

Katarzyna GRATA¹, Małgorzata NABRDALIK¹ and Adam LATAŁA¹

EVALUATION OF PROTEOLYTIC ACTIVITY OF *Bacillus mycooides* STRAINS

OCENA AKTYWNOŚCI PROTEOLITYCZNEJ SZCZEPÓW *Bacillus mycooides*

Abstract: The research has focused on evaluation the capability of *Bacillus mycooides* strain isolated from soil (designated as A134) and from water (designated as G3) to secrete proteolytic enzymes. The effect of temperatures (30÷60°C) and medium composition (albumin, casein, skim milk) on protease production was evaluated. The proteolytic activity was determined by using the spectrophotometric method and expressed as amounts of μmol tyrosine liberated by 1 cm^3 crude enzyme. In conducted research, the amount of tyrosine depended on the temperature, medium formulation and strain of *Bacillus mycooides*. It was found that milk and albumin media were proved to be best for protease production by soil strain A134 at 30°C (14.00 and 13.72 μmol of tyrosine, respectively), while casein medium at 60°C (11.44 μmol). Strain *B. mycooides* isolated from water G3 behaved differently from strain A134. The best temperature for enzyme production was 60°C but milk and albumin media (16.2 and 17.0 μmol , respectively), whereas 30°C on casein medium. Moreover, it was observed that this strain did not exhibit any enzyme expression on casein medium at 50 and 60°C.

Keywords: *Bacillus mycooides*, proteolytic activity, temperature

Proteases are enzymes that catalyse hydrolytic reactions in which protein molecules are degraded to peptides and amino acids [1, 2]. They constitute one of the most important groups of industrial enzymes and have applications in different industries such as detergent, food, feed, pharmaceutical, leather and waste processing. Microbial proteases account for approximately 60% of the total worldwide enzyme sales and are preferred to the enzymes from plant and animal sources, since they possess almost all the desired characteristics for biotechnological applications [1, 3]. Most commercial proteases, mainly neutral and alkaline are produced by organisms belonging to the genus *Bacillus* [4]. They are specific producers of extracellular proteases and attractive industrial tools for a variety of reasons, including their high growth rates leading to short fermentation cycle times, their capacity to secrete proteins into the extracellular media [5]. All these bacteria are easily isolated from natural environment.

Various nutritional, physical and chemical factors such as media composition, temperature, pH, aeration, incubation times, inoculum concentration significantly affect protease production. Interactions of these parameters are reported to have a significant influence on the production of the enzyme [3, 6, 7]. Bacterium from *Bacillus cereus* "group" such as *Bacillus mycooides* and *Bacillus cereus* were reported to produce proteolytic enzymes however, few studies have been done on proteolytic enzymes from *B. mycooides* [8].

The aim of presented research was the evaluation of the ability to synthesize protease by selected *Bacillus mycooides* strains, isolated from natural environment, depend on the temperature and medium formulation.

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

Materials and methods

The bacterial strains used in this study were *Bacillus mycoides* strains, isolated from soil samples - *B. mycoides* marked as A134 and from water of Turawa lake - *B. mycoides* marked as G3.

The growth media for protease production consisted of a basal salt solution containing [$\text{g} \cdot \text{dm}^{-3}$]: $(\text{NH}_4)_2\text{SO}_4$ - 2.0; K_2HPO_4 - 3.0; KH_2PO_4 - 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.5 that was enriched appropriate for albumin, casein and skim milk (1%). The pH of the medium was adjusted to 7.0. The cultures were maintained at 30°C for 48 h in a shaking incubator (110 rpm). At the end of incubation culture were centrifuged at 4000 rpm for 20 min then obtained cell-free supernatants were used as crude enzyme to determine proteolytic activity. The effect of temperature on protease production was defined by incubating the reaction mixtures at different temperatures (30, 40, 50 and 60°C). Protease activity was determined in the presence of 1% casein hydrolysate solution using the spectrophotometric method. For these studies protease activity was expressed as amounts of tyrosine liberated from casein per 10 min by 1 cm^3 enzyme solution under the assay conditions. The amount of tyrosine was calculated according to Tyrosine Standard Curve [9].

Results and discussion

In conducted research, it was found that each strain has its own special conditions for maximum enzyme production and the amount of released micromoles of tyrosine dependent on the temperature and the medium composition. The ability of *Bacillus mycoides* strains to protease production at the varied range temperature in different medium formulation are presented in the Figures 1-3.

Strain *Bacillus mycoides* G3, isolated from water maximum activity obtained at 60°C on albumin medium (17.00 μmol) and 4-fold lesser at 30°C (4.4 μmol). In contrast to this strain *Bacillus mycoides* A134 isolated from soil, showed the highest level of protease activity at 30°C (13.72 μmol) and to a lesser extent at 60°C (10.52 μmol). However this strain did not exhibit any activity at 40°C (Fig. 1).

