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Effect of Different Carbon Sources on the Enhancing of Glucosidase using *Bacillus* sp. RL1

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Abstract : A glucosidase producing bacteria was isolated from soil and identified as new strain *Bacillus* sp. RL1. The influence of different carbon sources (Pine apple, corn steam, corn cob, wheat bran and CMC were evaluated as carbon sources at different concentrations for production glucosidase by these bacteria. Pine apple gave the highly yield followed by Corn steam and Corn cob. CMC gave the lowest enzyme activity. Maximum enzyme production was achieved by 2.5% (w/v) carbon source with agitation speed 200 rpm..
Keywords: *Bacillus* sp. RL1, glucosidase , Carbon Sources. Enzyme assay.

Introduction

Globally the estimated quantity of the wastes generation was 12 billion tones in a year 2002 of which 11 billion tones were industrial wastes and 1.6 billion tones were municipal solid waste. About 90 billion tones solid wastes are expected to be generated annually by the year 2025. Annually, Asia alone generates 4.4 billion tones of solid wastes and municipal solid waste comparises 790 million tones of which about 48 million tones are generated in India. Unscientific disposal of waste causes an adverse impact on all components of the environment and human health (1). Agricultural waste is also caused is also caused environmental pollution. Their conversion into useful products may ameliorate the problems they cause.

Cellulase is an industrially important enzyme, which is extensively used for increasing yield of juice in food industry, decreasing discoloration and fuzzing effects of cloth in textile industry, strengthening and whitening of paper pulp in paper industry, and to bio-fuel generation through saccharification process (2-3,15,23). Cellulose is composed of glucose units linked together by a 1.4-D-b glycoside bond (2). Cellulase is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds. It has three major components viz. endo 1.4-b-glucanase (E.C.3.2.1.4), exo 1.4-b-glucanase (E.C.3.2.1.91), and b-glucosidase (E.C.3.2.1.21), on which endoglucanase is known as a well-recognized component (3,4,5,6) Many cellulolytic aerobic and anaerobic bacteria (2) have been isolated from decomposed soil, for biotechnological applications. For more than fifty years one of the main areas of biotechnology research into lignocellulose has been driven by the need to isolate and identify organisms which are either hyper- producers and/or sufficiently robust to withstand conditions of the intended application and/or are producers of novel lignocellulolytic enzymes. In term of enzyme novelty from an applications perspective, interest is focused on not only finding enzymes which could break down lignocellulose much more rapidly but also enzymes which could withstand pH, temperature and inhibitory agents more resiliently depending on the intended application. Since,the present paper investigated how the different carbon sources and culture culture conditions on strain *Bacillus* sp. RL1 for enhancing-glucosidase yield (7).

Materials and Methods:

Chemicals，Media and Media Components All chemicals , media and media components used were of analytical grade obtained from Sigma Chemicals Ltd, USA and other chemical from Shanghai Chemicals Ltd, China

