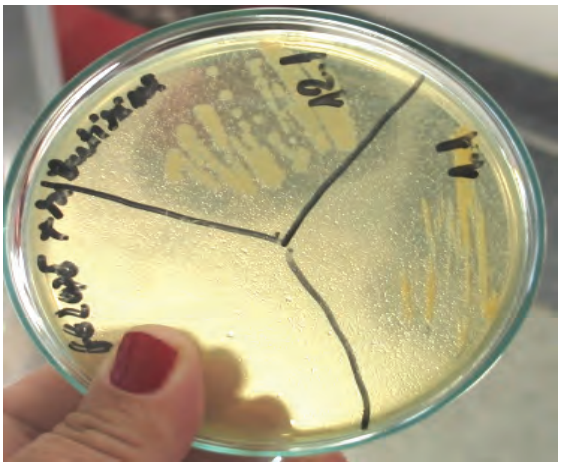

Figure 1. Bacterial strains cultivated on agar medium supplemented with 1% tributyrin (original).

In assessing the capacity of the strains isolated from the aquatic sediment of the Danube lakes to synthesize amylase, it was found that 80% of them had this ability. For this, the bacterial strains were grown on starch nutrient agar for 24 hours. After incubation, the starch hydrolysis is signaled by flooding the culture plate with Lugol solution (Fig. 2) (a yellowish ring around the bacterial culture is observed, the rest of the medium being dark).

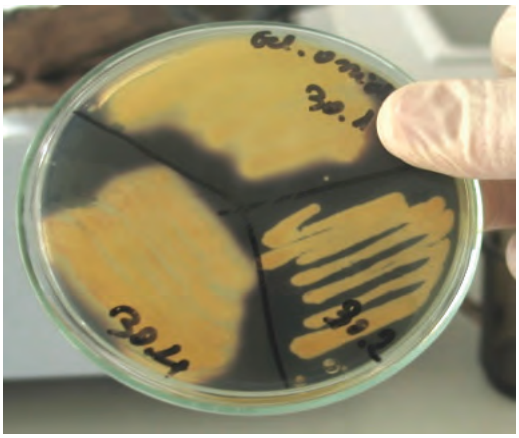

Figure 2. Bacterial strains cultivated on agar medium with starch (original).

The presence of urease and esterase, respectively, was assessed by cultivating the fifteen studied strains on Christensen medium and on an agar medium with Tween 80, respectively. All fifteen bacterial strains showed a negative reaction. Absence of the urease enzyme is a first indication that the pathogenic potential of these strains is reduced, indicating their possible use in biotechnological synthesis processes.

Another aspect of this study was the selection of bacterial strains that have the ability to metabolize citrate. To metabolize this compound, microorganisms synthesize a permease type enzyme (permease citrate). Selection was performed on Simmons medium containing bromothymol blue, a pH indicator. The colour turns from green to blue at a pH over 7.6. The increase in pH is due to the metabolisation of citrate (LAZĂR et al., 2004).

Out of the fifteen tested strains, eight have the ability to metabolize citrate (Table 1; Fig. 3), these being Gram negative bacteria because Simmons medium is used to differentiate gram-negative bacteria based on citrate usage (***. DIFCO, 2009). These strains can have applicative potential in food industry.

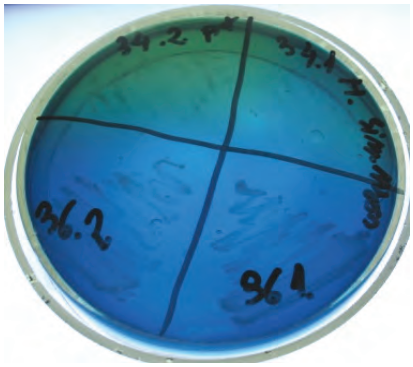

Figure 3. Bacterial strains cultivated on Simmons medium (original).

One last aspect investigated in this study was to signal the ability to synthesize intracellular polyhydroxyalkanoates (Fig. 4).

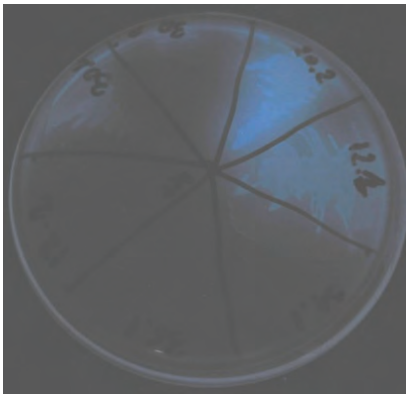

Figure 4. Bacterial strains cultivated on agarified mineral medium
(SPIEKERMANN et al., 1999) supplemented with 1% Nile Blue, UV view (original).

These biodegradable products with plastic-like features and properties were synthesized by 8 bacterial strains, representing approximately 53% of the strains under test. We believe that these strains are of real technological interest, as they can replace plastic, helping to reduce pollution.

It has also been found that S20.2 and S36.2 strains have the ability to synthesize, in addition to polyhydroxyalkanoates, also tributyrinase, urease, amylase and citrate permease enzymes. In other tested strains, three, two or one product of interest with technological applicative potential were spotted.

CONCLUSIONS

Two out of the five evaluated strains have the ability to synthesize four of the six metabolic products of interest.
Tween esterase and urease enzymes were not reported in any bacterial strains.
Eight bacterial strains have the ability to synthesize polyhydroxyalkanoates.

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