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## INFLUENCE OF ENVIRONMENTAL CONDITIONS ON LIPOLYTIC ACTIVITY OF *Bacillus* sp. STRAINS

### WPLYW WARUNKÓW ŚRODOWISKA NA AKTYWNOŚĆ LIPOLITYCZNA SZCZEPÓW *Bacillus* sp.

**Abstract:** The aim of the research was the evaluation of lipolytic activity of *B. cereus* and *B. mycooides* strains, in reference to carbon source, pH and the temperature. In the research, two strains of *Bacillus cereus* and *Bacillus mycooides* each, isolated from the soil and water, were applied. The sources of carbon in culture media were fatty substrates: tributyrin, Tween 40, Tween 60, Tween 80 and glucose. The lipolytic activity was measured by means of titration in pH ranging from 5 to 8 and the temperature ranging from 30 to 60°C. The results were noted as the amount of liberated  $\mu$ moles of fatty acids. In the conducted research, the amount of liberated  $\mu$ moles of fatty acids depended on the type of fatty substrate in the medium, pH and the temperature. The strains under study showed the lowest activity at pH 5 and 6, and the highest activity at pH 7 and 8. In these conditions, most of the strains showed the lipolytic activity, even in case of the lack of fatty substrate in the medium. The highest amount of  $\mu$ moles of fatty acids was liberated at pH 8 in the medium with Tween 40, and the highest results (52.5  $\mu$ mol) were noted for the soil strain *B. cereus*. When analysing the influence of the temperature on the lipolytic activity, it was found that the highest amount of  $\mu$ moles was noted at 30 and 40°C, and the lowest at 50 and 60°C. The best results were obtained for most of the strains at 30°C, in medium with Tween 40, and the most active was the soil strain of *B. mycooides* (52.5  $\mu$ mol). The exception is *B. cereus*, as it liberated 82.5  $\mu$ mol, in the medium with glucose. Taking into account all analysed sources of carbon and parameters, it seems that the most active were *B. mycooides* strains.

**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 are produced by plants, animals and microorganisms, of which the last group remains in the centre of attention. Many kinds of bacteria possess the ability to produce them, among others bacteria of *Bacillus* kind [1].

A common interest in bacterial lipases is connected with their role as biocatalysts in many biochemical processes. They are used, among others to produce detergents, food, paper, pharmaceuticals and in the environmental protection. As shown by data in literature, they are varied in terms of their enzymatic activity, which depends on the species of microbes and the culturing conditions (eg pH of the growth medium, temperature, source of nitrogen and presence of lipids in the medium) [1-3].

The aim of undertaken research was the evaluation of lipolytic activity of *B. cereus* and *B. mycooides*, isolated from the natural environment, in reference to carbon source, pH and the temperature.

### Materials and methods

The objects of the study were 4 *Bacillus* strains:

- 2 *Bacillus cereus* strains marked as: A96 and G10, isolated from soil and water, respectively;

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- 2 *Bacillus mycoides* strains marked as: A134 and G3, isolated from soil and water respectively.

The sources of carbon in culture media were the following fatty substrates: tributyrin, Tween 40, Tween 60, Tween 80 and glucose. The cultures were maintained in Erlenmeyer flasks of 250 cm<sup>3</sup> capacity containing 50 cm<sup>3</sup> of respective growth medium with an inoculum of density equal to E = 2, obtained from the 48-hour culture on a nutrient broth. Incubation was conducted on a rotary shaker for 2 days at 30°C.

Samples were collected after 2 days of culturing and centrifugated for 20 minutes at 4000 rpm. The extracellular lipolytic activity was determined in the obtained supernatant by means of titration towards the same substrates as the ones added to the growth media (the proper treatment). In the control treatment the supernatant was replaced with water. Lipolytic activity was estimated at pH ranging from 5 to 8, and at temperature ranging from 30 to 60°C. The amount of liberated fatty acids was determined 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  $\mu$ moles of fatty acids.

## Results

In presented paper 4 bacterial strains of *Bacillus* kind were screened for their ability to synthesize lipolytic enzymes on the growth media containing different sources of carbon, at pH ranging from 5 to 8, and the temperature ranging from 30 to 60°C.

In conducted research, the amount of liberated  $\mu$ moles of fatty acids depended on the carbon source in the growth medium, pH and the temperature.

The strains of *B. cereus* and *B. mycoides* under study were the least active at pH 5 and 6. However, the highest activity was noted at pH 7 and 8 (Figs. 1 and 2). Most of the strains showed lipolytic activity under these conditions, even in case of the lack of fatty substrates in the medium. The exception is *B. mycoides* A134, which did not show activity at pH 7, and in the presence of glucose (Fig. 1).