1. **Microorganism and culture :** The glucosidase producing bacterial strain (*Bacillus* sp. RL1) was isolated from soil (Jin Yun mountain，1800 m) using serial dilution up to 10^{-9} and pour plate technique Single colonies on the plates were isolated and purified by transferring them five times onto CMC agar plates. Primary screening was performed by growing the isolate on CMC agar medium-containing-(g/L), KH2PO4, 2; (NH4) 2SO4,4; MgSO4, 0.5 ; Peptone, 10; agar agar, 20; and distilled water, supplemented with 1% carboxy methyl cellulose (CMC) at 30°C for 24 hrs .
2. **Identification of the isolates:** The bacterial isolates obtained after the Primary screening was maintained in pure culture on CMC agar slants. All the agar slants were refrigerated at 4 °C until used. Study of colony morphology of the isolated culture was carried out followed by gram"s staining and endospore staining. Physical and Biochemical characterization of the isolated colonies was carried out using standard protocols (8). Identification was carried out according to Burges's Manual (7th Ed.).
3. **Phylogenetic analysis:**The 16S rRNA gene sequence of *Bacillus* sp.RL1 was determined by direct sequencing of the purified PCR-amplified 16SrRNA gene fragment as described previously by (9). Genomic DNA was extracted by the CTAB protocol and was used as the PCR template. PCR was performed with universal bacterial primers complementary to conserved regions of the 5 and 3ends of the 16S rRNA gene, 27F (forward) (5 –AGAGTTTGATCCTGGCTCAG -3 and 1492R (reverse) (5 –GGTTACCTTGTTACGACTT -3. PCR was performed using ampliTaq gold (Applied Bio systems). The PCR products were purified with a qiaquick PCR purification kit (QIAGEN) according to the manufacturer's instruction. The purified 16S rRNA gene was sequenced directly using the ABI prism big dye terminator cycle sequencing ready reaction kit (Applied Bio systems) and an ABI prism model 377 genetic analyzer (Applied Biosystems). The obtained 16S rRNA gene sequences of isolated bacteria were compared with those from the DDBJ nucleotide sequence database using the program BLAST.
4. **Secondary screening:** A secondary screening for cellulolytic activity was conducted by using Congo red test. The bacterial isolates were grown on serial CMC agar plates containing (g/L), KH2PO4, 2; (NH4)2SO4, 4; MgSO4, 0.5; Peptone, 10; agar agar, 20; and distilled water, supplemented with 1% carboxy methyl cellulose (CMC) at 30°C for 24 hrs to allow the secretion of glucosidase. Following incubation, the agar media was flooded with an aqueous solution of Congo red solution (1% w/v) and left for 15 min, the stain poured off and the plates were washed with 1M NaCl for 20 min, the solution poured off. The formation of a clear zone of hydrolysis indicated cellulose degradation (10).
5. **Fermentation sugar:** The culture was grown aerobically in a 50ml Erlenmeyer flask that contain 30ml of CMC medium containing (g/L) KH2PO4, 2; (NH4) 2SO4,4; MgSO4, 0.5 ; Peptone, 10 and distilled water, supplemented with 1% carboxy methyl cellulose (CMC) without agar, at 35°C, pH7.0 for 72hrs. At the end of the incubation period, culture was centrifuged at 10'000 rpmfor 10 min and glucosidase activity was measured in the culture supernatant.
6. **Enzyme Activity Assay:**
7. **glucosidase assay:** Glucosidase activity was measured by monitoring the release of glucose or reducing sugar from salicin (11). One unit of glucosidase activity is defined as the amount of enzyme required to catalyze hydrolysis of the substrate in 1 min to generate 1µmol glucose at pH 7.0 and 35°C.The above extracts was made an assay of the activity of glucosidase against salicin at 35◦C, respectively. All Data recorded in this work were average outcome of three replications.
8. **Effect of carbon sources on glucosidase activity:** Under optimized temperature, pH and incubation period. Five different carbon sources (wheat bran, corn cob, corn stem, pineapple and CMC) were tested at the different concentrations range 0.5, 1.0, 1.5, 2.0 and 2.5 % (w/v), on glucosidase production.
9. **Effect of agitation speed on glucosidase activity :** *Bacillus* sp. RL1 was inoculated into production medium and incubated at 35°C for 72 hours in stationary phase conditions at three different conditions as follows; static, 100rpm and 200rpm using 50ml shaker flask. The glucosidase activity was measured under standard enzyme assay condition.

Results:

Effect of carbon source (Wheat bran%) on glucosidase production:

The glucosidase producing *Bacillus* sp. RL1 strain was isolated from soil and identified to be a new strain with help of 16S rRNA sequence homology of *Bacillus* sp. RL1 (Data not shown). Data presented in Figure.1 shown that glucosidase producation by *Bcillus* sp. RL1 was influenced by concentration of carbon source in basal medium (CMC) supplemented with agricultural carbon source wheat bran. glucosidase increased with increases of wheat bran concentration from 1.0 to 2.5% (w/v).

