Carboxymethyl cellulase production from a *Paenibacillus* sp.

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Abstract

Cellulases are industrially important enzymes with a potential to convert cellulose into fermentable sugars. Novel bacterial isolate *Paenibacillus* sp. CKS1 was tested for cellulase activity and the optimal conditions for carboxymethyl cellulase (CMCase) production were determined. Maximum CMCase activity was obtained in the third passage of the bacterial culture after 3 days of incubation at 30 °C. Cellobiose and yeast extract was the optimal source of carbon and nitrogen for induction of CMCase activity. In addition, with initial pH 7 of the medium and 40 ml of working volume in 500 ml culture flasks with shaking at 150 rpm, the maximum CMCase activity in a crude culture supernatant reached value of 0.532±0.006 U/ml. For crude CMCase, optimal temperature was 50 °C and optimal pH 4.8, respectively. HPLC analysis confirmed the bacterium is capable to hydrolise CMC to glucose and other soluble sugars.

Keywords: *Paenibacillus* sp., cellulose, CMCase production, optimal conditions.

Available online at the Journal website: http://www.ache.org.rs/HI/

SCIENTIFIC PAPER

UDC 577.15:661.728.8.094.941: 543:579

Hem. Ind. **70** (3) 329–338 (2016)

doi: 10.2298/HEMIND150222038M

Cellulose is the most abundant natural biopolymer on Earth and the most dominant component of agricultural waste [1]. Cellulosic biomass is a renewable and an abundant resource that can be used for production of biofuels and animal feed [1,2]. Because of its potential applications in industry, microbial conversion of cellulose into simple sugars or bioethanol has received excessive attention in the past decades [3,4]. Active research on cellulases began in the early 1950s and lead to an increasing application of cellulases in biotechnological processes in various industries including food, brewery and wine, animal feed, textile and laundry, pulp and paper, and agriculture [5]. The major source of cellulases are microorganisms that produce these enzymes while growing on cellulosic materials [5,6]. Cellulase is a family of at least 3 groups of enzymes, endo-(1,4)-β-D-glucanase (EC 3.2.1.4), exo-(1,4)--β-D-glucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21), that hydrolyze cellulose while the mechanism of enzymatic activity differs between the different enzyme classes: endoglucanases (carboxymethyl cellulases), exoglucanases (avicelases) and β-glucosidases [5–7]. Endoglucanases cut the amorphous cellulose polysaccharide chain at random internal sites and generate oligosaccharides of various lengths [1]. Exoglucanases are active on the reducing or non-reducing ends of the cellulose polysaccharide chains and liberate either glucose or cellobiose as major products [1]. β-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose and act on the non-reducing ends [1]. Most cellulases are secreted by microbial strains belonging to fungi, or bacterial strains within the *Bacilli* and *Actinomycetes* classes although fungal cellulases have been studied the most [3]. Nevertheless, the studying of bacterial ceullulases is very promising since isolation, screening and selection have enabled the discovery of numerous novel cellulase-producing bacteria from a wide variety of environments [8]. The aim of this study was to select a cellulolytic strain from a culture collection of bacterial soil isolates. A *Paenibacillus* strain has been identified as a potent producer of cellulases as it has shown a notable cellulolytic activity on carboxymethyl cellulose (CMC) agar plate. The conditions that enabled maximal carboxymethyl cellulase (CMCase) production were optimized with emphasis of the following parameters: passaging of the culture, incubation time, carbon and nitrogen source, medium pH, medium volume/surface ratio and aeration. Finally, the crude CMCase was characterized for the optimal temperature and pH.

MATERIAL AND METHODS

Microorganism and chemicals

The microorganism was isolated from the soil sample and screened for cellulase production and identified based on the following characteristics: Gram stain, aerobic growth, morphological characteristics of colonies and bacterial cells, spore formation, appearance and shape. Identification of the strain was done

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Paper received: 22 February, 2015

Paper accepted: 3 July, 2015

by 16S rRNA encoding gene (ARB database, gene accession number KP715850).

Chemicals used for preparation of cultivation media were purchased from Torlak Institute of Immunology and Virology (Belgrade, Serbia) except for the casein hydrolisate that was purchased from Fluka. CMC and cellobiose was purchased from Sigma, Avicel from Merck and Croscarmellose-Na from J. Rettenmaier & Sohne.

Screening for cellulase producing bacteria

Screening for cellulase producing bacteria was performed by growing bacterial strains on CMC agar plates (per liter: CMC 1 g, yeast extract 3 g, K_2HPO_4 3 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g and agar 6 g). An overnight bacterial culture was performed in a liquid CMC medium (the same composition as CMC agar medium without addition of agar). A 4% of bacterial culture was inoculated into fresh CMC liquid medium and incubated for 24 h on 30 °C, with shaking at 150 rpm. After overnight growth, 5 µl of liquid bacterial culture was spot plated on CMC agar plates. After incubation for 24–48 h at 30 °C, plates were flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 min. Clear zones appearing around growing bacterial colonies indicated cellulose hydrolysis [9].

