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PECTIN LYASE ACTIVITY OF *PENICILLIUM SP.* 25/5B STRAIN GROWN ON BEET-PULP EXTRACTS. PART I. SUBMERGED CULTURES

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Key words: submerged culture, pectin lyase activity, *Penicillium sp.*, beet-pulp extracts.

The pectin lyase (PL 4.2.2.10) (4) activity of *Penicillium sp.* 25/5B on beet-pulp water extracts, supplemented with nitrogen salts was studied. The highest PL activity and biomass production was obtained on a 4% beet-pulp water extract supplemented with $(NH_4)_2HPO_4$ and additionally buffered with a phosphate buffer, pH = 6.0 (Fig. 3).

INTRODUCTION

The biosynthesis of enzyme preparations is usually based on culture media containing by-products of the food industry [2, 3, 6]. In the case of pectolytic enzymes, pectin containing substrates such as beet, apple or grape pulps are usually used [4, 7, 9, 11]. These kinds of culture media can be enriched by the addition of some organic compounds such as malts sprouts or inorganic compounds i.e. mineral salts [5, 8, 10]. The nitrogen and phosphate salts can perform an important role in the biosynthesis of pectin enzymes.

In this paper we discuss the influence of culture media composed of beet-pulp water extracts, enriched with different concentrations of nitrogen and phosphate salts, on the biosynthesis of pectin lyase by *Penicillium sp.* 25/5B strain.

MATERIALS AND METHODS

Strain: *Penicillium sp.* 25/5B was used, isolated from beet-pulp and having the ability to synthesize pectin lyase (PL 4.2.2.10). Media: the fungus was kept on mineral Czapek-Doxa-Pectin-Agar (CzPA) slants, contain-

ing pectin (2%) as the only source of carbon. Inoculum was prepared in the above media without agar (CzP), distributed at the rate of 100 ml in 300 ml conical flasks and sterilized at 121°C for 30 min.
The following media were tested:

A — beet-pulp water extracts which contained from 1.5 to 6.0% dry matter;

B — beet-pulp water extracts with a 4% dry matter content and enriched with the following nitrogen salts: $NaNO_3$; NH_4NO_3; $(NH_4)_2SO_4$; $(NH_4)_2HPO_4$; in an amount equivalent to 0.033 to 0.528% nitrogen;

C — beet-pulp water extracts (see B) additionally enriched with a 0.05 M phosphate buffer (pH = 6.0).

Media A, B and C (100 ml of each) were poured into 500 ml conical flask and sterilized at 121°C for 30 min.

Chemical composition of beet-pulp used: components in mg/g in terms of dry weight — pectin (as Ca-pectinate) — 114.00, nitrogen — 16.30, P_2O_5 2.20, K_2O — 6.53, Na_2O — 0.92, CaO — 5.30, MgO — 2.23.

Preparation of beet-pulp water extracts: a weighed sample of beet-pulp was milled in a ball mill and than suspended in water and kept in an open steaming autoclave for 20 min. The steamed mass obtained was pressed through several layers of cheese cloth. After that the concentration of dry matter was fixed. Inoculation: conical flasks with a CzP medium were inoculated with a spore — mycelium suspension from 7 days old slants culture. Each flask was inoculated with a suspension obtained from two slants cultures. The cultures were incubated at room temperature for 7 days. After that the mycelium was washed several times in sterile water and homogenized with glass-pellets. Spore mycelium suspension prepared in this way to the amount of 4 ml per 100 ml of A, B or C media was used.

Culture conditions: the submerged cultures were grown on a rotary shaker (160 rpm) for 72 h at 20°C-22°C. Next, cell-free culture filtrates (CF) for enzyme assays were prepared by filtration of the culture through several layers of cheese cloth and centrifugated for 10 min at 12 000 g. The mycelium obtained was washed in 200 ml of distilled water and used to determine the dry matter of biomass produced, by drying it at 80°C.

All experiments were repeated 3-5 times. Enzyme assay: the pectin lyase activity was estimated using CF fluids, spectrophotometrically by the semimicro thiobarbarbituric acid test, measuring the absorption at 550 nm [1].