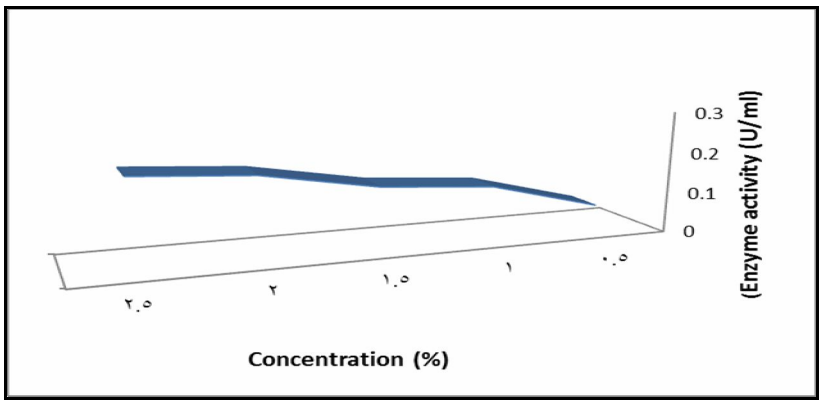

Figure. (1) Effect of carbon source (Wheat bran%) on glucosidase production (U/ml) by *Bacillus* sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Effect of carbon source (Corn cob%) on glucosidase production:

In the present study, it was found that all of these corn cob concentrations leaded to increase of enzyme activity with the increase the concentration 0.5, 1.0, 1.5, 2.0, 2.5% (w/v). 2.5% as sole carbon source strongly affected glucosidase production by *Bacillus* sp. RL1. (Figure.2).

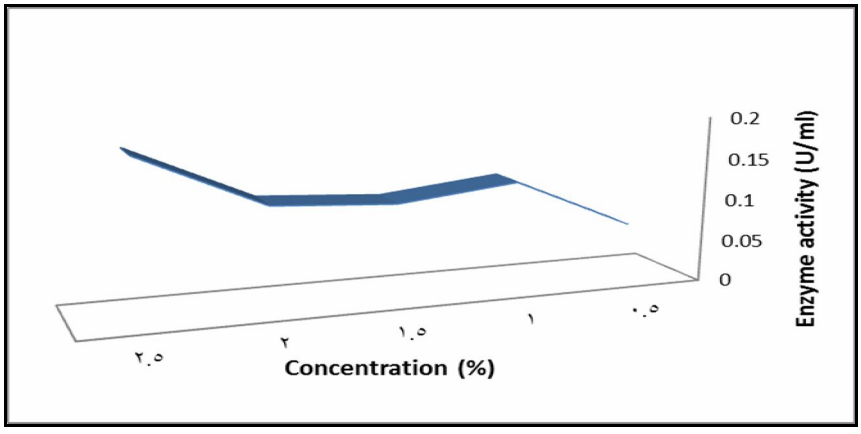

Figure. (2) Effect of carbon source (Corn cob%) on glucosidase production (U/ml) by *Bacillus* sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Effect of carbon source (Corn stem%) on glucosidase production:

The glucosidase production by *Bacillus* sp. RL1 at different concentrations of corn stem showed that the enzyme activity was increased with the increase of corn stem concentration from 0.5 to 2.5%, 0.0605, 0.1289, 0.1485, 0.1953 and 0.2123, respectively. (Figure.3).

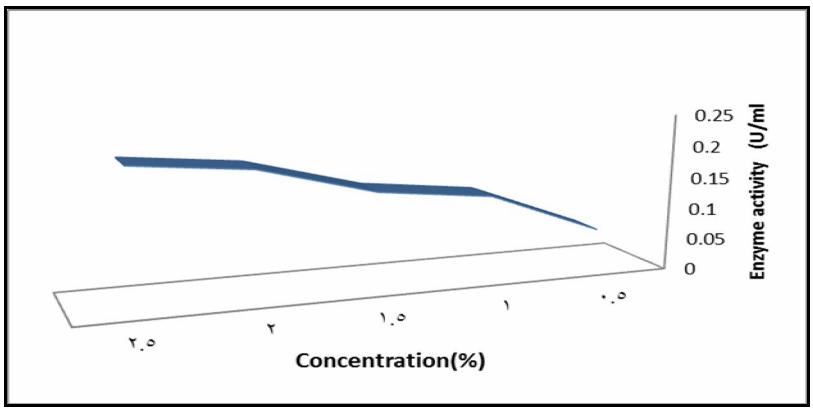

Figure. (3) Effect of carbon source (Corn stem%) on glucosidase production (U/ml) by *Bacillus* sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Effect of carbon source (Pineapple%) on glucosidase production:

