DOI: 10.2428/ecea.2013.20(01)004 ECOL CHEM ENG A. 2013;20(1):39-46

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EFFECT OF DIFFERENT CARBON SOURCES ON AMYLOLYTIC ACTIVITY OF *Bacillus* sp.

WPŁYW RÓŻNYCH ŹRÓDEŁ WĘGLA NA AKTYWNOŚĆ AMYLOLITYCZNĄ *Bacillus* sp.

Abstract: Studies on the amylolytic activity were carried out with used ten (10) *Bacillus* strains (*B. pumilus*, *B. cereus*, *B. mycoides* and *B. subtilis*), isolated from soil samples and water of Turawa Lake. The amylolytic activity was estimated on the basis of reduction in the intensity of the blue colour resulting from enzymatic hydrolysis of starch, in depending on the carbon sources and their concentration. The cultures were maintained at 30ŞC with the following substrates as carbon sources: potato starch, corn starch, maltose and glucose. Conducted research indicate, that among an analyzed strains the most active appear the *B. mycoides* G3 and *B. subtilis* G2. They preferred the maltose as the source of the carbon. Moreover, in comparison with all examined strains, *B. subtilis G2* showed the amylolytic activity on all tested media.

Keywords: *Bacillus* sp., amylolytic activity, potato starch, corn starch, maltose, glucose

Enzymes, including amylases have been reported to occur in microorganisms, although they are also found in plants and animals [1]. Most enzymes today (and probably nearly all in the future) are produced by microorganisms, such as bacteria, yeast and fungi. Among bacteria several species of *Bacillus* (*B. subtilis*, *B. licheniformis*, *B. cereus*, *B. amyloliquefaciens*) produce very active enzymes with ability to degradation of substrates such as cellulose, pectin, chitin and starch. All these bacteria are easily isolate from the natural environment. Amylase represent of the most important enzyme group and are great significance in present day for biotechnology [2–4].

Spectrum of applications of amylases has used in many sectors such as medical, and analytical chemistry [4–5]. They have also applied in the food processing, textile, paper and distilling industries, in agriculture and environmental protection [4, 6–7]. These enzymes account for about 30 % of the world's enzyme production [8].

The effect of the medium composition (the source of carbon and his concentration) on the level of amylase synthesis by selected *Bacillus* strains was examined.

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Materials and methods

The amylolytic bacteria were isolated from soil samples and water of Turawa Lake and screened for amylase production on Waksmana medium consisting of ($g \cdot dm^{-3}$): agar – 20, soluble starch – 10, K_2HPO_4 – 1, NaCl – 1, $(NH_4)_2SO_4$ – 2, $CaCO_3$ – 5, pH 6.2. Amylolytic isolates were selected by flooding the agar plates with Gram's iodine solution. The selected strains (only the positive and the better zone formed strains) were taken for further experiments and kept on the nutrient agar at 4 ^oC. Bacterial phenotypic characterization by physiological and biochemical tests were performed according to the Bergey's Manual of Systematic Bacteriology [9] and API 50CHB system (bioMerieux, France).

Various carbon sources such as potato starch (P), corn starch (C) or maltose (M) in the range 1–5 % and glucose (G) (1 %) were evaluated for their influence on amylase production by supplemented as individual components to the production media. The medium (50 cm^3) was inoculated with a concentrated suspension of *Bacillus* strains (optical density of 2.0 at λ = 560 nm) and incubated at 30 ^oC on a rotary shaker at 110 rpm. After centrifugation at 4000 rpm for 20 min the supernatant of the culture was used to determine the amylolytic activity.

The amylolytic activity was estimated on the basis of reduction in the intensity of the blue colour resulting from enzymatic hydrolysis of starch according to the modified Fenela method [10]. The reaction mixture containing 0.2 % starch dissolved in potassium phosphate buffer (pH 7.0) and 2 cm^3 0.85 % NaCl was incubated at 30 ^oC for 5 min, following was added 1 cm^3 or 0.1 cm^3 of culture filtrate, then incubated at 37 ^oC for 30 min in a water bath. To this, 5 cm^3 of Lugols iodine was added and amylolytic activity was estimated after appropriate dilution and the absorbency was measured at λ = 560 nm against substrate blank with a spectrophotometer. On the basis of the obtained results, the amount of degrading of starch per 30 min at 37 ^oC was evaluated. One enzyme unit [U/cm^3] was defined as the amount of enzyme that catalysed the degrading of starch per min under the assay conditions.

