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PRODUCTION OF PENICILLIN ACYLASE BY *BACILLUS SUBTILIS*

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ABSTRACT

Strain of Bacillus Subtilis capable of producing penicillin acylase was isolated from soil sample. Optimum time for penicillin acylse production was 48hrs. Increased temperature up to 40°C better suited penicillin acylase production. Glucose increased penicillin acylase production. Penicillin acylase worked optimally at neutral to slightly alkaline pH and within 40 to 50°C. Penicillin acylase was more active against ampicillin.

KEY WORDS

Ampicillin, Penicillin acylase, Bacillus Subtilis.

INTRODUCTION:

penicillin acylase or penicillin amidohydrolase (EC 3.5.1.11) is one of the most significant enzyme useful in the pharmaceutical industry for large scale production of 6-aminopenicillanic acid [1] which is starting material for synthesis of semisynthetic penicillin [2] . Penicillin acylases are produce by yeast, bacteria and fungi. Among them, enzyme produced by *E. coli* is the most well-characterized and common one for industrial application. Due to high industrial importance of penicillin acylase, numerous efforts have been made towards screening for strains overproducing this enzyme [3]. Penicillin acylase has also been used for synthesis of amoxicillin [4]. The *E. coli* penicillin acylase being intracellular is quite difficult to purify. Even if whole cell is used for catalysis it would pose substrate/product diffusion problem thereby putting break on speed of reaction. The present research work describes our findings on penicillin acylase production by *Bacillus Subtilis*. isolated from soil sample. Very few reports of penicillin acylase of this *Bacillus Subtilis* are available. Thus, we have also optimized few production parameters.

MATERIALS AND METHODS

Isolation of Penicillin acylase producing bacteria: For enrichment 1g of soil sample was added to nutrient broth tubes with increasing pH (7, 8, 9 and 10). The broth was supplemented with 0.2% benzathine penicillin. For isolation loop full enriched sample was streaked on nutrient agar + benzyl penicillin of respective pH.

Screening of Penicillin Acylase Producers: Two isolates B1and B2 were grown for 24 hrs on nutrient medium composed of g/L, MgS04 0.2; KH2P04 3.0; K2HP04 7.0; Yeast extract 5.0; Peptone 20.0; Benzylpenicillin 10; Agar agar 20. Cultures were then screened for PAG activity by acidimetric method (5). The test reagent was composed of Penicillin G, 600mg + Phenol red (0.5%), 0.5mL + Sterile distilled water, 4.5 mL. The test reagent was adjusted to pH 8.5 with 1.0 M NaOH giving violet color to the solution. Three drops of freshly prepared reagent were added to 0.5mL cell suspension of test cultures. The positive cultures used for further study.

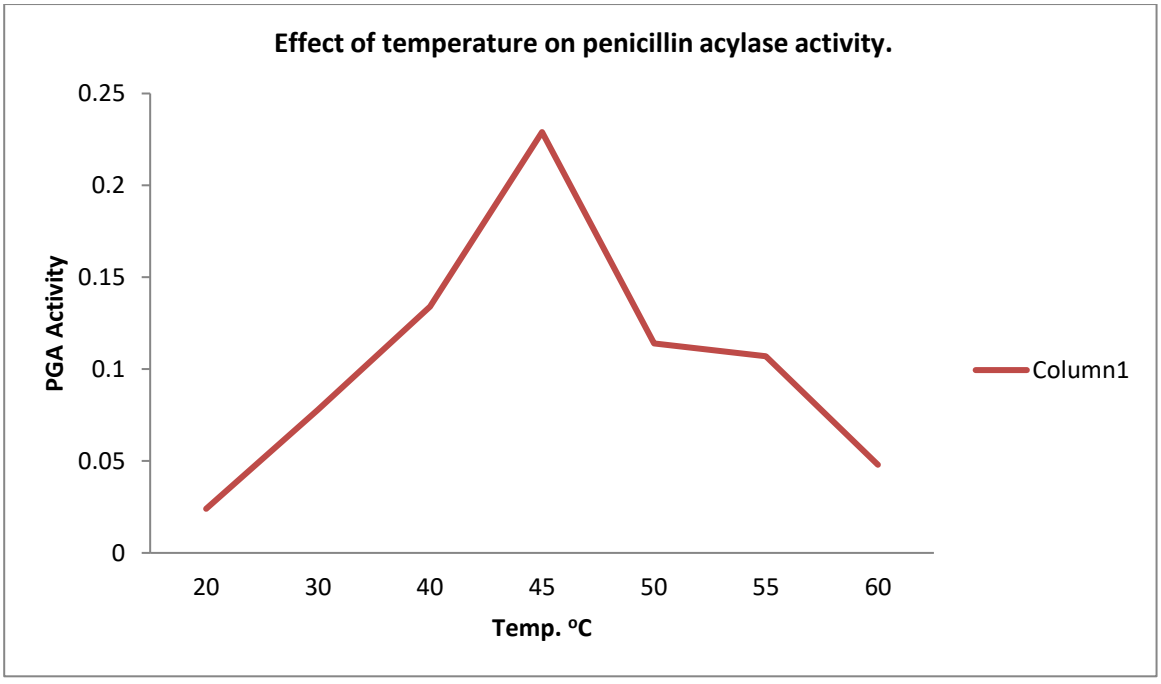
Penicillin Acylase Assay: Penicillin acylase assay was done by p-Di-Methyl Amino Benzyldehyde (PDAB) method [6]. The PDAB reagent composed of PDAB 1.5g, Acetic acid 100mL, Methanol 60mL, and water 40mL. Reaction mixture composed of Cell suspension, 1mL +

