

Małgorzata NABRDALIK¹, Katarzyna GRATA¹ and Adam LATAŁA¹

EVALUATION OF LIPOLYTIC ACTIVITY OF *Bacillus* sp. ISOLATED FROM THE NATURAL ENVIRONMENT

OCENA AKTYWNOŚCI LIPOLITYCZNEJ *Bacillus* sp. WYIZOLOWANYCH ZE ŚRODOWISKA NATURALNEGO

Summary: The aim of the research was the evaluation of lipolytic activity of selected *Bacillus* sp. strains depending on the source of fatty substrate and incubation time. Strains of *Bacillus pumilus* presented the highest activity on the medium with tributyrin, and the lowest on the medium with addition of Tween 60. The most active were strains marked: *M1*, *A115* and *G8*, and the least active *Tw3*. *Bacillus subtilis* *G2* strain showed similar activity to the most active strains of *Bacillus pumilus*. Compared activity among *Bacillus cereus* showed that the strain marked *G10* was liberating the highest amount of μ moles of fatty acids on the medium with tributyrin and Tween 60. Bacterial strain marked *A96* produced the highest amount of fatty acids on medium with Tween 40 and Tween 80. *Bacillus mycoides* *G3* showed 2-3 fold lower activity than found in case of *Bacillus cereus* strains. The last analysed strain, *Bacillus firmus*, confirms that extracellular secretion of lipases is the most intense on the medium with tributyrin, Tween 40 and Tween 80, and the least intense on the medium with Tween 60.

Keywords: *Bacillus* sp., lipases, tributyrin, Tween

Lipases, defined as hydrolases of glycerol esters EC 3.1.1.3, are the enzymes of high catalytical potential. They catalyse the hydrolysis and trans-esterification of triacylglycerols, enantioselective synthesis, and hydrolysis of a variety of esters. They are produced by plants, animals and microorganisms, of which the last group remains in the centre of attention. Many bacteria have the ability to produce them but the most important in the process are the species: *Pseudomonas*, *Staphylococcus* and *Bacillus* [1].

A wide interest in bacterial lipases is linked to their role as biocatalysts in many biochemical processes. They are used, among others to produce detergents, food, paper, pharmaceuticals and in the environmental protection. However, according to data from literature [1, 2] the microorganisms are varied in terms of their enzymatic activity, which depends on the species of the microbes and the culturing conditions (eg pH of the growth medium, temperature, source of nitrogen and presence of lipids in the medium). Therefore, bearing in mind immense application abilities of microbiological lipases, there has been research done in order to find new strains, able to synthesize significant amounts of highly active enzymes.

The aim of presented research was the evaluation of the ability to synthesize extracellular lipases by selected *Bacillus* sp. strains isolated from the natural environment, depending on the source of fatty substrate.

Materials and methods

The objects of the study were the following 10 bacteria strains:

- 5 *Bacillus pumilus* strains marked as: *M1*, *Tw3*, *A115*, *G4*, *G8*,
- *Bacillus subtilis* *G2*,
- 2 *Bacillus cereus* strains marked as: *A96* and *G10*,

¹ Biotechnology and Molecular Biology Department, University of Opole, ul. kard. B. Kominka 4, 45-035 Opole, tel. 077 401 60 56, email: mnabrdalik@uni.opole.pl

- *Bacillus mycoides* G3,
- *Bacillus firmus* Tw4.

The source of fatty substrates in the growth media were 10% solutions of the following: tributyrin, Tween 40, Tween 60 and Tween 80. The cultures were maintained in Erlenmeyer flasks of 250 cm³ capacity containing 50 cm³ of the respective growth medium and placed on a rotary shaker for 15 days at 30°C. The cultures were introduced with an inoculum of density equal to E = 2 (standardized with the use of a spectrophotometer) obtained from the 48-hour culture on a nutrient broth.

Samples for the analysis were collected after 2, 4, 8 and 15 days of culturing and centrifuged for 20 minutes at 4000 rpm. In the obtained supernatant the extracellular lipolytic activity was determined by means of titration towards the same substrates previously added to the growth medium (the proper treatment). In the control treatment the supernatant was replaced with water. The amount of liberated fatty acids was estimated by titration with 0.05 M NaOH solution against 2% phenolphthalein as an indicator, and calculated as a subtraction between the proper treatment and the control treatment results. The result was presented as the amount of liberated μ moles of fatty acids.

Results

In the presented paper 10 bacterial strains of *Bacillus* kind were screened, within 15 days, for their ability to synthesize lipolytic enzymes on the growth media containing different sources of fatty substrates. The obtained results proved the variety among tested *Bacillus* strains in terms of extracellular lipases production according to the source of fatty substrate in the growth medium.