Results and discussion

In this paper, *Bacillus* strains that are capable of growing on starch as the sole carbon source have been isolated. Based on its ability to production of amylase, *Bacillus* strains were chosen to study the performance of amylase during degradation of starch. The bacterium were identified as strains of *B. mycoides* (2 strains), *B. cereus* (3 strains), *B. pumilus* (4 strains) and *B. subtilis* (1 strain).

Potato starch, corn starch, maltose and glucose are major substrate considered for enzyme production in this study. The effect of different carbon sources on amylase production showed that each bacteria behaved differently. Figure 1 shows the enzymatic activity of the amylolytic *Bacillus* species that are capable of decomposition of potato starch.

From *Bacillus cereus* "group", the *B. mycoides G3* showed high enzyme release and 2.5 % concentration of potato starch was found to be optimum for the production of

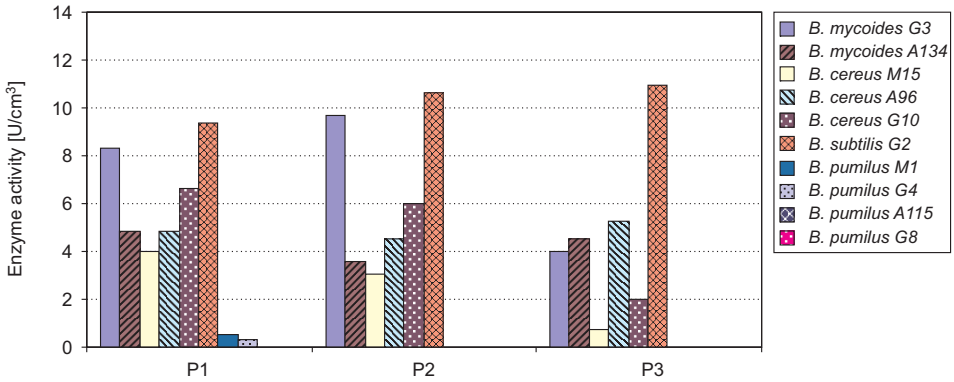

Fig. 1. Effect of different concentrations of potato starch (P1 – 1 %, P2 – 2.5 %, P3 – 5 %) on the amylolytic activity of *Bacillus* strains

amylase (9.66 U/cm^3). The amount of starch degrading was from 0.12 to 0.29 mg/cm^3 (Table 1). In the case of *Bacillus subtilis* "group" strain *B. subtilis G2* produced the highest yield of amylases (9.33–11.0 U/cm^3), spreading from 0.28 to 0.33 mg/cm^3 of the starch (Table1). In contrast to the other *Bacillus* strains, *B. pumilus G8* and *A115* strains did not produced amylase on this medium, whereas *B. pumilus* marked *M1* and *G4* appear the ability to degradation of starch only at 1 % concentration of potato starch (0.53 U/cm^3 and 0.33 U/ cm^3, respectively) (Fig. 1).

Table 1

Effect of different concentrations of potato starch and corn starch on amylolytic activity of *Bacillus* strains [mg/cm^3]

Bacillus strains	Medium with potato starch			Medium with corn starch		
	1 %	2.5 %	5 %	1 %	2.5 %	5 %
Bacillus mycoides G3	0.25	0.29	0.12	0.23	0.22	0.36
Bacillus mycoides A134	0.15	0.11	0.14	0.20	0.07	0.17
Bacillus cereus M15	0.12	0.09	0.02	0	0.03	0.04
Bacillus cereus A 96	0.15	0.14	0.16	0.14	0.12	0.11
Bacillus cereus G10	0.20	0.18	0.06	0.12	0.07	0.12
Bacillus subtilis G2	0.28	0.32	0.33	0.29	0.26	0.25
Bacillus pumilus M1	0.02	0	0	0.03	0.01	0.03
Bacillus pumilus G4	0.01	0	0	0	0	0
Bacillus pumilus A115	0	0	0	0	0	0
Bacillus pumilus G8	0	0	0	0	0	0

The studies with different concentrations of corn starch were similar to the results obtained on the medium enrichment in potato starch. The effect of corn starch on amylase synthesis by *Bacillus* sp. was also investigated (Fig. 2).

