

Małgorzata NABRDALIK¹, Katarzyna GRATA¹ and Adam LATAŁA¹

PROTEOLYTIC ACTIVITY OF *Bacillus cereus* STRAINS

AKTYWNOŚĆ PROTEOLITYCZNA SZCZEPÓW *Bacillus cereus*

Abstract: The aim of conducted research was to assess proteolytic activity of *Bacillus cereus* strains, depending on the source of proteins in a growth medium and temperature. Two *Bacillus cereus* strains: G10 and A96, isolated from soil and water respectively, were applied in the research. The source of carbon in the growth media was provided by the protein substrates as follows: albumin, casein and skimmed milk. The temperature ranged between 30 and 60°C. The proteolytic activity was determined with the use of a spectrophotometric method with 2% casein as a substrate, at the wavelength $\lambda = 560$ nm. In the experiment, the proteolytic activity depended on the type of protein substrate in the growth medium and the temperature. *B. cereus* G10 strain showed the highest activity at 30°C in case of albumin and the lowest in the growth medium with skimmed milk. At temperatures 40 and 50°C the most favourable medium was with an addition of skimmed milk and the least favourable with albumin. Proteolytic activity was not recorded when the temperature was the highest and the medium contained skimmed milk, whereas at the same temperature the lowest activity was noted again in the medium with albumin. *Bacillus cereus* A96 strain showed, however, different activity. The highest activity was noted at temperatures of 30 and 50°C in the medium with skimmed milk, and the lowest in the medium with casein. The opposite was noted at 40°C, as no proteolytic activity was recorded in the presence of skimmed milk while the highest was recorded in the presence of casein. When analysing the influence of the temperature on the proteolytic activity, it has been noted that strains of *Bacillus cereus* are the most vigorous at the temperature of 30 and 40°C, and the least vigorous at 50 and 60°C. The highest recorded values for the screened strains have been obtained at 30 and 40°C in the medium with skimmed milk.

Keywords: *Bacillus cereus*, proteolytic activity, albumin, casein, skimmed milk

Proteases are an important group of enzymes both physiologically and commercially. Proteases constitute nearly 65% of the global industrial enzyme market most of which are alkaline proteases [1]. Most of these find applications in the food industry, in the meat tenderization process, peptide synthesis, for infant formula preparations, baking and brewing. Furthermore, they are used in pharmaceuticals and medical diagnosis, in the detergent industry as additives, as well as in textile industry in the process of dehairing and leather and silk processing [2].

The genus *Bacillus* contains a number of industrially important species and an approximately half of the present commercial production of bulk enzymes derives from the strains of *Bacillus* spp. The *Bacillus cereus* group is the major source of commercial alkaline protease production worldwide [3, 4].

The aim of conducted research was to assess proteolytic activity of *Bacillus cereus* strains, depending on the source of proteins in a growth medium and temperature.

Materials and methods

In the research the supernates of *B. cereus* strains marked as G10 and A96, isolated prior to the experiment from water and soil respectively, were used. The supernates had been obtained from the bacterial culture grown in the medium containing [g/dm³]: (NH₄)₂SO₄ 2.0, K₂HPO₄ 3.0, KH₂PO₄ 2.0, MgSO₄×7H₂O 0.5. The basic culture medium

¹ Independent Department of Biotechnology and Molecular Biology, Opole University, ul. kard. B. Kominka 4, 45-035 Opole, tel. 77 401 60 56, email: mnabrdalik@uni.opole.pl

was supplemented with 1% source of carbon in the form of protein substrates such as: albumin, casein and skimmed milk. Incubation was carried out at 30°C for 48 hour.

Proteolytic activity was determined in the presence of 2% casein as a substrate. The mixture of supernate and casein was incubated at temperature range between 30 and 60°C for 10 min. The reaction was stopped by adding 5% trichloroacetic acid and the mixture was then left for 15 min and centrifuged at 10 000 rpm for 10 min. Obtained supernates were mixed with 6% Na₂CO₃ and Folin's reagent. The absorbance was measured at the wavelength $\lambda = 560$ nm. Enzymatic activity was expressed as the number of micromoles of tyrosin liberated by 1 cm³ of the bacterial culture.

Results

In presented paper, *Bacillus cereus* strains G10 and A96, were screened for production of proteolytic enzymes in culture media containing different sources of protein and at temperature range between 30 and 60°C.

In conducted research, the number of liberated micromoles of tyrosine depended on the type of protein substrate in the culture medium and the temperature.