In extracellular lipases biosynthesis the most effective source of the fatty substrate was tributyrin (Fig. 1) in case of all the strains. The highest amount of fatty acids on this medium was liberated by the *Bacillus cereus* G10 strain and amounted to 97.5 μ mol, and the lowest amount (13.125 μ mol) was liberated by the strain *Bacillus pumilus* Tw3. However, the other strains of *B. pumilus* under study liberated much more of fatty acids which ranged from 30.625 to 50 μ moles for strains G4 and M1, respectively (Fig. 1).

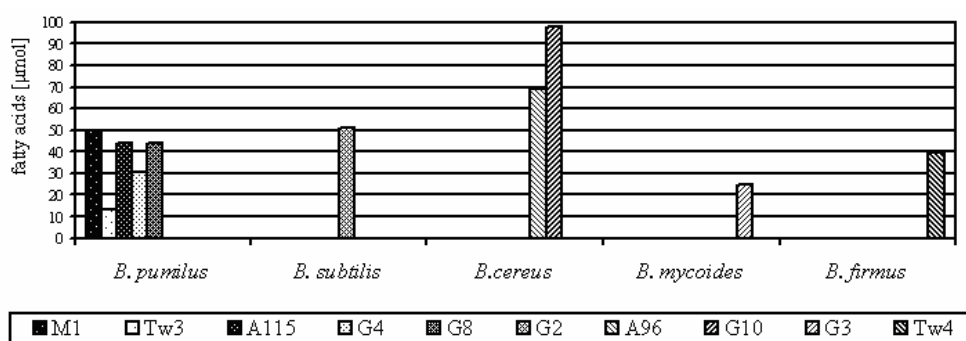


Fig. 1. The influence of tributyrin on extracellular lipases production by selected *Bacillus* strain

The growth medium containing Tween 40 as the fatty substrate also turned out to be a favourable medium for extracellular lipases production. In this case *B. subtilis* G2 strain

produced the highest amount of fatty acids (34.375 μmol) followed by *B. cereus* A96 and G10 strains (31.875 and 29.375 μmol respectively) and *B. pumilus* G4 and G8 strains (30 and 31.25 μmol). The least amount of fatty acids was liberated by the following strains: *B. firmus* Tw 4 16.25 μmol and again *B. pumilus* Tw3 14.375 μmol (Fig. 2).

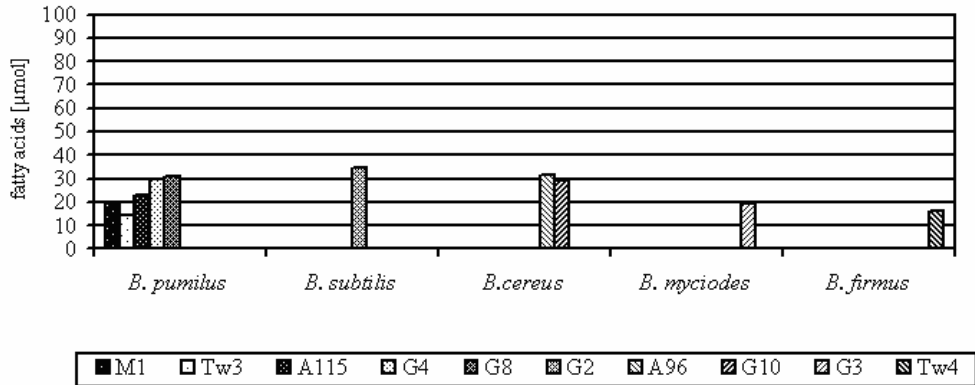


Fig. 2. The influence of Tween 40 on extracellular lipases production by selected *Bacillus* strains

Tween 60 appeared to be the least favourable source of the fatty substrate in the biosynthesis process of the extracellular lipases. The lowest values of liberated μmol of fatty acids were obtained in case of all the strains when compared with the other fatty substrates. The amount of liberated fatty acids was not higher than 30 μmol (Fig. 3).

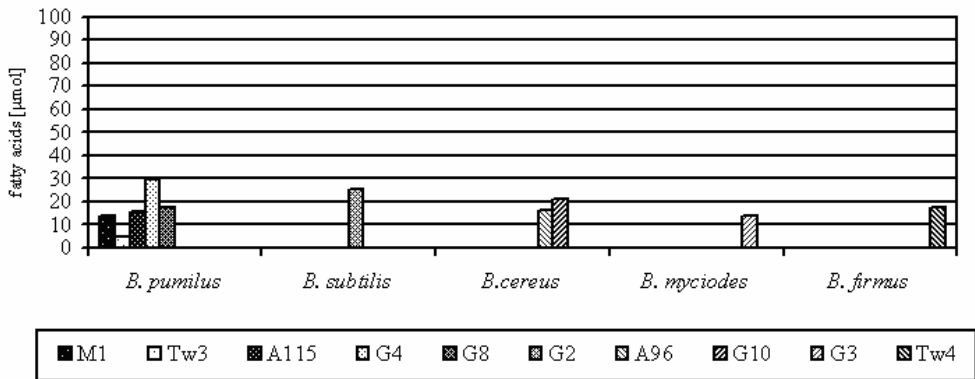


Fig. 3. The influence of Tween 60 on the production of extracellular lipases by selected *Bacillus* strains

The last source of fatty substrate under study - Tween 80 was promoting extracellular lipases production. During the experiment the most vigorous strain was *B. subtilis* G2 liberating 33.125 μmol . The amount of liberated μmol of fatty acids fluctuated around 20 in case of other strains. However, the lowest amounts were obtained for the following strains: *B. mycoides* G3 and *B. pumilus* A115 and amounted 15.625 and 15.0 μmol , respectively (Fig. 4).