Enzyme assay for CMCase

Carboxymethyl cellulase (CMCase) activity was measured by reduction of 3,5-dinitrosalicylic acid (DNS) in the presence of glucose released by enzymatic hydrolysis of cellulose according to the method of Müller [10]. CMCase activity was determined as follows: 500 µl of enzyme solution (crude bacterial supernatant) was added in test tubes with 500 µl of 1 % CMC in 0.1 M acetate buffer at pH 4.80 and incubated at 50 °C for 30 min in a rotary shaker with rotation speed of 150 rpm. After incubation, 1 ml DNS reagent was added. The reaction mixture was boiled for 5 min in a water bath. After cooling at room temperature 5 ml of distilled water was added to each tube and absorbance of the solution was measured at 540 nm on spectrophotometer (Ultrospec 3300 *pro* Amersham Bioscience). CMCase activity was determined by using a calibration curve for glucose. One unit of CMCase activity was defined as the amount of enzyme that released 1 µmol of glucose equivalent per minute. All assays were carried out in triplicate, while the results are presented as mean value with given standard deviation.

Effect of temperature and pH on CMCase activity

The optimal temperature and pH of crude enzyme was determined using the following procedure: the crude enzyme (bacterial supernatant) was incubated with 1 % CMC in 0.1 M acetate buffer pH 4.80 for 30 min at temperatures between 30 and 70 °C with steps of 10 °C. After incubation, the CMCase activity was measured using the method of Müller [10]. To determine the optimum pH of the enzyme, the crude enzyme was mixed with substrates prepared in following buffer solutions: 100 mM citrate buffer (pH 3.0, 4.0 and 4.8), 100 mM sodium phosphate buffer (pH 6.0 and 7.0), 100 mM Tris–HCl (pH 8.0 and 9.0) and incubated at the optimum temperature of 50 °C for 30 min. CMCase activity was measured according to the method of Müller [10]. The maximum CMCase activity obtained at different temperatures and pH was considered to be 100%.

Influence of various factors on CMCase production

CMCase production was measured in liquid non-optimized medium composed by mixing CMC 5.0 g/l, yeast extract 3.0 g/l, KH_2PO_4 4.0g/l, Na_2HPO_4 4.0g/l, $MgSO_4 \cdot 7H_2O$ 0.2g/l, $CaCl_2 \cdot 2H_2O$ 0.001g/l and $FeSO_4 \cdot 7H_2O$ 0.004 g/l. The pH of the medium was adjusted to 7 before autoclaving. After sterilization at 121 °C for 20 min, a 4% of an overnight bacterial culture was inoculated into fresh medium in a rotary shaker with mixing speed of 150 rpm at 30 °C. The culture medium was centrifuged at 6000*g* for 15 min to remove the cells. The crude cell-free supernatant (pH 5.15) was analyzed for CMCase activity.

The influence of the following parameters on the crude enzyme activty were tested: bacterial passaging, incubation time, pH of the growth medium, various carbon sources, concentration of the optimal carbon source, various nitrogen sources, concentration of the optimal nitrogen source and different medium volumes and agitation speeds.

Influence of the bacterial passaging on CMCase production

The effect of the bacterial culture passaging on CMCase production was examinated by measuring CMCase activity after transfering the bacterial culture every 24 h into fresh medium (passaging) which contained CMC as an inducer for CMCase production. Each passage was monitored for CMCase activity for 4 days.

Influence of the incubation time on CMCase production

The effect of incubation time on CMCase production was investigated by taking samples every 24 h, for five days, and CMCase activity was determinated.

Influence of the pH of the growth medium on CMCase production

The effect of initial pH of the growth medium was examinated by adjusting the pH of the medium to 4, 5, 6, 7, 8, 9 and 10 with 1 M NaOH or 1 M HCl before

autoclaving. CMCase activity was determinated on a third day of incubation.

Influence of the different carbon sources on CMCase production

Different carbon sources including CMC, Avicel, cellobiose and crosscarmellose were added to the growth medium in concentration of 2.5 g/l to evaluate the effect on the CMCase production. For the optimal carbon source (cellobiose), influence of different concentrations (1.5, 2.5, 5.0, 6.0 and 7.0 g/l) on CMCase production was examined.

Influence of the different nitrogen sources on CMCase production

Different nitrogen sources: yeast extract, tripton, meat extract and NH_4NO_3 were added to the growth medium in concentration of 3 g/l. The influence of different cocentrations(2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 g/l) of the optimal nitrogen source (yeast extract) on CMCase production was measured.