Among the five different substrate concentration, the enzyme activity was more in 2.5% Pineapple as a sole carbon source. While the minimum activity was recorded at 0.5%. (Figure.4).

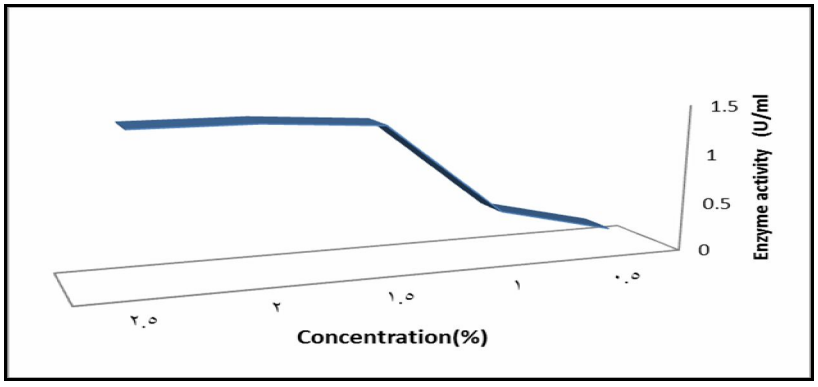

Figure. (4) Effect of carbon source (Pineapple%) on glucosidase production (U/ml) by *Bacillus* sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Effect of carbon source (CMC%) on glucosidase production:

To evaluate the effect of carbon source on glucosidase production, CMC was used as a sole carbon source with five concentrations (0.5 – 2.5%). Enzyme production increased with the increases of CMC concentration. Maximum yield of enzyme was obtained at 2%(w/v) and yield of glucosidase decreased when CMC 2.5% was used. (Figure.5).

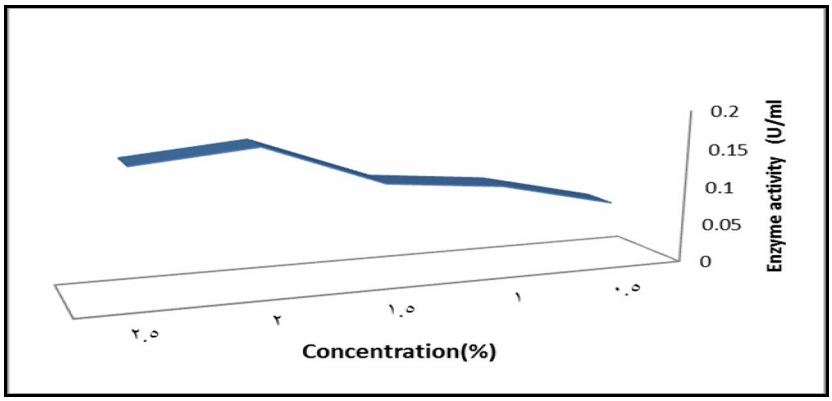

Figure. (5) Effect of carbon source (CMC%) on glucosidase production (U/ml) by *Bacillus* sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Effect of shaking (rpm) on glucosidase production

With operating temperature, pH and time course 35°C, 7 and 72 hrs, respectively, the effect of agitation speed was investigated by comparing the performance of the agitation rate at three agitation rates namely 0rpm (static), 100rpm and 200rpm. Results shows a remarkable increase in fermentation medium under shaking conditions compared to static. It was observed higher enzyme activity at 200 rpm with different carbon sources wheat bran, corn cob, corn stem and CMC, 0.1982, 0.2280, 0.4051 and 0.1947 IU/ml, respectively. Table (1 – 5).