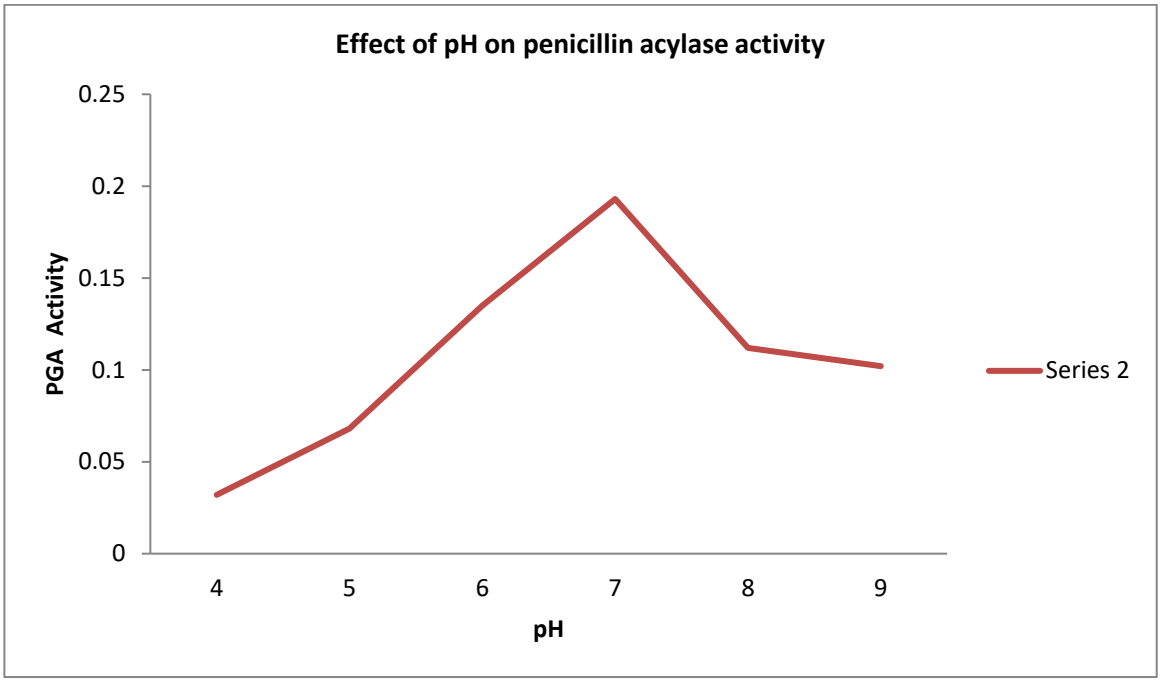
0.5% Penicillin G in 0.1M Phosphate buffer, 5mL. After incubation at 40ºC for 40 min 1.5 mL PDAB reagent was added. The 6-APA produced was measured spectrophotometrically at 415 nm. One unit of penicillin acylase was taken as amount of enzyme required to liberate 1 µMol of 6-amino penicillanic acid per minute under assay conditions.