The reaction mixtures were prepared using Mc-Ilvaine buffer, pH = = 5.80. One unit of pectin lyase activity was defined as the amount of enzyme which caused an increase of 0.01 in absorbance at 550 nm per hour under the specified conditions.

RESULTS

The submerged cultures of *Penicillium sp.* 25/5B on beet-pulp water extracts have shown (Fig. 1) that the best growth of the strain tested and the highest activity of PL were obtained on beet-pulp water extracts containing 4% dry matter. On these media the production of biomass exceeded 0.8% and the PL activity was 8 units/ml. The pH values during the growth of tested fungi were reduced from pH 4.7 to pH 4.1, when extracts containing over 4% dry matter were used.

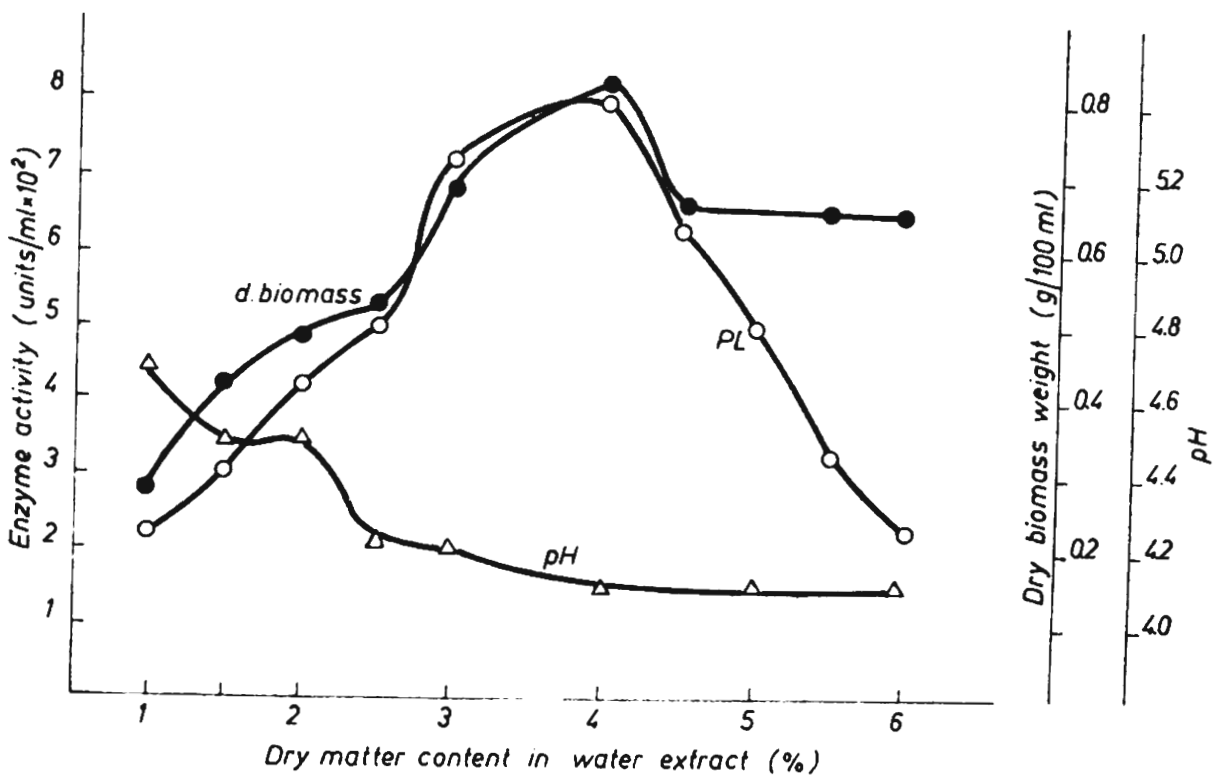

Fig. 1. Effect of different concentrations of beet-pulp water extracts in culture media on the activity of pectin lyase and the growth of *Penicillium sp.* 25/5B

The addition of nitrogen salts to the optimal beet-pulp extract (with 4% d.m.) influenced the growth of the *Penicillium* strain tested as well as the PL activity and the pH values of the culture media (Fig. 2). All the nitrogen salts tested (with the exception of $(NH_4)_2SO_4$ stimulated biomass production by 50 to 100% at the most, as compared with a culture medium without any addition of salts (Fig. 2a). In the presence of $(NH_4)_2HPO_4$ in an amount corresponding to 0.264-0.528% nitrogen, and $NaNO_3$ in an amount corresponding to 0.132% nitrogen, the PL activity increased by 8 to 12 units/ml. All other concentrations of these salts, as well as of $(NH_4)_2SO_4$ and NH_4NO_3 caused a decrease of PL activity even to 2 units/ml (Fig. 2b).

