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RESEARCH ARTICLE

A Novel Promising Thermotolerant Cellulase-Producing *Bacillus licheniformis* 1-1v Strain Suitable for Composting of Rice Straw

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Abstract

Thirty nine thermotolerant cellulose decomposing bacteria were isolated from different natural sources. Amongst those, isolate 1-1v exhibited the highest avicelase, carboxymethyl cellulase and filter paperase activities at 50ºC using rice straw as a sole carbon source. This isolate was identified as *Bacillus licheniformis* 1-1v based on morphological, physiological, biochemical characteristics using VITEK 2 system, and 16S rRNA sequence analysis. Factors affecting cellulase productivities and rice straw utilization were investigated. The optimum rice straw concentration, pH value and temperature for the highest cellulase production were 50 % (*w/v*), 6.0, and 50ºC, respectively. Interestingly, the isolate showed high activity at a wide range of temperatures and pH values. The effect of some compost additives on cellulase production were also investigated. These additives include rock phosphate, feldspar, dolomite, and natural powder of iron, zinc, manganese salts, gypsum, lime and some nitrogen sources (sodium nitrate, ammonium sulphate, ammonium nitrate and urea). Amongst all, feldspar has significantly enhanced cellulase production. The highest cellulase production was obtained by supplementation of 0.75 % (*w/v*) of feldspare at 131.1 U/g-dry rice straw. The optimal inoculum size for cellulase production were 1 % that exhibited cellulase production at 139.4 ±6.76 U/g-dry rice straw compared to 20.8 U/g-dry rice straw before optimization (~ 7 folds increase) These result indicated the potentiality of the selected strain for rapid biodegradation of rice straw and its possible application in enhancement of composting process.

INTRODUCTION

Rice is the third most important produced grain crop in the world next to wheat and corn. About 1-1.5 kg of straw is produced from the harvesting of 1 kg rice grain. It is estimated that about 650–975 million tons of rice straw produced per year globally **(Biond *et al*., 2010)**. A part of this is going as animal feed and rest as waste. Therefore, rice straw is one of the most abundant lignocellulosic waste materials in the world. The disposal of rice straw is a problematic due to the high bulk material, slow degradation rate, creation of environmental pollution problem, and disease distribution. For the sustainable development of human being, the effective utilization of these materials for production of bio-based products is necessary **(Abdel-Rahman *et al*., 2011).** Rice straw has high cellulose content (32–47%), hemicellulose (19–27%) and lignin (5–24%) that can be hydrolyzed into fermentable sugars **(Saha, 2003)**.

Rice straw compost is supplemented to fields at different countries to improve soil fertility and increase yield. The compost can serve as an excellent source of nutrients in organic farming to reduce the negative effect of increasing chemical fertilizer use. To make the rice straw composting process economically viable, lignocellulolytic microbes based biodegradation is necessary for solid state fermentation process. The *in situ* inoculation of effective cellulose decomposing microorganisms might improve the composting process in term of composting time and compost quality.

The main objective of the present study is to isolate and characterize potential thermotolerant cellulase-producing bacterial isolates for efficient utilization of rice straw to be applicable in composting process in a trial to shorten the composting time and/or increase the product quality.

2. MATERIAL AND METHODS

2.1 Isolation sources and media used

Different natural sources of soil, cattle manure, chicken manure, compost, paper residues, and decomposed plant were collected from different governorates in Egypt. These samples were used as isolation source of cellulase producing bacteria in the present study. A modified inorganic starch nitrate salts medium that composed of (g/L): cellulose substrate (Avicel pH 101 or carboxymethyl cellulose (CMC)), 20; $NaNO_3$, 2.0; K_2HPO_4 (anhydrous basis), 1.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.7H_2O$, 0.01; yeast extract 1.0 was used for bacterial cultivation and enzyme production. Medium pH was adjusted at 7.0 otherwise mentioned. The media were sterilized at 121°C for 20 min.

2.2 Isolation, purification and screening of cellulase producing bacteria

For bacterial isolation, one gram of each natural source was inoculated into Mineral salt medium supplemented with (2%) avicel or CMC as sole carbon source in 100 ml flasks containing 50 ml working volume. The flasks were incubated separately at 45 and 50°C for 72h. A loopful was streaked onto agar plates containing same medium. Agar streak plate method was used for purification of bacterial isolates under investigation.