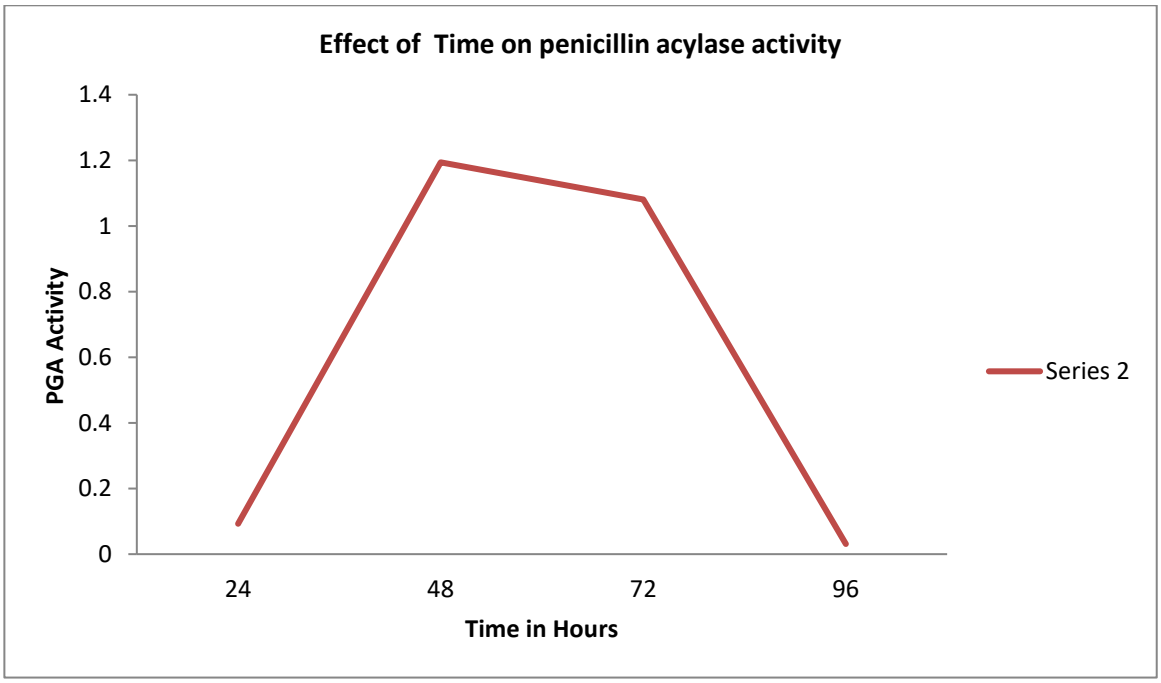
Optimization of Growth Conditions for Penicillin Acylase Production: For time course and temperature optimization the production media used composed of g/L, Beef extract 3.0; Bacteriological peptone 5.0; NaCl 5.0; Benzylpenicillin 10; pH 7. For time course optimization the 5% inoculums of test organism was inoculated in production medium. Penicillin acylase activity was determined after every 24hrs for 4days. For temperature optimization inoculated production medium was incubated at different temperatures (20, 30, 40, 50 and 60ºC). Effect of glucose, lactose and sucrose on penicillin acylase production was studied. For this the production media was supplemented with 0.2% of test sugar.

RESULT

Two isolates were obtained from plate with pH 7 and 8, no growth was observed on agar plates of pH 9 and 10. Isolates were given codes as B1 and B2. In acidimetric test we found that these cultures changed the color of phenol red from violet to deep red this confirms cultures are penicillin acylase positive. Morphological appearance and biochemical activities of two bacteria are performed, results were compared with Bergey's manual of Determinative Bacteriology. From this it can be concluded that B1 & B2 are strains of Staphylococcus spp. Penicillin acylase activity of both strains were showing almost double in supernatant than in pellet. This shows that the enzyme is largely extracellular. The penicillin acylase production conditions for all cultures were optimized. Penicillin acylase production reached maximum at 48 hours of incubation. In case of optimum production temperature, we found penicillin acylase at higher level when grown at 45°C. Production medium when supplemented with sucrose penicillin acylase production was stimulated. The penicillin acylase temperature optimum was found to be at 45°C. Penicillin acylase of *Bacillus Subtilis* was working best at pH 7.