Nitrogen salts which had a stimulating effect on PL synthesis, also influenced the pH of the culture media during growth, changing it from pH 4.0 to pH 6.0, whereas the $(NH_4)_2SO_4$ and NH_4NO_3 salts caused an increase of acidity, lowering the pH to 3.2-3.4 (Fig. 2c).

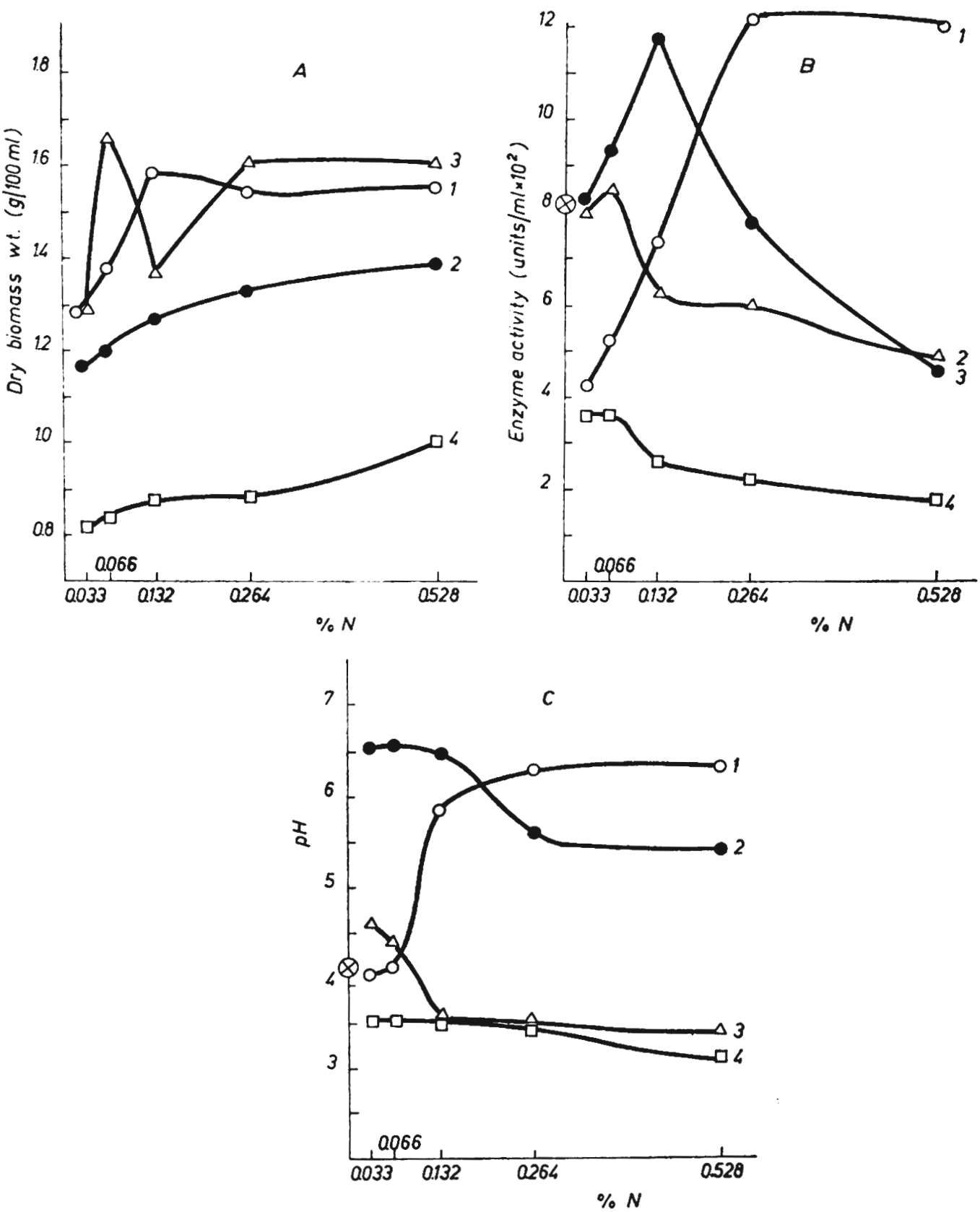

Fig. 2. Pectin lyase activity and growth rate of *Penicillium sp.* 25/5B after culturing on beet-pulp water extracts enriched with different concentrations of nitrogen salts: Fig. 2a — changes in growth rate, 2b — changes in pectin lyase activity, 2c — pH changes in culture media

In further experiments in which the effect of pH stability during the growth of *Penicillium sp.* in the above media was tested, it was shown that and addition of phosphate buffer to the media caused a further increase of PL activity as well as biomass production (Fig. 3). The biomass increased from 1.3% (buffered culture media without salts) to nearly 1.8%