For qualitative screening tests, solid agar plate media contained the main ingredients mineral salt medium supplemented with cellulose substrate (avicel or CMC) separately were prepared. Each plate was inoculated in the center with bacterial isolate, incubated at 50°C for 72h. After incubation, avicelase and CMCase activity visualized by flooding plates with Grams iodine solution. The appearance of clear zones around bacterial growth were investigated and taken as criteria for determining the exo- and endo-glucanase activities as shown in **Fig. 1a &1b.**

For quantitative screening tests, the amount of avicelase, CMCase, and filter paperase (FPase) were investigated in broth media rice straw (2%, *w/v*) investigated. Cellulase activities were measured by calculating the reducing sugar concentration using dinitrosalcylic acid (DNS) method **(Ghose, 1987)**. One unit of enzyme activity was defined as the amount of enzyme that released 1 µmol of glucose per minute. Avicelase and CMCase assay were measured as described by **Miller, (1959)**. Filter paper assay (FPU Assay) was measured as described by **Ghose, 1987**.

2.3 Identification of the most potent bacterial isolate

VITEK 2 test was performed in Theodor Bilharz Research Institute located in Giza, Egypt. Partial 16S ribosomal deoxyribonucleic acid (16S rDNA) region of the isolate, corresponding to positions 8– 1510 of *Escherichia coli* 16S rDNA, was analyzed using a set of universal primer 27^f and 1492^r. The sequences of the primers are as follows: 27^f, 5′-AGAGTTTGATCCTGGCTCAG-3′; and 1492^r, 5-GGTTACCTTGTTACGACTT -3′. Total genomic DNA was extracted from cells using protocol of Gene Jet genomic DNA purification Kit (Fermentas). Genomic DNA was used as a polymerase chain reaction (PCR) template. The sequencing for the PCR product was carried out in GATC Biotech Company by use ABI 3730xl DNA sequencer. Similarity search was performed in the GenBank database using the BLAST algorithm.

2.4 Factors controlling cellulase(s) activities by isolate 1-1v

2.4.1 Effect of rice straw concentrations

Mineral salt media supplemented with different concentrations of rice straw *viz.*, 20, 30, 40, and 50 % (*w/v*) were inoculated by at 10 % from preculture medium. The flasks were then incubated at 50°C for 72 hrs. At the end of incubation, 10 ml of distilled water was added to each flask and mixed well. Then, enzymes was extracted by filtration and filtrate was further centrifuged at 6000 rpm, 4°C for 10 min. The enzymatic activities were determined (IU/ml) as described before.

2.4.2 Effect of different pH values

Rice straw (50%, *w/v*) mineral medium was prepared and inoculate at 10 % from bacterial preculture in 100-ml flasks under solid state fermentation. Each flask contains 5 g of dried rice straw and 10 ml medium. The medium pH were adjusted at 5.0, 6.0, 7.0 and 8.0 with 1N HCL and 1N NaOH using pH meter. The flasks were incubated at 50°C. At the end of incubation, 10 ml of distilled water was added to each flask and mixed well. Then, enzymes was extracted by filtration and filtrate was further centrifuged at 6000 rpm, 4°C for 10 min. The enzymatic activities were determined (IU/ml).

2.4.3 Effect of different incubation temperatures

Rice straw (50%, *w/v*) mineral medium was prepared and inoculate at 10 % from preculture medium in 100-ml flasks under solid state fermentation. Each flask contains 5 g of dried rice straw and 10 ml medium. The flasks were incubated at various temperatures *Viz*, 30, 35, 40, 50, 55 and 60°C for 72 hrs. At the end of incubation, enzymes were extracted by filtration and assayed as described above.

2.4.4 Effect of compost additives on the activity of bacterial isolate and enzymes production

All compost additives were obtained from Al-Ahram mining company for organic fertilizers, Cairo, Egypt. The effect of natural powder of iron, zinc and manganese salts on the microbial growth and enzymatic activities were investigated using different concentrations *viz.*, 0, 0.025, 0.050 and 0.075%. Rock phosphate (18% P_2O_5), Feldspar (12% K_2O), and dolomite were used at 0, 0.5, 0.75 and 1% to the production medium. Gypsum or lime were applied at 0, 5, 7.5 and 10%. Nitrogen sources (sodium nitrate, ammonium sulphate, ammonium nitrate and urea) were incorporated individually into the production medium at the concentration of 1, 2 and 3% (*w/v)*. The exclusion of nitrogen sources was also investigated. All optimal condition were taken into consideration. At the end of incubation periods, cellulase(s) productivities were measured as previously mentioned.