Among the all *Bacillus* sp. tested, only two strains *B. mycoides G3* and *B. subtilis G2* revealed the enzymatic abilities in the highest degree. The enzymatic activity ranged

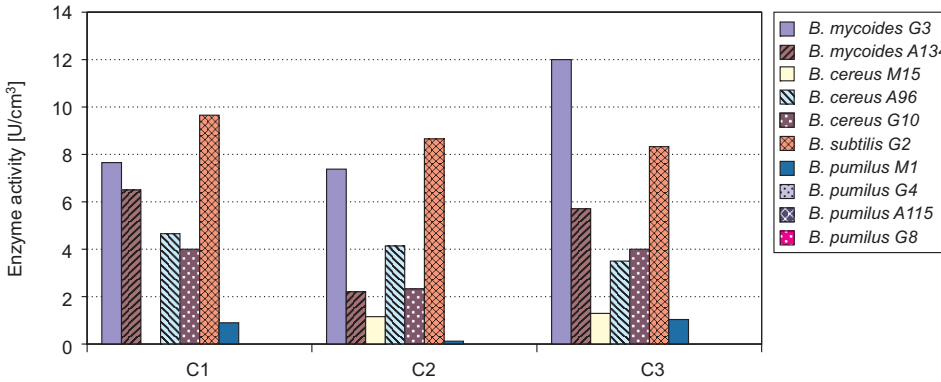

Fig. 2. Effect of different concentrations of corn starch (C1 – 1 %, C2 – 2.5 %, C3 – 5 %) on the amylolytic activity of *Bacillus* strains

from 7.33 U/cm^3 to 12.0 U/cm^3 and from 8.33 U/cm^3 to 9.66 U/cm^3, respectively. The maximum degree of starch decomposition were observed in the presence of 5 % (0.36 mg/cm^3) and 1 % (0.29 mg/cm^3) concentration of corn starch, respectively. It has also been found that *B. pumilus* strains marked *A115, G4* and *G8* did not secrete of the enzymes on this medium (Fig. 2, Table 1).

The influence of the maltose on the decomposition of starch by *Bacillus* sp. are presented in the Fig. 3 and Table 2. Based on the obtained results, the highest ability to the amylase synthesis were found for *B. subtilis G2* and *B. mycoides G3*. The decomposition of starch by this strains were from 0.18 mg/cm^3 to 0.40 mg/cm^3 and from 0.30 mg/cm^3 to 0.40 mg/cm^3, respectively. Moreover, the maximum level of amylase synthesis was observed on the medium supplemented with 1 % (13.33 U/cm^3) and 5 % (13.33 U/cm^3) maltose, and it proved to be a good substrate for this enzyme synthesis. It was found that the decrease in the release of the enzyme by *B. mycoides* G3 and increase in the case of *B. subtilis G2*, occurred when the concentration of maltose

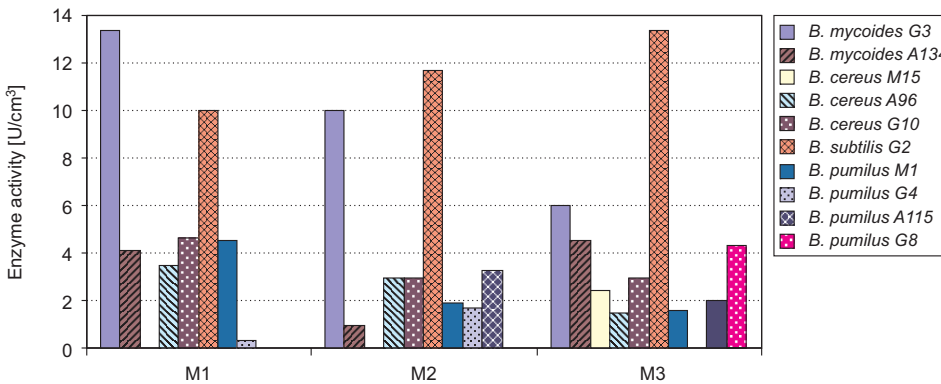

Fig. 3. Effect of different concentrations of maltose (M1 – 1 %, M2 – 2.5 %, M3 – 5 %) on the amylolytic activity of *Bacillus* strains

was increased. However, strains of *B. cereus M15* and *B. pumilus G8* revealed the enzymatic activity when maltose was added at 5 % concentration (2.46 U/cm^3 and 4.33 U/cm^3, respectively). Other *Bacillus* strains showed 2–3 fold lower amylolytic activity than two the most active strains.