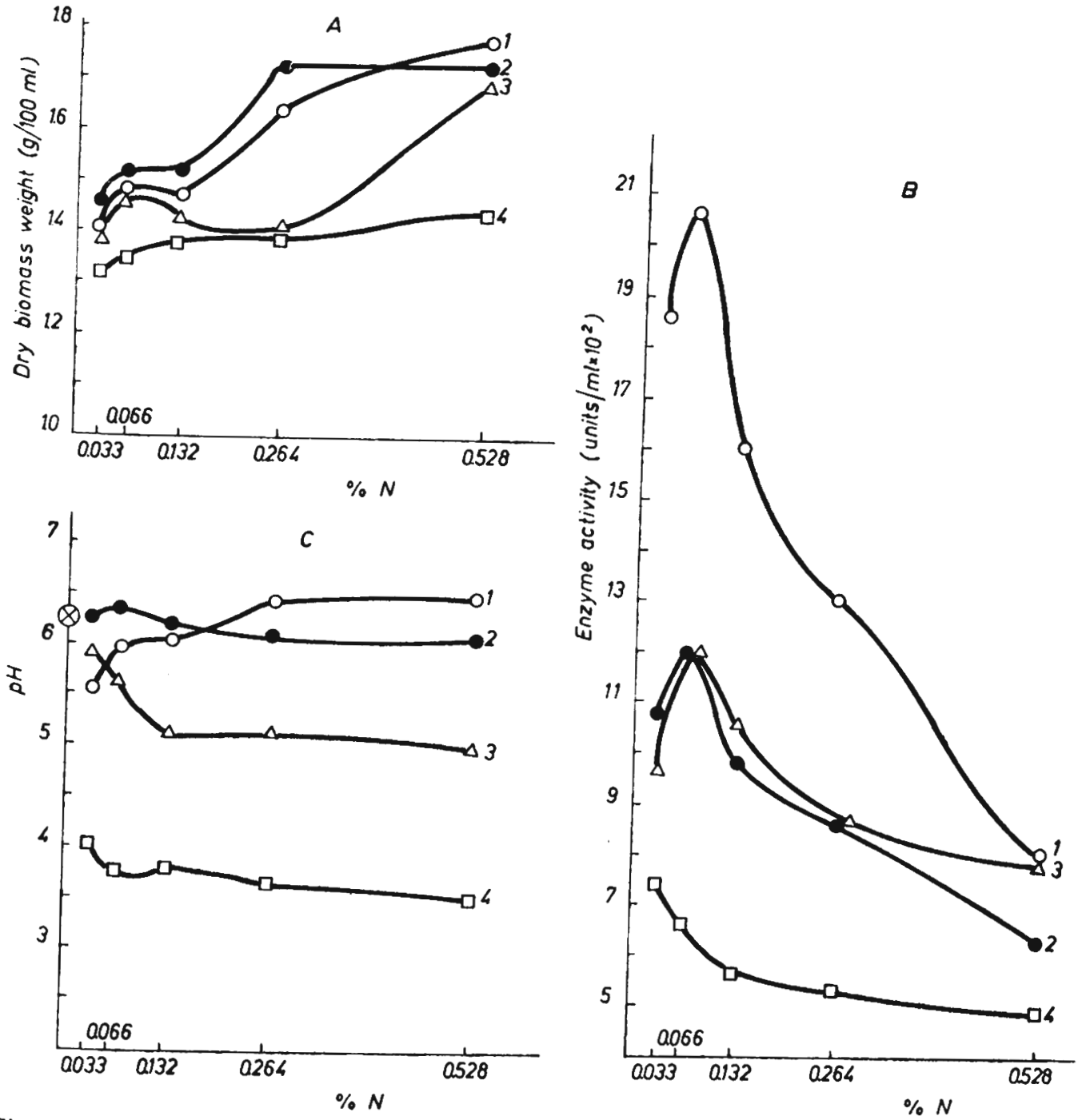

Fig. 3. Pectin lyase activity and growth rate of *Penicillium sp.* 25/5B after culturing on buffered beet-pulp water extracts enriched with different concentrations of nitrogen salts: Fig. 3a — changes in growth rate, 3b — changes in pectin lyase activity, 3c — pH changes in culture media; explanation to Fig. 2 and 3: 1 = $(NH_4)_2HPO_4$: 2 = = $NaNO_3$: 3 = NH_4NO_3 : 4 = $(NH_4)_2SO_4$

at an optimal concentration of nitrogen in the medium (Fig. 3a). The results presented in Fig. 3b show that the addition of phosphate buffer to the culture media, especially to media containing $(NH_4)_2HPO_4$ caused an increase of PL activity to 21 units/ml and in experiments with $NaNO_3$ and NH_4NO_3 to 12 units/ml compared to 9 units/ml found in the control media. It is interesting to note, that the highest PL activity was found in culture media with an amount of nitrogen equivalent to 0.066% (Fig. 3b).

The presence of nitrogen salts which stimulated growth and PL activity had no influence in the buffered media on pH, keeping it at the level of pH = 6.0-6.5. Only in experiments with $(NH_4)_2SO_4$ despite the addition of a buffer pH decreased to 4.0-3.5 (Fig. 3c).

CONCLUSIONS

1. The strain *Penicillium sp.* 25/5B was able to synthesise pectin lyase enzymes in submerged culture conditions on beet-pulp water extracts.
2. From among the four nitrogen salts tested in non buffered media, $(NH_4)_2HPO_4$ and $NaNO_3$ stimulated the activity of pectin lyase to about 50%.