Influence of different medium volumes (surface/ /volume ratio) and agitation speeds on CMCase production

The effect of different working medium volumes 40, 100 and 150 ml in 500 ml flask (surface/volume ratio 78.5/40; 78.5/100 and 78.5/150) with shaking speed at 150 rpm during fermentation was investigated for CMCase production. Finally, the effect of different agitation speeds 100, 120 and 150 rpm on CMC-ase production at a constant working volume of 40 ml in 500 ml flask was investigated.

HPLC analyses of the CMC hydrolysis

The CMC hydrolysis product, obtained from optimized medium with maximum CMCase activity, was analyzed by high performance liquid chromatography (HPLC). 5.0 ml of enzyme solution (crude bacterial supernatant) was incubated at 50 °C with 5.0 ml of 1% (*w*/*V*) CMC in 0.1 M acetate buffer (pH 4.80) in a rotary shaker with rotation speed of 150 rpm. After 30 min, hydrolysis was stopped by boiling the sample for 5 min. The sample was then filtered through a 0.22 μm membrane filter.

For quantitative analysis of the obtained sample, the Dionex Ultimate 3000 Thermo Scientific (Waltham, USA) HPLC system was used. A carbohydrate column (Hyper REZ XP Carbohydrate Ca^{2+}, 300 mm×7.7 mm, 8μm) on 80 °C was employed. Water (HPLC grade, JT Baker (USA)) was used as sole mobile phase with an elution rate 0.6 ml/min during the analysis. Detection was performed by RI detector (RefractoMax 520, ERC, Germany). All data acquisition and processing was done using Chromeleon Software. The separated hydrolysis products were identified by comparison with glucose standard.

Statistical analysis

Mean values of various experiments were compared by the analysis of variance. All statistical analyses were performed using the Origin Pro 8 software.

RESULTS AND DISCUSSION

Screening for cellulase producing microorganism

Screening for cellulase production was tested on CMC agar plate by the appearance of a halo around the bacterial colony. Based on physiological characteristics, optimal temperature for growth of the strain was 30 °C, thus this temperature was used for incubation. The strain with cellulolytic activity was identified as member of the *Paenibacillus* genus based on the following characteristics: Gram positive reaction, aerobic growth, spore formation with characteristic ellipsoidal spores that were larger than the viable cells. The bacterial strain with cellulolytic activity designated as *Paenibacillus* sp. CKS1 was identified as *Paenibacillus chitinolyticus* based on the almost full-length 16S rRNA gene sequence. The sequence was deposited to the GeneBank database under accession number KP715850.

CMCase production

Cellulase systems consist of endoglucanase, exoglucanase, and β-glucosidase and the synergy of all these enzymes makes hydrolysis of cellulose to glucose possible [11]. CMC is an example of an amorphous cellulose and is generally used as a substrate for the study of endoglucanases or CMCases [12]. In our study, the most potent cellulolytic isolate strain *Paenibacillus* sp. CKS1 was grown on amorphous cellulose (CMC). The strain *P.chitinolyticus* CKS1 showed greater catalytic affinity for CMC, so the cellulases secreted by this isolate could be categorized as endoglucanase or CMCases.

Subculturing (passaging) of an microorganism in a medium of essentially the same composition as that employed for final culture showed to be an effective way to enhance a desired property [13]. Particularly for large enzyme complexes, such as cellulases, such adaptation of a microorganism is expected to enhance enzyme synthesis. In contrast to this expectation, Beckord *et al*. [14] found that one subculture to a medium similar to the final culture medium had a beneficial effect, while subculturing more than once had no significant influence on the production of enzyme. In order to define if the adaptation of the microorganism to the specific cultivation medium had an impact on CMCase activity, the influence of passaging of bacterial culture was examined. The results showed that CMCase activity increased with culture passaging and

with the incubation time (Figure 1). The highest CMCase activity was detected at the third passage and at the third day of incubation (0.197±0.019 U/ml). CMCase activity increased with culture passaging and the highest CMCase activity was detected at the third passage, thus third passage was applied to all further experiments. In further tests the second passage was used as inoculum for further investigation of CMCase production. With regard to CMCase activity, which increased with culture passaging, the highest CMCase activity was detected at the third passage, thus third passage was applied to all further experiments.

When testing the influence of incubation time on the CMCase activity it was determined that the highest CMCase production was obtained after three days of incubation (Figure 2). After this period CMCase production decreased. The decrease of CMCase activity could be a consequence of changed conditions in the medium (pH change, production of inhibiting byproducts), or due to the depletion of nutrients in the fermentation medium as seen for other bacterial strains [15]. The timing of the optimal CMCase production is a strain dependent characteristic. Some cellulolytic *Paenibacillus* sp. had the optimal CMCase production as shorter as 24 h (*Paenibacillus* sp. P118 [16]) or similar to our strain *P.chitinolyticus* CKS1 of about 72 h (*Paenibacillus tarimensis* L88 [17] and *Paenibacillus* sp. ME-271 60 h [18]). In general, the increase in the enzyme activity during the incubation period depends on the culture characteristics and growth rate of selected microorganism. *Paenibacillus* sp. CKS1 produced CMCase during the earlier stage of fermentations, in a late exponential phase (data not shown), while the maximum CMCase production was achieved in the late stationary phase.