Table. (1) Effect of shaking (rpm) using (Wheat bran%) on glucosidase production (U/ml) by Bacillus sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Conc.(%)	0 rpm	100 rpm	200 rpm
0.5	0.0667	0.0623	0.0675
1.0	0.1287	0.1806	0.0940
1.5	0.1129	0.0758	0.01409
2.0	0.1212	0.1739	0.1496
2.5	0.1860	0.1196	0.1982

Table. (2) Effect of shaking(rpm) using (Corn cob%) on glucosidase production (U/ml) by Bacillus sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Conc.(%)	0 rpm	100rpm	200 rpm
0.5	0.0562	0.0471	0.0591
1.0	0.0761	0.0739	0.0947
1.5	0.1258	0.1134	0.1485
2.0	0.1503	0.1449	0.1875
2.5	0.1994	0.2040	0.2280

Table. (3) Effect of shaking(rpm) using (Corn stem%) on glucosidase production (U/ml) by Bacillus sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Conc.(%)	0 rpm	100 rpm	200 rpm
0.5	0.0605	0.0706	0.0706
1.0	0.1289	0.1169	0.1399
1.5	0.1484	0.1434	0.2371
2.0	0.1953	0.1921	0.3218
2.5	0.2128	0.2202	0.4051

Table. (4) Effect of shaking(rpm) using (pineapple%) on glucosidase production (U/ml) by Bacillus sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Conc (%)	0 rpm	100 rpm	200 rpm
0.5	0.1976	0.1450	0.2474
1.0	0.4694	0.3991	0.4248
1.5	1.2404	0.6764	0.7583
2.0	1.4815	1.3056	0.8266
2.5	1.4919	1.3673	1.0816

Table. (5) Effect of shaking(rpm) using (CMC%) on glucosidase production (U/ml) by Bacillus sp. RL1, Temperature35°C, pH 7 and 72 hours incubation period.

Conc. (%)	0 rpm	100 rpm	200 rbm
0.5	0.0758	0.0467	0.0633
1.0	0.1086	0.0636	0.0968
1.5	0.1221	0.0617	0.0978
2.0	0.1770	0.0967	0.1099
2.5	0.1630	0.0849	0.1947

Discussion:

B-glucosidase production was found to be dependent upon nature of carbon source used in culture media. To maximize enzyme yield, the effect of different agricultural carbon sources were used and CMC medium at five concentrations. The results of production medium have been examined and presented in Figure.(1-5) pure substrates are too be expensive to be employed for industrial production of cellulases, so alternative substrates particularly crude raw materials of agricultural origin have been explored as cost effective substrates. CMC of production medium was replaced with alternative substrates such as pine apple, wheat bran, corn cob and corn stem. Many other workers have been used agricultural carbon source to produce cellulases, crude raw materials of agricultural origin like industrial rsidues from soy production, wheat bran, hay and corn cobs have been used for cost effective production of industrially important enzyme. (2,12,13,14,15). It was quite interesting to observe that enzyme activity was enhanced on all of these crude substrates at different concentrations compared to that on pure CMC.(14,15), the results in current study were in correlation with the finding of many other workers whom found that maximum enzyme was obtained with crude agricultural waste.

From the obtained data it is best supported the importance of shaking to facilitate maintenance of homogenous conditions especially with respect to temperature and other parameters (16,17). As outlined earilier shaking plays an important role in increase the amount of dissolved oxygen and dispersion of macromolecules in the medium. It might contribute to the greater growth and better enzyme production noted in current investigation. However the shearing effect induced by the higher shaking speed on the cell and enzyme activity may contribute negatively towards cell growth and enzyme stability (18). In conclusion, the present study revealed that Pine apple powder is one of the best agricultural substrate as sole carbon source for glucosidase production by *Bacillus* sp.RL1, the organism was readily utilized this substrate and current study confirmed that 2.5% (w/v) is optimum concentration at 35°C, pH 7 and 72hrs incubation period for higher enzyme activity.

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