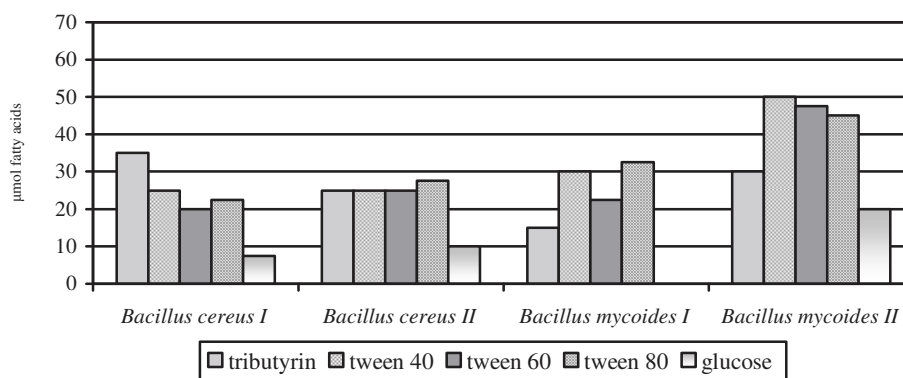


Fig. 1. Lipolytic activity of *B. cereus* and *B. mycoides* strains at pH 7

All strains under study showed the lowest activity at pH 7 on the medium with glucose as the source of carbon. Whereas the highest activity depended on the strain itself. *B. cereus* strain liberated the highest amount of fatty acids (35.0  $\mu\text{mol}$ ) in the presence of tributyrin, strains *B. cereus* G10 and *B. mycooides* A134 on the medium with the addition of Tween 80 (27.5 and 32.5  $\mu\text{mol}$  respectively), and *B. mycooides* G3 strain in the presence of Tween 40 - 50.0  $\mu\text{mol}$  (Fig. 1).

However, the highest amount of  $\mu\text{moles}$  of fatty acids was liberated at pH 8. All strains under study showed favourable activity towards the medium with Tween 40, and the highest amount (52.5  $\mu\text{mol}$ ) was obtained for the soil strain *B. cereus* A96. The exception was *B. mycooides* G3, which as at pH equal to 7, preferred the medium with Tween 60, and liberated 60.0  $\mu\text{mol}$  of fatty acids (Fig. 2).

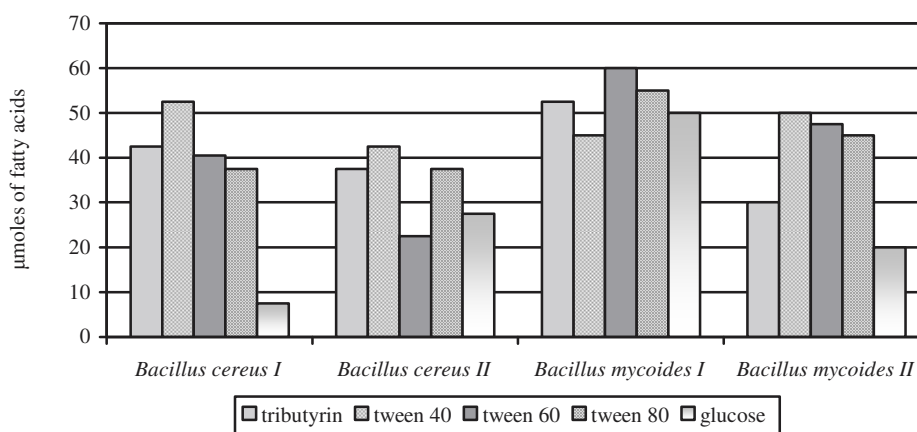


Fig. 2. Lipolytic activity of *B. cereus* and *B. mycooides* at pH 8

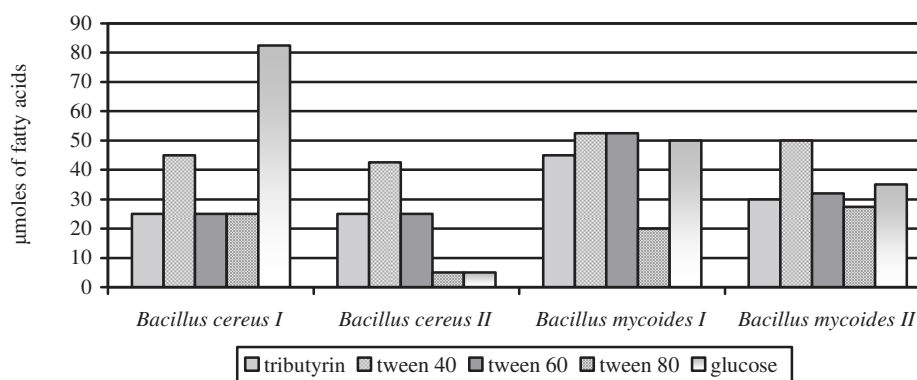


Fig. 3. Lipolytic activity of *B. cereus* and *B. mycooides* strains at 30°C

When analysing the influence of the temperature on the lipolytic activity, it was found that the highest amount of fatty acids was noted at 30 and 40°C (Figs. 3 and 4), and the

lowest at 50 and 60°C. The highest values, for most of the strains, were obtained at 30°C on the medium with Tween 40, and the most active was the soil strain *B. mycooides* (52.5 µmol). The exception is the soil strain of *B. cereus*, which liberated 82.5 µmol on the medium with glucose (Fig. 3).