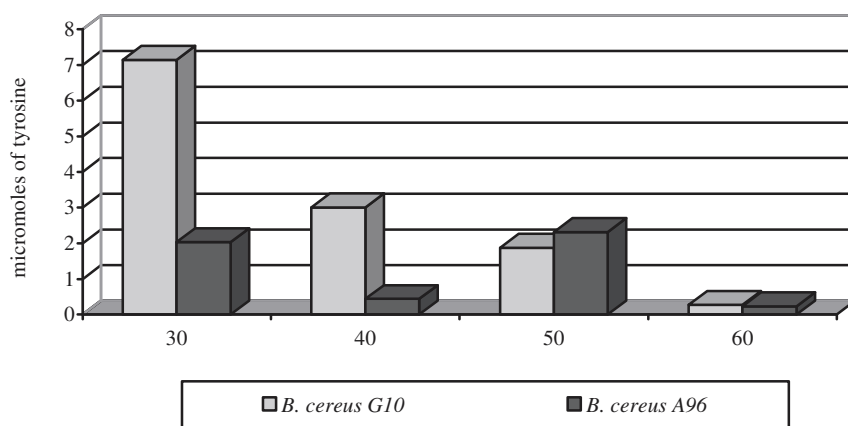


Fig. 1. Proteolytic activity of *B. cereus* strains in the medium with albumin

As shown in Figure 1, it can be stated that *B. cereus* G10 strain isolated from water showed higher proteolytic activity in the medium with albumin when compared with the soil strain *B. cereus* A96. The strain liberated over three times more tyrosine, that is 7.114 micromoles, at 30°C than the other bacterial strain under study. *B. cereus* A96 soil strain was slightly more active only at 50°C and liberated 2.316 micromoles of tyrosine. The lowest activity for both of the strains was noted at 60°C and obtained results were similar: 0.269 and 0.232 micromoles of tyrosine, respectively.

In case of medium with casein as the source of carbon (Fig. 2) it was noted that 30°C was the most favourable temperature for *B. cereus* G10 strain isolated from water and 40°C was the most favourable for the soil strain *B. cereus* A96. In the optimal temperature conditions, *B. cereus* G10 strain liberated 6.9 micromoles of tyrosine, which is 11-fold

higher value when compared with *B. cereus* A96 strain. It should be highlighted, that at 40°C *B. cereus* A96 strain liberated the highest amount of tyrosine of all recorded values in the experiment, which amounted 8.836 micromoles. Obtained value was twice higher than the value obtained in case of *B. cereus* G10 strain isolated from water. Similarly to the culture medium with albumin, also in the medium with casein proteolytic activity was the lowest at 60°C.

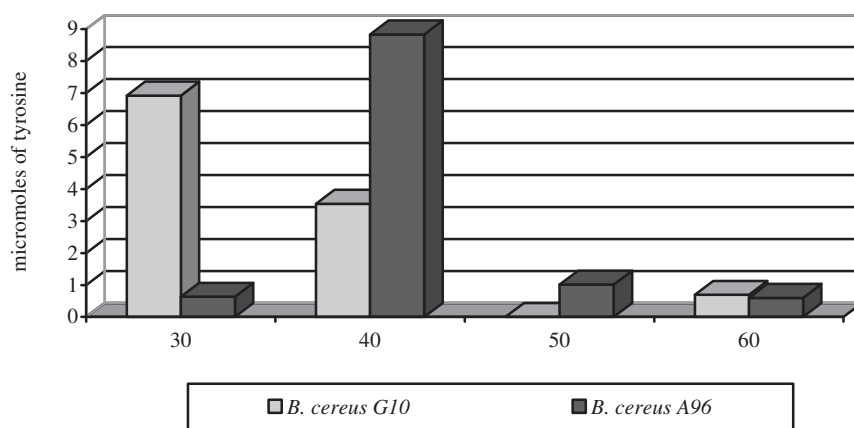


Fig. 2. Proteolytic activity of *B. cereus* strains in the medium with casein

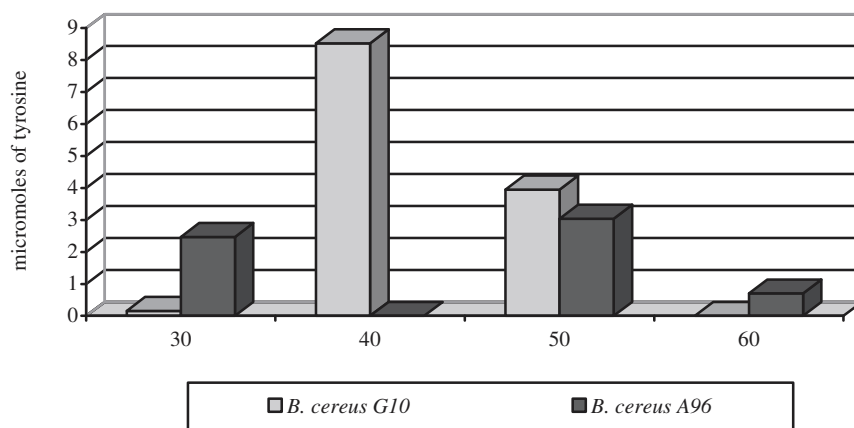


Fig. 3. Proteolytic activity of *B. cereus* strains in the medium with skimmed milk

The last analysed source of carbon was skimmed milk (Fig. 3), which turned out to be the most favourable for *B. cereus* G10 strain at 40°C. The obtained amount of micromoles of tyrosine was the highest for this culture medium and amounted 8.52 micromoles. The highest amount of tyrosine in case of *B. cereus* A96 was liberated at 50°C and amounted

3.036 micromoles, which was lower in comparison with the results noted for *B. cereus* G10 under the same conditions. Again, the lowest proteolytic activity was observed at 60°C.