2.4.5 Effect of different inoculum sizes

Different inocula sizes from pre-culture medium of strain 1-1v were applied *viz*. 1, 2, 5, 10, 20, 30, 40 and 50 % to the production medium. Each ml from precultue medium contains 6.8×10^7. All other optimal conditions were taken into consideration. At the end of incubation periods, the activity of cellulase enzyme were measured as previously mentioned.

3. RESULTS

3.1 Isolation and screening of bacteria

Thirty nine morphologically distinct isolates were obtained from natural sources collected from different localities in Egypt on avicel medium at 45 and 50°C. Single colonies from the obtained isolates were grown onto mineral salt agar plate medium containing avicel or CMC (2%, w/v) at 50°C for 72h. Appearance of clear zone around the bacterial growth using iodine soultion indicates cellulase activity. Amongst those isolates, 1-1V showed the highest avicelase and CMCase activities with 5.17 cm, and 4.75cm, respectively **(Fig. 1a &1b)**. Therefore isolate 1.1v was considered as the most potent isolates.

The quantitative analysis of cellulase(s) production for isolate 1-1v was also studied using mineral salt broth media supplemented with rice straw (2%) at initial pH 7.0, 50°C for 72h. Total cellulase (Filter paperase, FPase), exogluganse (avicelase), and

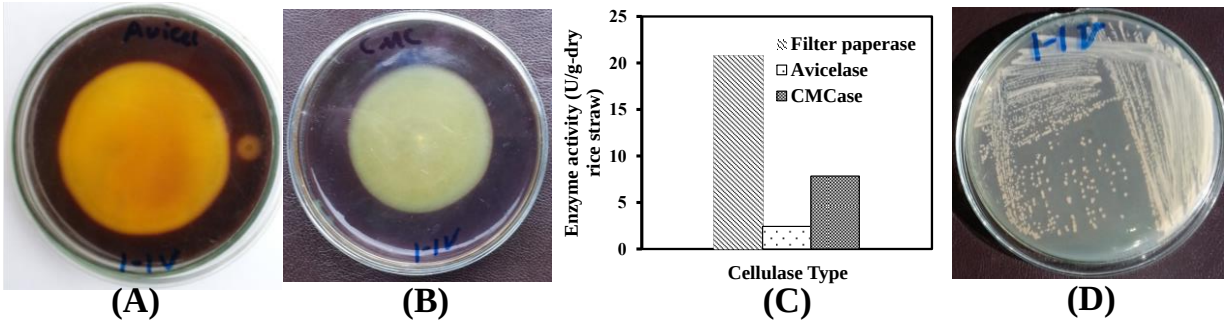

Fig. 1: (a) Avicelase, and (b) CMCase activity on agar plates. (C) Filter paperase, Avicelase, and CMCase production on mineral medium supplemented with rice straw (2%, *w/v*) by isolate 1-1V at 50 °C. (d) Culture characteristics for isolate 1-1V on agar plates.

endoglucanase (CMCase) activities were 20.8, 2.42 and 7.84 U/g-dry rice straw, respectively **(Fig.1c)**. Consequently, isolate 1-1v was chosen for additional experiments. The preliminary characterization of this isolate showed negative reaction with 3% KOH, Gram positive, catalase positive rod shapes (bacilli). The growth characteristics of this isolate on nutrient agar plates is shown in **Fig. (1d)**.

3.2 Identification of Isolates 1-1V using biochemical characteristics and 16S rRNA sequence

Isolate 1-1v was characterized by bacterial identification kit of VITEK 2 system using BCL colorimetric card, which tests some metabolic activities such as acidification, alkalinization, enzyme hydrolysis and growth in the presence of some inhibitory substances **(Table 1)**. Interestingly, the isolate showed β-glucosidase activities that can complete the hydrolysis of cellobiose in cellulosic materials. This indicate that the strain can produce all types of cellulases (endo-glucamnase, exo-glucanase, and β-glucosidase).