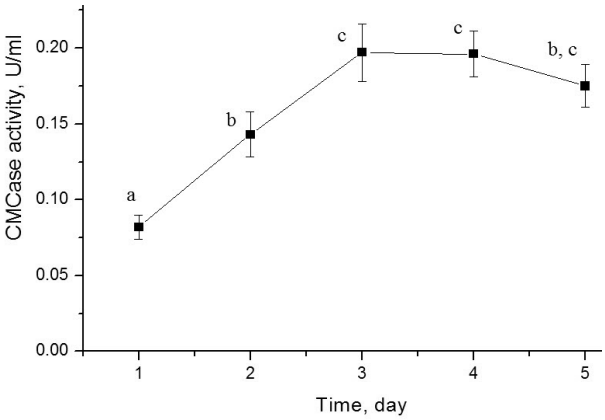

Figure 2. CMCase activity for the strain CKS1 during 5 days of incubation on 30 °C at 150 rpm with 40 ml of working volume in 500 ml flask.

It is well established that initial pH of the bacterial growth medium has an effect on the availability of certain metabolites and ions and influences the permeability of the cell membrane [4]. Our results also showed that the pH of the growth medium was an important factor affecting the CMCase activities. The optimum pH of the growth medium for maximum production of CMCase for the strain CKS1 was 7.0 (Figure 3).

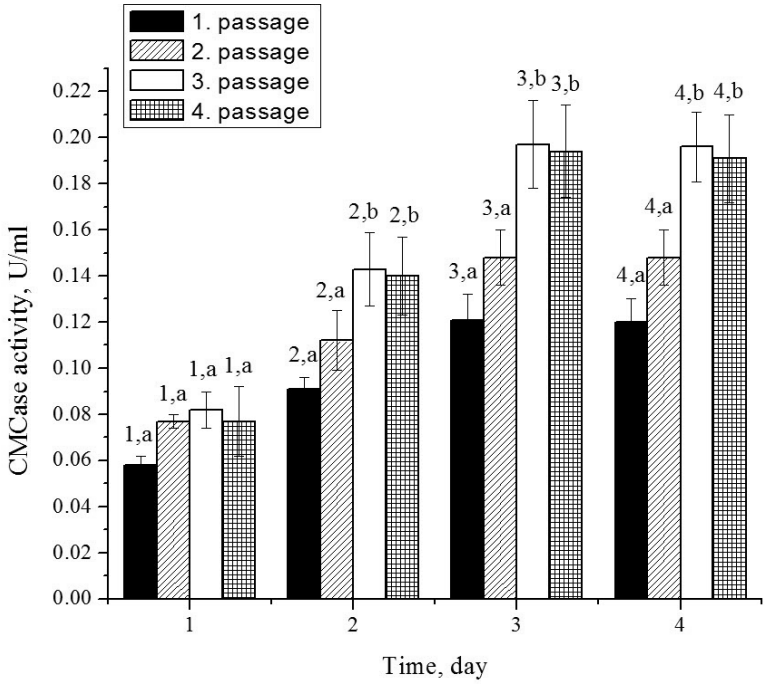

Figure 1. The influence of passaging culture on CMCase activity. A 4% of bacterial culture was inoculated in each passage for four days at 30 °C at 150 rpm.

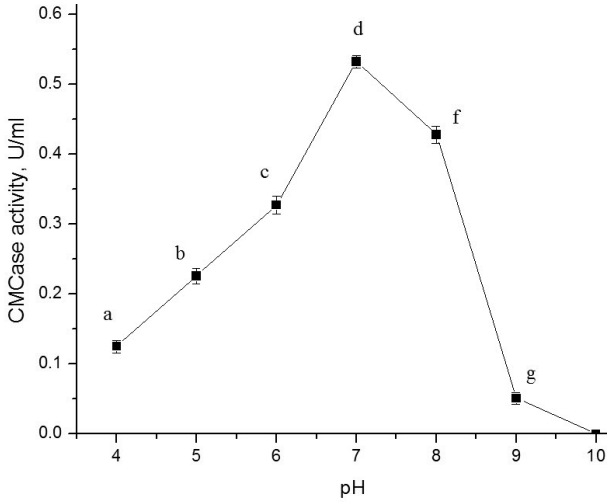

Figure 3. Effect of initial pH of the growth medium on CMCase production for the strain CKS1 on 30 ℃ at 150 rpm with 40 ml of working volume in 500 ml flask.