Effect of pH on penicillin acylase activity
PGA Activity
0.25
0.2
0.15
0.1
0.05
0
4
5
6
7
8
9
pH
Series 2


Effect of Time on penicillin acylase activity
PGA Activity
1.4
1.2
1
0.8
0.6
0.4
0.2
0
24
48
72
96
Time in Hours
Series 2

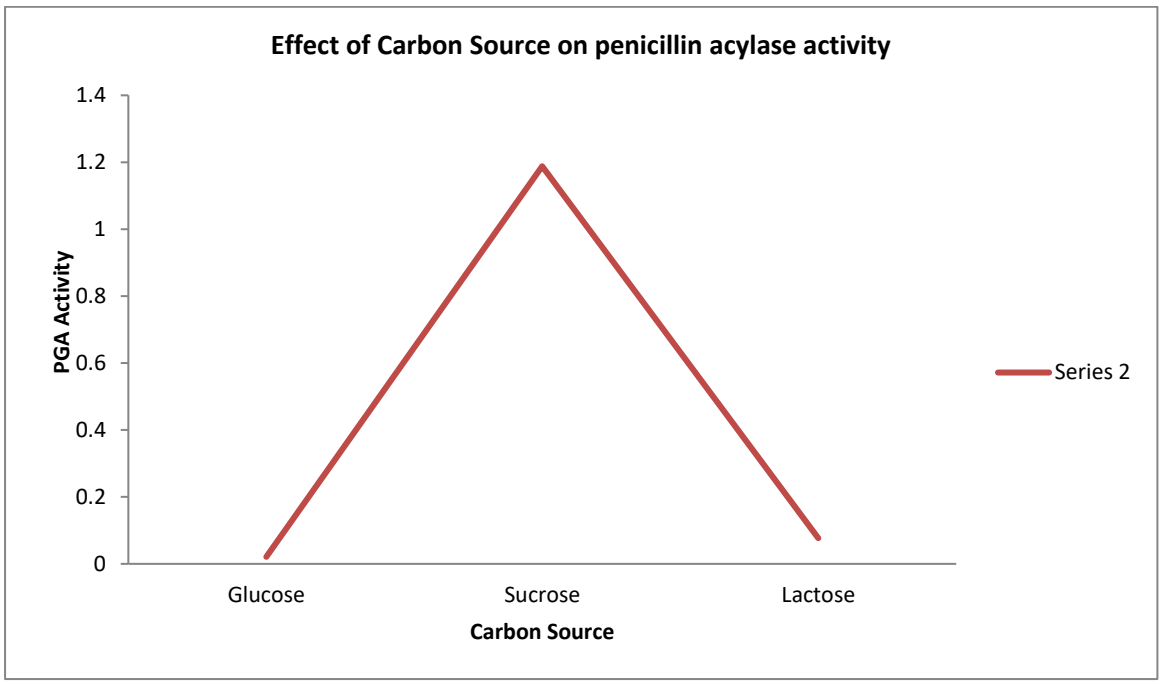


Table 1: Morphological appearance and Biochemical activities of penicillin acylase producers

Characters	SS1
Pigmentation	Off white
Shape of cells	Cocci
Arrangement	Clusters
Gram Nature	+Ve
Motility	-ve
Indole Production	-ve
Methyl red	-ve
Voges Proskeur Test	-ve
Citrate Utilization	-ve
Glucose Fermentation	-ve
Sucrose Fermentation	+ve
Lactose Fermentation	-ve
Catalase Test	+Ve

DISCUSSION

In present study *Bacillus Subtilis* extracellular penicillin acylase producers were isolated from soil sample. Few characteristics of enzymes like optimum temperature, pH, incubation time was determined, and few production parameters were optimized. Nam and Ryu, 1979 reported that penicillin acylase of *M. luteus* showed optimum activity at 36°C and 7.5 pH. Our findings are disagree with those pH optima but could tolerate higher temperature. Sedigheh Javadpour *et al.* 2002, characterized penicillin acylase of *E. coli* and they found that the pH and temperature optimum was 8 and 60°C. According to Hesham *et al*. 2009, penicillin acylase production by *E. coli* reaches maximum after 19 hrs of incubation remained stable up to 24hrs after which it declined. Thus, our isolates were slower compared to *E. coli* (7). Szentirmai, 1964, proposed the inhibitory effect of carbohydrates on penicillin acylse production when added to medium at higher concentration (8).

CONCLUSION

Bacillus Subtilis used here produce extracellular penicillin acylse. Production of enzyme is better at elevated temperature. Enzyme of both tolerated higher

temperature but pH should be near neutrality. Presence of sucrose at small concentration stimulated enzyme production. Penicillin acylase has broad substrate specificity.

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