The 16S rDNA gene of the isolate 1-1v was amplified by polymerase chain reaction (PCR) using the universal primers. The forward and reverse primers used for PCR amplification were 27^{f} (5′-AGAGTTTGATCCTGGCTCAG -3′) and 1492^{r} (5′-GGTTACCTTGTTACGACTT-3′) (16S rDNA universal primer). The sequencing for the PCR product was analyzed in GATC Company by use ABI 3730xl DNA sequencer. A sequence (1222 bp) showed 97% identity to that of reference strain *Bacillus licheniformis* strain DSM 13 16S ribosomal RNA gene (accession number NR-118996.1) available in the NCBI (http://www.ncbi.nlm.nih.gov). The phylogenetic analysis of the isolates was shown in **Fig. (2)**. Accordingly, we concluded that the isolate 1-1v was identified as *Bacillus licheniformis* 1-1.

3.3 Factors controlling the production of cellulase(s)

3.3.1 Effect of rice straw concentrations, pH values and temperatures on cellulase (s) activities

To characterize cellulase production by isolate 1-1v under various conditions, the effects of rice straw concentration, pH and temperature were investigated. Four levels of initial rice straw concentrations (ca. 20-50 %, *w/v*) at 50°C and pH 7.0 were conducted **(Fig. 3a)**. Cellulase production was increased with an increase of rice straw concentration achieving the

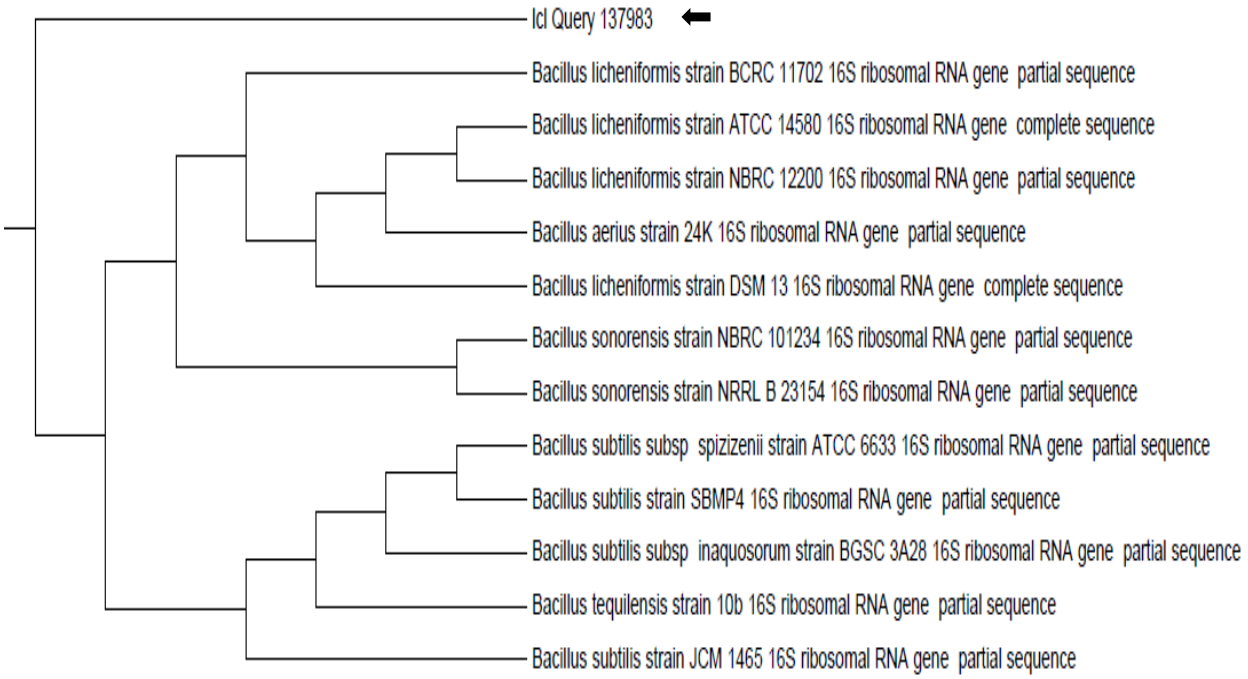

Fig. 2: Phylogenetic tree derived from 16S rRNA gene sequence of bacterial isolate 1-1v.