The amount of liberated fatty acids, on respective media, was slightly lower at 40°C, when compared with the amounts obtained at 30°C. Only the strains of *B. mycooides* A134 and G3, showed higher activity on the medium with glucose, liberating 87.5 and 42.5 µmol, respectively (Fig. 4).

The bacterial strains, at 40°C, used most favourably the fatty substrate in form of Tween 40, obtaining the highest values for the following strains of *B. mycooides*: A134 and G3, 52.5, noted as 52.5 and 45.0 µmol respectively. In the presence of Tween 80, examined strains liberated only 10 to 20 µmol, which proved their lowest lipolytic activity (Fig. 4).

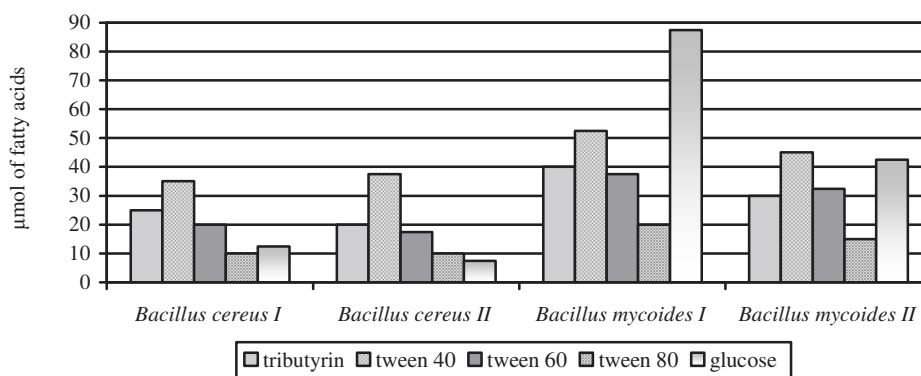


Fig. 4. Lipolytic activity of *B. cereus* and *B. mycooides* strains at 40°C

### Summary and conclusion

The research proved significant diversity of lipolytic activity of *Bacillus cereus* and *Bacillus mycooides* strains, towards the source of carbon, pH and the temperature analysed in the experiment. Based on the obtained results following conclusions were drawn:

1. The highest amount of fatty acids was liberated by the strains at 30°C and pH equal to 8. Under these conditions the most favourable medium was with the addition of Tween 40, as the source of fatty substrate.
2. Strains under study were active even if there was no fatty substrate in the growth medium.
3. The most active were *B. mycooides* strains.
4. Individual strains of *B. cereus* and *B. mycooides* showed diversity in terms of liberated µmoles of fatty acids, which was influenced by the environment from which they were isolated.

### References

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**Abstrakt:** Celem podjętych badań była ocena aktywności lipolitycznej szczepów *B. cereus* oraz *B. mycooides* w zależności od źródła węgla, pH oraz temperatury. Do badań wykorzystano 2 szczepy *Bacillus cereus* oraz 2 szczepy *Bacillus mycooides* wyizolowane z gleby i wody. Źródłem węgla w pożywkach były substraty tłuszczowe: tributryna, Tween 40, Tween 60, Tween 80 oraz glukoza. Aktywność lipolityczną oznaczono w zakresie pH od 5 do 8 oraz w temperaturach od 30 do 60°C. Oznaczenia aktywności lipolitycznej przeprowadzono metodą miareczkową, a wyniki podano jako ilość uwolnionych  $\mu\text{mol}$  kwasów tłuszczowych. W przeprowadzonym doświadczeniu ilość uwolnionych  $\mu\text{mol}$  kwasów tłuszczowych uzależniona była od rodzaju substancji tłuszczowej zawartej w podłożu, pH oraz temperatury. I tak, badane szczepy *B. cereus* oraz *B. mycooides* wykazywały najmniejszą aktywność przy pH 5 oraz 6, natomiast największą aktywność stwierdzono przy pH 7 i 8. W tych warunkach większość szczepów wykazywała aktywność lipolityczną nawet przy braku substratu tłuszczowego w podłożu. Najwięcej  $\mu\text{mol}$  kwasów tłuszczowych uwolniono przy pH 8 na podłożu z dodatkiem Tween 40, a największe wartości (52,5  $\mu\text{mol}$ ) uzyskano dla glebowego szczepu *B. cereus*. Analizując wpływ temperatury na aktywność lipolityczną, stwierdzono, iż najwięcej  $\mu\text{mol}$  kwasów tłuszczowych odnotowano w temperaturze 30 i 40°C, a najmniej w 50 i 60°C. Największe wartości dla większości szczepów uzyskano w temperaturze 30°C na podłożu z dodatkiem Tween 40, gdzie najbardziej aktywny okazał się glebowy *B. mycooides* (52,5  $\mu\text{mol}$ ). Wyjątek stanowi glebowy *B. cereus*, który na podłożu z dodatkiem glukozy uwolnił aż 82,5  $\mu\text{mol}$ . Uwzględniając wszystkie analizowane źródła węgla i parametry, należy stwierdzić, iż najaktywniejszymi były szczepy *B. mycooides*.

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