Similar to other parameters, various *Paenibacillus sp.* strains show the optimal CMCase activity under different medium acidity conditions. Some other species produce the highest levels of CMCase at neutral pH including *P. curdlanolyticus* B-6 (isolate from an anaerobic digester fed with pineapple wastes) and *P. cam-pinasensis* BL-11 (isolate from black liquor) [19,20], while others perform the best under alkali conditions such as *P. terrae* ME27-1 (pH 8) (isolate from soil sample from the subtropical region of China) [18], or acidic conditions such as *Paenibacillus polymyxa* an isolate from degrading citrus peel (pH 5.5) [21].

Generally, production of cellulases was shown to be inducible and was affected by the nature of the carbohydrate used in fermentation as a carbon source. Different commercial substrates have been used as inducers of CMCase production by *Penibacillus* sp. CKS1 (Figure 4a). According to this results it appeared that *Paenibacillus* sp. CKS1 produced the largest amount of CMCase (0.326±0.011 U/ml) while growing on cellobiose, although it produced CMCase on all tested substrates (Figure 4a). Mandels [22] and Paul [23] also reported that cellobiose is a good cellulase inducer for fungi and *Bacillus* sp. [24–26] but for some other *Paenibacillus* sp. the most potent inducer of the CMCase activity was CMC [18,27,28]. The tested *Paenibacillus* sp. CKS1 is the first reported *Paenibacillus* sp. for which cellobise is better inducer of CMCase production than CMC itself.

Cellobiose had the inducing effects in the concentration range of 1.5–7 g/l (Figure 4b). With cellobiose

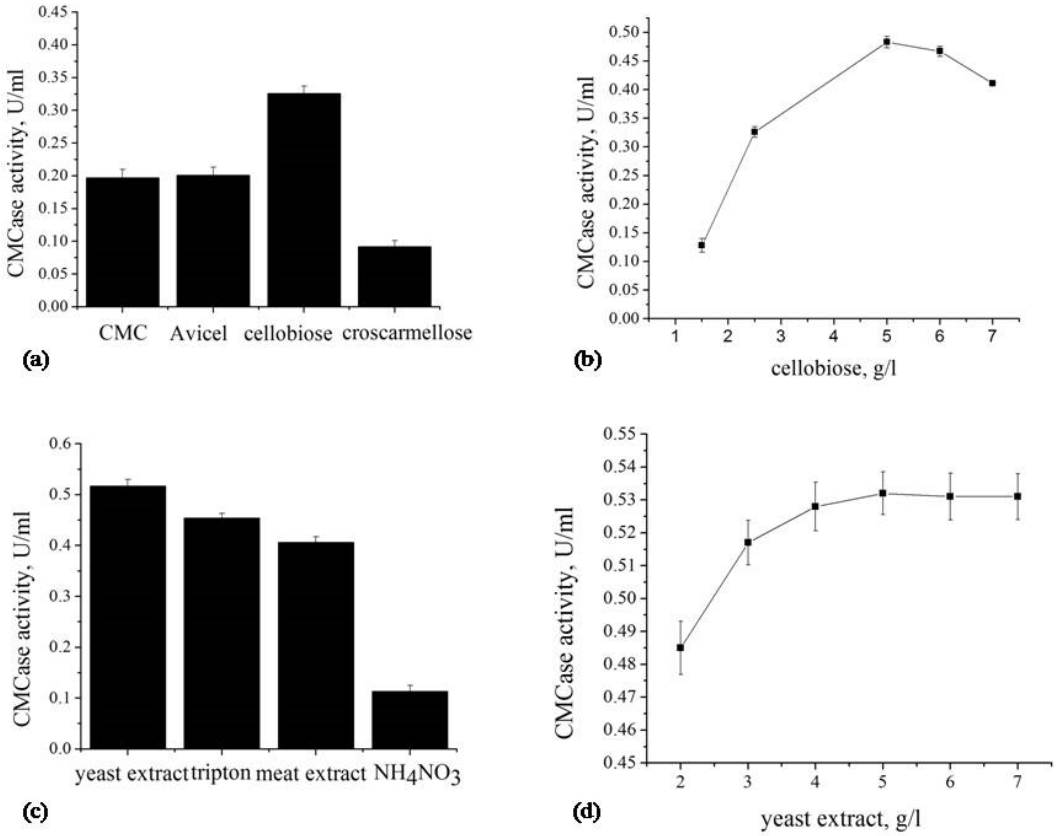

Figure 4. Effect of carbon and nitrogen sources on CMCase production by the strain CKS1 for 3 days of incubation. a) Different carbon sources: CMC, Avicel, cellobiose and croscarmellose, respectively. b) The concentration of cellobiose. c) Different nitrogen sources: yeast extract, tripton, meat extract and NH_4NO_3, respectively. d) The concentration of yeast extract.

concentration of 5 g/l the maximum CMCase activity (0.483±0.010 U/ml) was achieved. Further increase of the concentration leads to a slight decrease in CMCase activity.