Table 1: Biochemical characteristics of isolate 1-1v using the Biomerieux VITEK 2 system on BCL card.

Test	Mnemonic	Strain 1-1v	Test	Mnemonic	Strain 1-1v
Beta-Xylosidase	BXYL	–	D-Mannitol	D MAN	+
L-LYSINE-ARYLAMIDASE	Lys.A	–	D-MANNOSE	D.MNE	–
L-ASPARTATE ARYLAMIDASE	AspA	–	D-MELEZITOSE	D.MLZ	+
Leucine Arylamidase	Leu.A	+	N-Acetyl-D-Glucosamine	NAG	+
Phenylalanine Arylamidase	Phe A	+	Palatinose	PLE	+
L-Proline- Arylamidase	Pro A	–	L-Rhaminose	IRHA	–
BATA-GALACTOSIDASE	BGAL	+	Beta-Glucosidase	BGLU	+
L- PYROLYDONYL-ARYLAMIDASE	Pyr.A	+	Beta-Mannosidase	BMAN	+
Alpha-Galactosidase	AGAL	–	Phosphoryle Choline	PHC	–
Alanine Arylamidase	Ala.A	(–)	Pyruvate	PVATE	–
Tyrosine Arylamidase	Tyr A	+	Alpha-Glucosidase	AGLU	+
Beta-N-Acetyl-Glucosaminidase	BNAG	–	D-Tagatose	D TAG	+
Ala-phe-Pro-ARYLAMIDASE	APPA	–	D-Trehalose	D TRE	+
Cyclodextrin	CDEX	+	Inulin	INU	–
D-GALACTOSE	D.GAL	–	D-GLUCOSE	D.GLU	+
Glycogen	GLYG	(–)	D-RIBOSE	D.RIB	–
myo-Inositole	INO	+	Putrescine assimilation	PSCNa	+
Methyl-α-D-Glucopyranoside acidification	MdG	+	Growth in 6.5% NaCl	NaCL 6.5%	+
Ellman	ELLM	+	Kanamycin Resistance	KAN	–
Methyl-D-Xyloside	MdX	–	Oleandomycin Resistance	OLD	+
Alpha-Manosidase	AMAN	–	Esculin hydrolysis	ESC	+
Maltotriose	MTE	+	Tetrazolium Red	TTZ	+
Glycine Arylamidase	Gly A	–	Polymixin-B Resistance	POLYB R	+

highest cellulase production at 50% (*w/v*) with production of 41.3 U/g-dry rice straw.

In order to study the effect of pH values on cellulase production, fermentations were carried out under different intial pH at 5.0, 6.0, 7.0, or 8.0 to verify the possibility of increasing enzyme production at specific value **(Fig. 3b)**. *Bacillus licheniformis* 1-1v showed comparable cellulase production at all tested pH values. The optimum pH was in slightly acidic range of 6.0 at 43.50 U/g-dry rice straw. Interestingly, even at alkaline pH values (pH, 8.0) the isolate produced a considerable amount of cellulase at 41.4 U/g-dry rice straw.

To investigate the effect of incubation temperature on cellulase production, enzyme production was carried out at different temperatures. As shown in **Fig. 3c**, isolate 1-1v showed almost comparable cellulase production at wide range of temperatures (30–60ºC) with 43.1–53.1 U/g-dry rice straw. The optimal temperature was 50ºC with cellulase production of 53.1 U/g-dry rice straw.

3.3.2 Effect of compost additives on cellulase production by isolate 1-1V

3.3.2.1 Effect of natural powder of magnetite (iron), zinc powder, or manganese salt on cellulase production

In this experiment, different concentrations of magnetite (iron), zinc powder, or manganese salt were applied at 0.025, 0.050 and 0.075 % (*w/v*) to the production medium. Results recorded in **Fig. (4a)** proved that, the production of cellulase by *Bacillus licheniformis* 1-1v was gradually reduced by increasing the concentrations of Fe, Zn, or Mg as compared to control. The highest cellulase reduction was obtained by Mn where it reached down to 16.3–21.3 U/g-dry rice straw compared to control (63.2 U/g-dry rice straw). Cellulase production ranged (16.5–41.6 U/g-dry rice straw) by application of magnetite at 0.025–0.075 %. The application of Zn powdered induced slight cellulase reduction where the production was ranged 44.1–50.3 U/g-dry rice straw compared to 63.2 U/g-dry rice straw produced without Zn addition.