Table 2

Effect of different concentrations of maltose and glucose on amylolytic activity of *Bacillus* strains [mg/cm^3]

Bacillus strains	Medium with maltose			Medium with glucose
	1 %	2.5 %	5 %	1 %
Bacillus mycoides G3	0.40	0.30	0.18	0.23
Bacillus mycoides A 134	0.13	0.05	0.14	0.11
Bacillus cereus M15	0	0	0.08	0.07
Bacillus cereus A 96	0.11	0.09	0.04	0.15
Bacillus cereus G10	0.14	0.09	0.09	0.15
Bacillus subtilis G2	0.03	0.35	0.40	0.46
Bacillus pumilus M1	0.14	0.06	0.05	0.11
Bacillus pumilus G4	0.01	0.05	0	0.27
Bacillus pumilus A115	0	0.10	0.06	0.16
Bacillus pumilus G8	0	0	0.13	0.08

Figure 4 presented the yield of amylase produced by *Bacillus* sp. on medium with glucose.

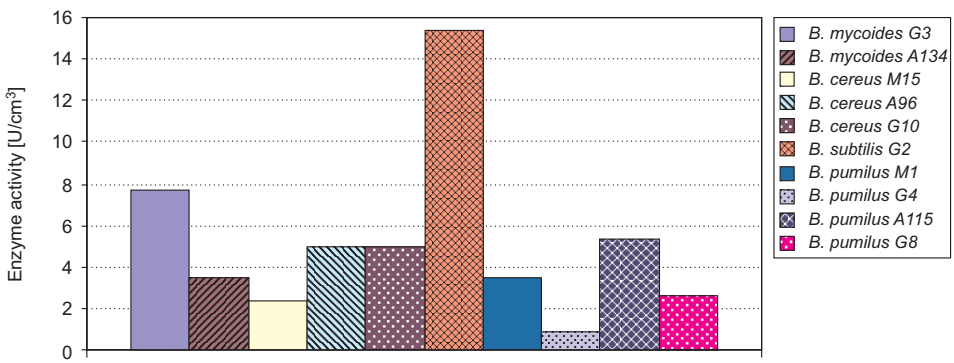

Fig. 4. Effect of 1 % concentrations of glucose on the amylolytic activity of *Bacillus* strains

Among the all strains of *Bacillus*, *B. subtilis G2* demonstrated the highest activity on the medium with glucose (15.33 U/cm^3). *Bacillus mycoides G3* that belong to the "cereus group" showed 2-fold lower activity (7.66 U/cm^3) than stated in case of *B. subtilis G2*. The amount of starch decomposed was 0.49 mg/cm^3 and 0.23 mg/cm^3, respectively. Moreover, we observed an over 3-fold decrease in the amylolytic activity

of the *B. cereus* strains marked *A96* and *G10* and *B. pumilus A 115*, but almost 4–5 fold decrease in the activity of the *B. cereus* strains marked *M15* and *G8* towards glucose as a substrate, compared with the most active strains (Fig. 4, Table 2).

The addition of carbon source in the form of either monosaccharides or polysaccharides could influence on the production of enzymes [4, 6, 11]. Use of media differenting in composition of nutritive component for cultivation of bacteria has allowed establishing that in the media rich in available monomers (glucose, amino acids) the bacteria absorb these monomers, whereas in poorer media they produce hydrolytic enzymes for degrading of highly molecular substrates [12]. Santhos et al [13] showed that with some carbon sources an inverse relationship exists between the growth and the amount of amylase produced. Maltose, starch and citrate did not conform to this relationship in that these substrates stimulated amylase formation. Similarly, it has been reported by Saxena et al [14] and Ajayi et al [6], that amount carbon sources, soluble starch, maltose, corn starch and xylose were found to support amylase production. These results are in agreement with the report of many authors [*eg* 2, 4] who reported maximum amylase production *eg* by *B. cereus*, *Bacillus K-12,* when starch was used as carbon source.