Regarding the influence of the different nitrogen sources, it was determined that yeast extract is the most stimulative for CMCase production as growth of the strain on the medium containing yeast extract led to CMCase activity of up to 0.517±0.006 U/ml (Figure 4c). The optimal concentration of the yeast extract in the medium was 5 g/l (Figure 4d) thereby providing a 0.532±0.006 U/ml of CMCase activity. Yeast extract is the main nutritional supplement which serves as a rich source of amino acids, vitamins, nitrogen and carbon for bacterial growth [29]. Based on our results, it is evident that *Paenibacillus* sp. CKS1 prefers organic nitrogen sources for growth and enzyme production. When growing on medium that contained inorganic nitrogen source, NH_4NO_3, *Paenibacillus* sp. CKS1 strain produced notably lower amount of CMCase compared to the used organic sources of nitrogen (Figure 4c). Our results are in line with previous reports for the majority of cellulolytic *Paenibacillus* strains [17,20,28] although some strains are able to produce CMCases in large quantities while growing in presence of inorganic nitrogen [19,27,30].

Oxygen in the medium may have great influence on the production of metabolites and enzymes, including extracellular enzymes [31]. CMCase production was significantly affected by medium volume since all tests were done in the same type of flasks and the medium volume had a direct effect on the surface/volume ratio. Given that surface/volume ration and the aeration area is relatively decreased when larger volumes are used for bacterial growth, we evaluated the influence of medium volume on CMCase production for our strain *Paenibacillus* sp. CKS1. The highest CMCase production was achieved when the culture medium volume was 40 ml in 500 ml volume flasks. Increasing the volume of media leads to significant decrease of the enzyme activity (Figure 5). The strain CKS1 grows under strictly aerobic conditions and any reduction in oxygen level leads to a decrease of the CMCase production.

Furthermore, agitation had a significant influence on the CMCase production. When the strain was grown under stationary conditions, the CMCase activity was under the detection level of the applied methodology (data not shown). The CMCase activity did not change significantly when agitation speed changed in the range from 120 to 150rpm.

The literature data available on the production of cellulase by *Paenibacillus* sp. are difficult to compare with each other due to different growing conditions of microorganisms, different substrates, ways the results of enzymatic activity is interpreted. Also, CMCase from the strain CKS1 are from crude, not purified culture supernatant which may have influence on a lower value of enzyme activity. Nevertheless, our results indicated that relatively higher aeration surface had a stimulative effect on CMCase activity of *Paenibacillus* sp. CKS1 indicating the positive effect of oxygen on the CMCase production and activity.

Effect of temperature and pH on CMCase activity

The influence of temperature on CMCase activity produced by the *Paenibacillus* sp. CKS1 was examined at various temperatures ranging from 30 to 70 °C at pH 4.8. Optimal temperature for CMCase activity was 50 °C at pH 4.8 (Figure 6a).

The optimal temperature for other CMCases produced by *Paenibacillus* strains is close to or slightly higher than the one determined for our strain as it values 50 °C for *Paenibacillus terrae* ME27-1 [18], 55 °C for *P. cookii* SS-24 [32], 60 °C for *Paenibacillus* sp. B39A

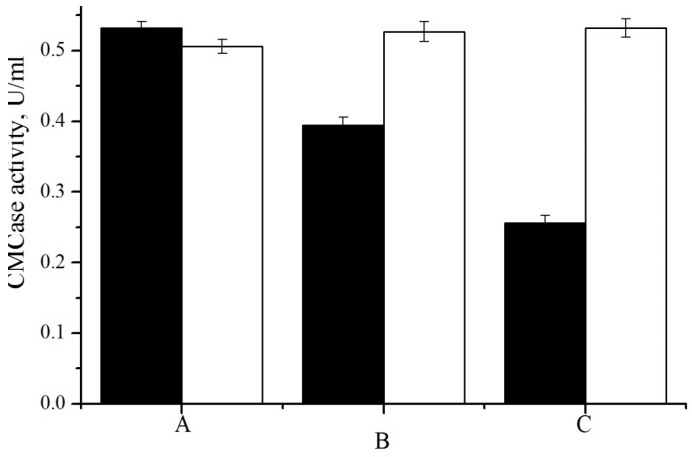

Figure 5. Effects of different medium volume (filled square) and agitation speeds (opensquare) on CMCase production from Paenibacillus sp. CKS1. The cultures were carried out at different working volumes in 500 ml flasks: A) 40; B) 100 and C) 150ml; with shaking at 150 rpm. The effect of agitation speeds: A) 100; B) 120 and C) 150 rpm on CMCase production was performed in a constant working volume (40 ml).

and *Paenibacillus elgii* [30,33] and 65 °C for *P. barcinonensis* CMCase [28].