3.3.2.2 Effect of gypsum and lime on cellulase production

The effect of gypsum and lime on cellulase production medium was recorded in **Fig. (4b).** Different concentration were applied *viz*., 5.0, 7.5, and 10% (w/v) of the production medium. Addition of gypsum did not apparently exert a significant effect on cellulase production. Cellulase production was ranged 52.4–60.9 U/g-dry rice straw compared to 63.2 U/g-dry rice straw. On the other hand, addition of lime decreased cellulase production. Addition of 5-10 % of lime leaded to higher than 50 % cellulase reduction by *Bacillus licheniformis* 1-1v that ranged (23.9–25.6 U/g-dry rice straw) compared to 63.2 U/g-dry rice straw.

3.3.2.3 Effect of rock phosphate, feldspar, or dolomite concentrations

In order to give a more clear characterization of the isolate regarding the enzyme production using compost additives, we applied different concentrations of rock phosphate, feldspar, or dolomite separately at 0, 0.5, 0.75 and 1% (*w/v*) to the production medium as shown in **Fig. (4c)**. Supplementation of rock phosphate (0.5-1%) to a medium inoculated with *Bacillus licheniformis* 1-1v resulted in a significant decrease (almost 50%) in cellulase production that ranged 28.5–34.1 U/g-dry rice straw compared to control experiment (63.2 U/g-dry rice straw). Similarly, supplementation of dolomite (a natural sources for magnesium) to the production medium have significantly reduced cellulase production. On the other hand, addition of feldspar (a natural source for potassium) has significantly enhanced cellulase production. The highest cellulase production was

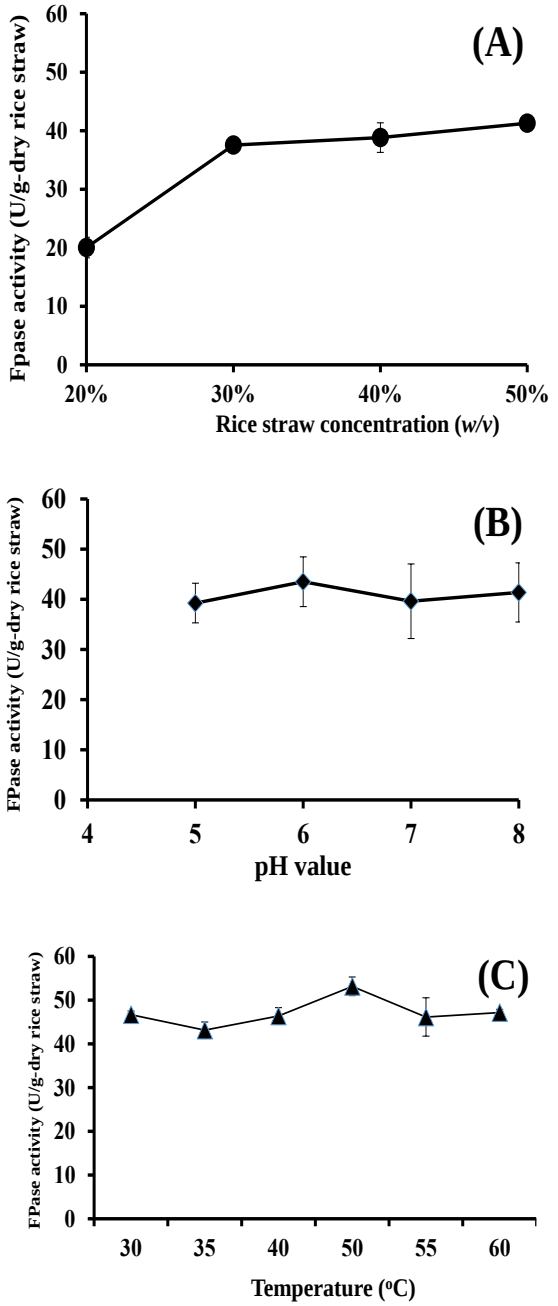

Fig. 3: Effect of (A) Rice straw concentration, (B) pH value, and (C) temperatures on cellulase production by *Bacillus licheniformis* 1-1v.

obtained by supplementation of 0.75 % (*w/v*) of feldspar at 131.1 U/g-dry rice straw.