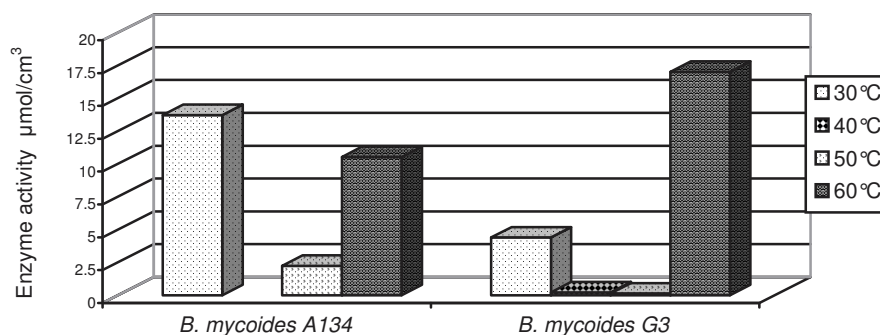


Fig. 1. Effect different temperature on protease activity of *Bacillus mycoides* strains on albumin medium

Casein medium was proved to be good medium for protease production by *Bacillus mycoides* A 134 that showed high level of released micromoles of tyrosine at 60°C

(11.44 μmol) followed 2-fold lower activity at 50°C than found in case 60°C. Lower temperature was found to have adverse effect on the metabolic activities of the microorganisms. In the remainder temperatures 30÷40°C the amount of tyrosine released was lower from 86.36% till 91.96% respectively, compared with 60°C. The second strain designated as G3 showed high protease expression at 30°C but merely 4.12 μmol of tyrosine but did not secrete enzymes in higher temperatures 50÷60°C (Fig. 2).

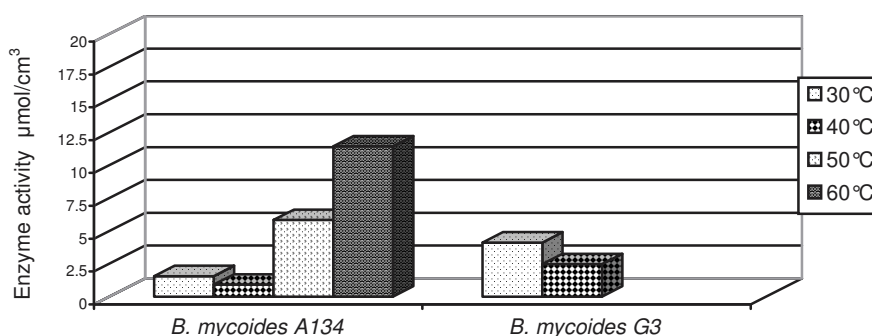


Fig. 2. Effect of temperature on protease activity of *Bacillus mycooides* strains on casein medium

The least analysed medium with skim milk, showed that extracellular secretion of proteases was most intense by *Bacillus mycooides* marked as G3 at 60°C (16.2 μmol) and lower by 37.37% at 30°C. It was found that temperature 50°C did not affect protease production. The high protease activity was found in case of *B. mycooides* A134 at 30°C (14.00 μmol) and less in the others temperatures from 82.3 till 95.8%, compared with the lowest temperature (Fig. 3).

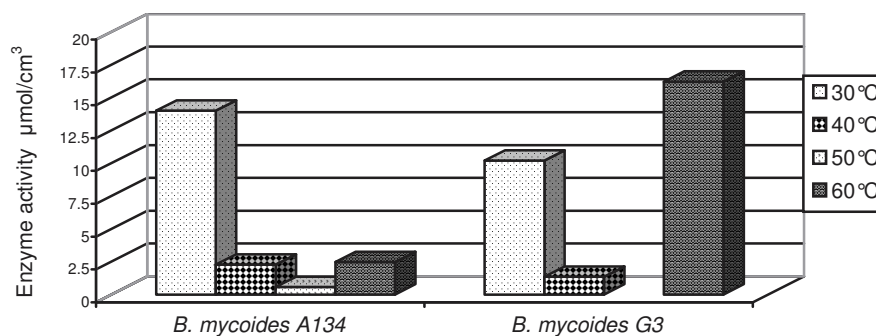


Fig. 3. Effect of temperature on protease activity of *Bacillus mycooides* strains on milk medium

Conclusions

Conducted research proved diversity between *Bacillus mycooides* strains in terms of their protease production, when different temperature and media composition were applied and enabled to draw the conclusions:

1. Biosynthesis of protease catalysed by *Bacillus mycooides* at different temperatures was the most diversified in depending of strain and media formulation.
2. The highest amount of tyrosine was released by *Bacillus mycooides* G3 at 60°C on albumin and milk medium.
3. Biosynthesis of proteases catalysed by *Bacillus mycooides* A134 was the most intense at 30°C on albumin and milk medium.

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OCENA AKTYWNOŚCI PROTEOLITYCZNEJ SZCZEPÓW *Bacillus mycooides*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

Abstrakt: Celem badań była ocena potencjalnej zdolności proteolitycznej szczepu *Bacillus mycooides* A134 wyizolowanego z gleby i szczepu G3 wyizolowanego z wody. Oceniono wpływ temperatury (30÷60°C) i składu podłoża zawierającego albuminę, kazeinę lub odtłuszczone mleko na aktywność proteolityczną. Oznaczenie aktywności proteolitycznej przeprowadzono metodą spektrofotometryczną, a wyniki podano jako ilość uwolnionych mikromoli tyrozyny. W przeprowadzonym doświadczeniu ilość mikromoli tyrozyny uzależniona była od temperatury i składu podłoża, a także szczepu. Stwierdzono, że *Bacillus mycooides* A134 uwolnił największą ilość w temperaturze 30°C na podłożach z mlekiem odtłuszczonym lub albuminą (14,00 oraz 13,72 μmoli tyrozyny, odpowiednio), natomiast w 60°C na podłożu z kazeiną (11,44 μmol). W porównaniu do niego szczep wyizolowany z wody G3 wykazywał odmienną aktywność. Największą ilość mikromoli tyrozyny stwierdzono w temperaturze 60°C na podłożach z mlekiem lub albuminą (16,2 oraz 17,0 μmoli odpowiednio), a 30°C na podłożu z kazeiną. Ponadto nie stwierdzono żadnej aktywności proteolitycznej na podłożu z kazeiną w 50 i 60°C.

Słowa kluczowe: *Bacillus mycooides*, aktywność proteolityczna, temperatura