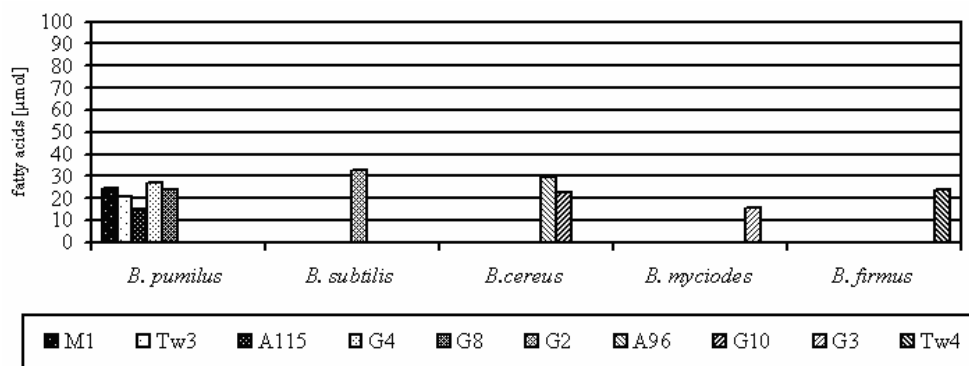


Fig. 4. The influence of Tween 80 on the production of extracellular lipases by selected *Bacillus* strains

Summary and conclusion

Conducted research proved significant diversity between particular *Bacillus* strains in terms of their lipolytic activity, when different sources of fatty acids were considered and enabled to conclude that:

1. Biosynthesis of lipases catalysed by *Bacillus* strains was the most intense on the medium with tributyrin, and the least intense on the medium with the addition of Tween 60. It proved that biosynthesis of these enzymes is the reaction induced by the lipids.
2. Taking into account all of the sources of fatty acids the most vigorous were the strains: *Bacillus cereus* G10 and A96 and the least vigorous was *Bacillus pumilus* Tw3.

References

- [1] Mroziak A., Hubert-Kocurek K. and Łabużek S.: *Lipazy bakterii Pseudomonas i Burkholderia oraz ich wykorzystanie w biotechnologii*. Post. Mikrobiol., 2006, **45**(1), 19-26.
- [2] Sharma R., Chisti Y. and Banerjee U.Ch.: *Production, purification, characterization, and applications of lipases*. Biotechnol. Adv., 2001, **19**, 627-662.

OCENA AKTYWNOŚCI LIPOLITYCZNEJ *Bacillus* sp. WYIZOLOWANYCH ZE ŚRODOWISKA NATURALNEGO

Streszczenie: Celem podjętych badań była ocena aktywności lipolitycznej wybranych szczepów *Bacillus* sp. w zależności od źródła substancji tłuszczowej oraz czasu inkubacji. Szczepy *Bacillus pumilus* wykazywały największą aktywność na podłożu z tributyriną, a najmniejszą na podłożu z dodatkiem Tween 60. Przy czym najbardziej aktywne były szczepy oznaczone M1, A115 i G8, a najmniej Tw3. Natomiast należący do tej samej grupy co *Bacillus pumilus* badany szczep *Bacillus subtilis* G2 wykazywał zbliżoną aktywność do najaktywniejszych szczepów *Bacillus pumilus*. Porównując pomiędzy sobą aktywność *Bacillus cereus*, stwierdzono, iż szczep oznaczony G10 uwalniał najwięcej μmoli kwasów tłuszczowych na pożywkę z tributyriną oraz Tween 60. Z kolei szczep A96 najwięcej μmoli kwasów tłuszczowych wytwarzał na pożywkach Tween 40 i Tween 80. *Bacillus mycoides* G3 wykazywał 2-3-krotnie mniejszą aktywność niż stwierdzona u szczepów *Bacillus cereus*. Ostatni z badanych szczepów *Bacillus firmus* potwierdza, iż wydzielanie zewnątrzkomórkowych lipaz najintensywniej przebiega na podłożu z tributyriną, w następnej kolejności na podłożach z Tween 40 i Tween 80, a najslabiej na pożywkę z dodatkiem Tween 60.

Słowa kluczowe: *Bacillus* sp., lipazy, tributyrina, Tween