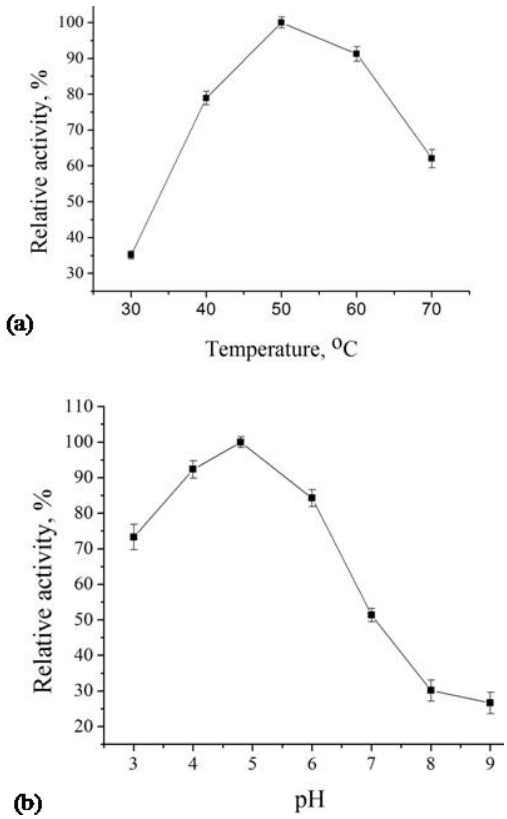

Figure 6. Effect of a) temperature and b) pH on CMCase activity.

The optimal pH for CMCase activity of *Paenibacillus* CKS 1 was 4.8 (Figure 6b) therefore the tested CMCase can be considered as acidophilic enzyme. This feature is in contrast to the majority of CMCases reported in the literature, since these enzymes typically favor neutral or alkaline conditions [18,20,33,34] although there are exception like *P. polymyxa* that shows optimal activity at slightly acidic conditions of pH 5.5 [21].

Hydrolysis products of CMCase

Paenibacillus sp. CKS1 CMCase hydrolyzed CMC to form glucose and a number of oligosaccharides assuming cellobiose, cellotriose, cellotetraose (Figure 7). In addition to these small molecules, some complex components, most likely larger oligosaccharide residues seem to be present in the hydrolysate. The presence of glucose and other olighosaharides indicates the existence of the enzyme complex of *Paenibacillus* sp. CKS1 which is responsible for degradation of CMC. The formation of glucose and cellobiose suggests the sequential action of endoglucanase and exoglucanase [35]. Many cellulases have been reported to show both endo- and exo-glucanase activities [12,20,30,36]. *Paenibacillus* sp. CKS1 could hydrolyse cellulose, amorphous-CMC and microcrystalline cellulose-Avicel (data not shown) suggesting that it produces both endo- and exo-glucanase.