The reports of many authors show that the efficiency of the amylase synthesis of some microorganisms is significantly inhibited by the presence of monosaccharydes or disaccharydes. The enzyme formation with the other carbon sources (glucose, lactose, mannose, galactose) were much lower compared with that with starch and maltose [13]. Sudharhsan et al [15] observed that glucose and fructose represses the production of amylase. Similarly, it has been reported by Santhos et al [13] and Lin et al [16] that synthesis of carbohydrate degrading enzyme in most species of genus *Bacillus* sp. leads to catabolic repression by readily metabolizable substrates such as glucose and fructose. Teodoro et al [17] found, that the addition of glucose (0.5 %) to the culture diminished greatly the synthesis of amylase. These results are similar to the findings of Aiyer [18], who observed that nonmetabolizable sugars like arabinose, raffinose, sucrose and galactose did not support amylase production.

Ability to the degradation of the starch depends not only on the kind of the source of carbon and its concentration, but also from species or the *Bacillus* strain. The effect of different carbon sources on amylase production applied in own investigations showed that each bacteria behaved differently. Similar results have also been obtained by other researchers. Jamuna et al [19] reported that α-amylase production by *B. subtilis* was higher with glucose than with starch. According Sarikaya et al [20], *B. amyloliquefaciens I* intensively produces amylolytic enzymes with starch, moreover *B. amyloliquefaciens II* with sucrose as the sole carbon source. In contrast, carbon sources such as glucose, maltose and starch did not enhance α-amylase production by thermophilic *B. coagulans* [21]. Moreover, Aiyer [18] using 1 % of different soluble sugars, amylase production by *B. licheniformis* was highest in fructose and maltose medium and in using 1 % of potato starch. Therefore, our results are in good agreement with the findings in these studies.

Conclusion

The obtained results indicate that ability to the decomposition of the starch depends not only on the kind of the source of carbon and its concentration, but also from species or the *Bacillus* strain. The effects of potato starch, corn starch and maltose in the range 1–5 % and glucose (1 %) on the enzyme production showed that each bacteria behaved differently. In the medium containing potato starch, corn starch, maltose or glucose the maximum production of amylase were archived for *B. subtilis G2* and *B. mycoides G3* from all *Bacillus* strains tested. Among the carbon sources added to basal medium, maltose was found as the best substrate for enzyme production by *B. mycoides G3*, however glucose and maltose for *B. subtilis G2*. Whereas, the lowest activity were observed for *B. pumilus A115* and *G8* strains. They demonstrated the lowest activity only on the medium with maltose, but did not show any activity on the medium containing of potato starch or corn starch.

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WPŁYW RÓŻNYCH ŹRÓDEŁ WĘGLA NA AKTYWNOŚĆ AMYLOLITYCZNĄ *Bacillus* sp.

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Abstrakt: Celem badań była ocena aktywności amylolitycznej 10 szczepów *Bacillus* sp. (*B. pumilus*, *B. cereus*, *B. mycoides* i *B. subtilis*) wyizolowanych z gleby i wody z jeziora Turawa. Na podstawie stopnia zmniejszenia się zabarwienia z jodem oznaczono ilość rozłożonej skrobi, w zależności od źródła węgla i jego koncentracji. Hodowle prowadzono w temperaturze 30 ^{o}C z zastosowaniem następujących źródeł węgla: skrobia ziemniaczana, skrobia kukurydziana, maltoza oraz glukoza. Uzyskane wyniki badań wykazały, iż spośród badanych szczepów *Bacillus sp*. najbardziej aktywnymi okazały się *B. mycoides G3* i *B. subtilis G2*, które preferowały maltozę jako źródło węgla. Ponadto, w porównaniu do wszystkich szczepów, *Bacillus subtilis G2* wykazał największą aktywność na wszystkich testowanych podłożach.

Słowa kluczowe: *Bacillus* sp., aktywność amylolityczna, skrobia ziemniaczana, skrobia kukurydziana, maltoza, glukoza