Summary and conclusion

The results obtained in the present study showed significant variety of proteolytic activity of individual *B. cereus* strains, in terms of different sources of protein substrate applied in the research and allowed to conclude the following:

1. There has been no distinct correlation between the temperature of incubation and proteolytic activity noted, which proves that analysed ability is specific for individual strains and depends on the protein substrate in the culture medium as well as the place the strain had been isolated from.
2. High specificity of exocellular proteases liberated by the strains under study, in relation to analysed protein substrates, proves that the process of their biosynthesis is induced by proteins.
3. Analysing the influence of temperature on proteolytic activity, it has been found that *B. cereus* strains showed the highest activity at 30 and 40°C, and the lowest at 50 and 60°C.
4. The highest recorded values for the strain isolated from water have been obtained in the medium with albumin and casein at 30°C and in the medium with skimmed milk at 40°C.
5. The strain isolated from soil showed the highest activity at 40°C in the medium with skimmed milk.

References

- [1] Banerjee C.U., Sani R.K., Azmi W. and Soni R.: *Thermostable alkaline protease from Bacillus brevis and its characterization as a laundry detergent additive*. Process Biochem., 1999, **35**, 213-219.
- [2] Joo H.S., Kumar C.G., Park G.C., Paik S.R. and Chang C.S.: *Oxidant and SDS stable alkaline protease from Bacillus clausii 1-52: production and some properties*. J. Appl. Microbiol., 2003, **95**, 267-272.
- [3] Gupta R., Beg Q.K. and Lorenz P.: *Bacterial alkaline proteases: molecular approaches and industrial applications*. Appl. Microbiol. Biotechnol., 2002, **59**, 15-32.
- [4] Joo H.S., Kumar C.G., Park G.C., Kim K.T., Paik S.R. and Chang C.S.: *Optimization of the production of an extra cellular alkaline protease from Bacillus horikoshii*. Process Biochem., 2002, **38**, 155-159.

AKTYWNOŚĆ PROTEOLITYCZNA SZCZEPÓW *Bacillus cereus*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

Abstrakt: Celem podjętych badań była ocena aktywności proteolitycznej szczepów *Bacillus cereus* w zależności od źródła białka zawartego w podłożu oraz temperatury. Do badań wykorzystano 2 szczepy *Bacillus cereus* G10 oraz A96 wyizolowane odpowiednio z gleby i wody. Źródłem węgla w pożywkach były substraty białkowe: albumina, kazeina oraz odtłuszczone mleko. Aktywność proteolityczną oznaczono w zakresie temperatur od 30 do 60°C. Oznaczenia aktywności proteolitycznej przeprowadzono metodą spektrofotometryczną, używając jako substratu 2% kazeiny po 10-minutowej inkubacji, przy długości fali $\lambda = 560$ nm. W przeprowadzonym doświadczeniu aktywność proteolityczna uzależniona była od rodzaju substancji białkowej zawartej w podłożu oraz temperatury. I tak, badany szczep *B. cereus* G10 w temperaturze 30°C największą aktywność proteolityczną wykazywał w obecności albuminy, a najniższą w obecności odtłuszczonego mleka. W temperaturach 40 i 50°C najkorzystniejszym podłożem okazało się podłoże zawierające odtłuszczone mleko, a najmniej korzystne zawierające albuminę. W najwyższej temperaturze nie stwierdzono aktywności proteolitycznej w obecności odtłuszczonego mleka, zaś najniższą aktywność odnotowano ponownie w środowisku albuminy. Z kolei drugi

z badanych szczepów, *Bacillus cereus* A96, charakteryzował się odmienną aktywnością. W temperaturach 30 i 50°C najwyższą aktywność proteolityczną odnotowano w podłożu zawierającym odtłuszczone mleko, a najniższą w środowisku kazeiny. Natomiast w temperaturze 40°C zaobserwowano odwrotną sytuację, gdyż nie stwierdzono aktywności proteolitycznej w obecności odtłuszczonego mleka, a najwyższą aktywność uzyskano w obecności kazeiny. Analizując wpływ temperatury na aktywność proteolityczną, stwierdzono, iż największą aktywność badane szczepy *Bacillus cereus* wykazują w temperaturach 30 i 40°C, a najmniejszą w 50 i 60°C. Najwyższe wartości dla badanych szczepów uzyskano w temperaturach 30 i 40°C na podłożu z dodatkiem odtłuszczonego mleka.

Słowa kluczowe: *Bacillus cereus*, aktywność proteolityczna, albumina, kazeina, odtłuszczone mleko