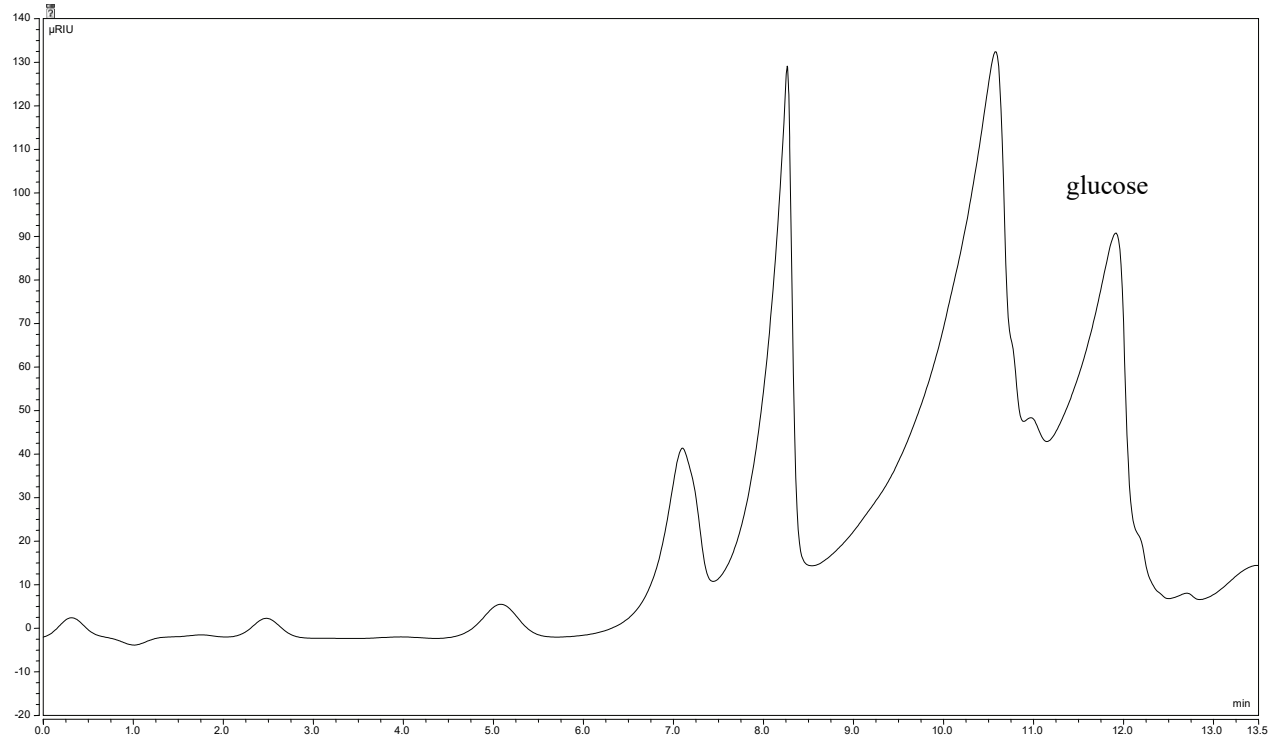

Figure 7. HPLC analysis of hydrolysis CMC.

CONCLUSION

In this study, we have identified three novel *Paenibacillus* strains as potent cellulolytic bacteria. Among them, *Paenibacillus* sp. CKS1 showed the highest cellulolytic potential. Culture conditions were optimized to enable the highest CMCase production by this strain. HPLC analysis confirmed the degradation of CMC into glucose and other oligosaccharides. *Paenibacillus* sp. CKS1 is a novel candidate for the CMCase production facilitating its potential use in industrial applications. Due to its acidophilic nature (pH 4.8) and relatively good tolerance to high temperatures, our CMCase might be a useful enzyme for industrial applications such as animal feed industry, clarification of fruit juices, biofuels production or in stonewashing and biopolishing in jeans industry.

Acknowledgements

The financial support for this investigation given by Ministry Education, Science and Technological Development of the Republic of Serbia under the project TR 31035 is gratefully acknowledged.

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IZVOD

PRODUKCIJA KARBOKSIMETIL CELULAZA POMOĆU SOJA *Paenibacillus* SP.

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(Naučni rad)

Celulaze su enzimi koji katalizuju hidrolizu β-1,4-glikozidne veze u molekulu celuloze. Spadaju u veoma važnu grupu enzima koje se koriste u različitim sferama industrije. Najvažniji enzimski proces je hidroliza biljne biomase pomoću celulaza u cilju dobijanja glukoze koja se može koristiti direktno kao proizvod za životinjsku i humanu primenu ili kao polazna sirovina za proizvodnju alkohola, aminokiselina, organskih kiselina, biogoriva i mnogih drugih korisnih proizvoda. U prehrambenoj industriji se celulaze uglavnom koriste u pekarstvu, u proizvodnji voća i povrća, piva i sokova. Takođe, se koriste u industriji deterdženata. *Paenibacillus* sp. predstavlja Gram pozitivne, aerobne i endosporoformirajuće bakterije. Značajan broj *Paenibacillus* vrsta su od industrijskog kao i poljoprivrednog značaja zbog sposobnosti degradacije složenih ugljovodonika i produkcije različitih enzima celulaza, amilaza, hitinaza. S tim u vezi, ispitivana je mogućnost produkcije enzima celulaze iz soja *Paenibacillus* sp. Ispitivanje celulolitičke aktivnosti vršeno ja kvalitativno i kvantitativno. Tokom kvalitativnog ispitivanja na karboksimetil celuloznoj agarnoj podlozi, kao najbolji producent celulaza, medju ispitivanim sojevim, pokazao se soj CKS1. U radu su određeni optimalni uslovi za produkciju karboksimetil celulaza uključujući efekat pasažiranja ispitivane kulture, vreme inkubacije, izvori ugljenika i azota, uticaj početnog pH, kao i uticaj zapremiene mediuma i brzine mešanja na produkciju karboksimetil celulaza. Najveća aktivnost karboksiemtil celulaze dobijena je u trećem pasažu nakon 72 h pri temperaturi od 30 °C. Kao jedini izvor ugljenika korišćena je celobioza (5 g/l) a kao izvor azota kvaščev ekstrakt (5 g/l). Takođe, podešavanjem početnog pH podloge na 7 i korišćenjem 40 ml radne zapremine podloge u erlenmajeru od 500 ml, na tresilici sa 150 rpm, postiže se maksimalna karboksimetil celulazna aktivnost od 0.532±0.006 U/ml. Na ovakav način dobijena sirova karboksimetil celulaza pokazuje maksimum svoje aktivnosti pri temperature od 50 °C i pH 4.8. HPLC analizom krajnjih produkta hidrolize CMC utvrđeno je prisustvo glukoze i ostalih oligosaharida. Soj CKS1 može naći primenu u industrijskoj proizvodnji karboksimetil celulaza kao i u proizvodnji bioetanola.

Ključne reči: *Paenibacillus* sp. • Celuloza • Karboksimetil celulaza • Produkcija • Optimalni uslovi