3. The highest stimulation of pectin lyase (to about 260%) was observed after cultivation of the *Penicillium* strain in media containing water extracts from beet-pulp with a 4% d.m. content, enriched with $(NH_4)_2HPO_4$ and buffered with a phosphate buffer pH = 6.0.

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AKTYWNOŚĆ LIAZY PEKTYNOWEJ SZCZEPU *PENICILLIUM SP.* 25/5B NA WYCIĄGACH Z WYSŁODKÓW BURACZANYCH. CZ. I. HODOWLE WGŁĘBNE

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Streszczenie

Przebadano aktywność liazy pektynowej (PL 4.2.2.10.) szczepu *Penicillium sp.* po hodowli na następujących podłożach:

A — na wodnych wyciągach z wysłodków buraczanych o zawartości suchej masy od 1,5 do 6,0%,

B — na wodnych wyciągach z wysłodków buraczanych o zawartości 4% s.m. wzbogaconych następującymi solami: $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, $(NH_4)_2HSO_4$, w ilościach odpowiadających zawartości azotu w tych związkach od 0,033 do 0,528%,

C — na wodnych wyciągach z wysłodków (jak w punkcie B) dodatkowo zbuforowanych 0,05 M buforem fosforanowym o pH = 6,0.

Na podstawie przeprowadzonych badań stwierdzono, że badany szczep był zdolny do syntezy enzymów typu PL w hodowlach wgłębnych na wodnych wyciągach z wysłodków buraczanych.

Z czterech przebadanych soli azotowych, kwaśny fosforan amonu i azotan sodu dodane do pożywek niezbuforowanych wpływały na wzrost aktywności enzymów PL i powodowały widoczne zmiany w pH płynów hodowlanych. Najwyższe aktywności PL stwierdzono na zbuforowanych (pH = 6,0) 4% wodnych wyciągów z wysłodków buraczanych wzbogaconych dodatkiem kwaśnego fosforanu amonowego.