3.3.2.4 Effect of nitrogen sources on cellulase production

To study the effect of organic or inorganic nitrogen supplementations, different concentrations of ammonium sulphate, ammonium nitrate, or urea were applied at 1, 2 and 3% to the production medium. Production medium without any nitrogen source are also investigated and compared to the original media (Mineral medium that contained 2 g/L of $NaNO_3$, and 1 g/L yeast extract as sources of nitrogen) as a control.

Data recorded in **Table (2)** showed that supplementation of nitrogen sources decreased cellulase production as compared to control experiment or without N- supplementations. Cellulases production were ranged 14.5–19.8, 10.5–12.4, and 23.0–25.2 U/g-dry rice straw compared to control (63.2 U/g-dry rice straw) or without N-supplementation (62.4 U/g-dry rice straw) by addition ammonium sulphate, ammonium nitrate, or urea at (1-3% w/v), respectively.

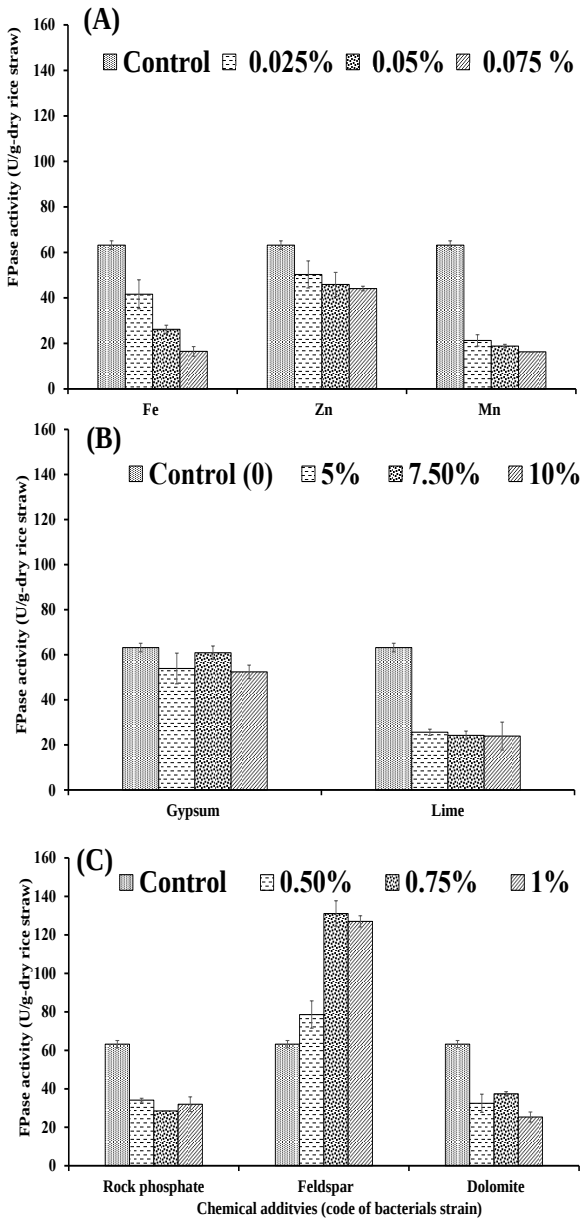

Fig. 4: Effect of some compost additives on cellulase(s) activities by *Bacillus licheniformis* 1-1v (a) Effect of magnetite, Zn powder and, manganesium, (b) Effect gypsum and lime and (c) Effect of rock phosphate, feldspar and, dolomite.

3.3.3 Effect of different inoculum sizes on cellulase production

In order to approach the best inoculum size for cellulase production, different inocula were applied viz, 1, 2, 5, 10, 20, 30, 40 and 50 % (v/v) of the production medium. The study was carried out with keeping all other conditions at their optimum levels (temp, pH, rice straw) and without supplementation of nitrogen sources. Data were recorded in **Fig. (5).** Interestingly, the optimal inoculum size needed to produce the maximum yield of cellulase production were 1 % for *Bacillus licheniformis* 1-1v. At this optimal inoculums size the highest yield were achieved at 139.4 ±6.76 U/g-dry rice straw. Higher inocula sized leaded to reduced cellulase production.

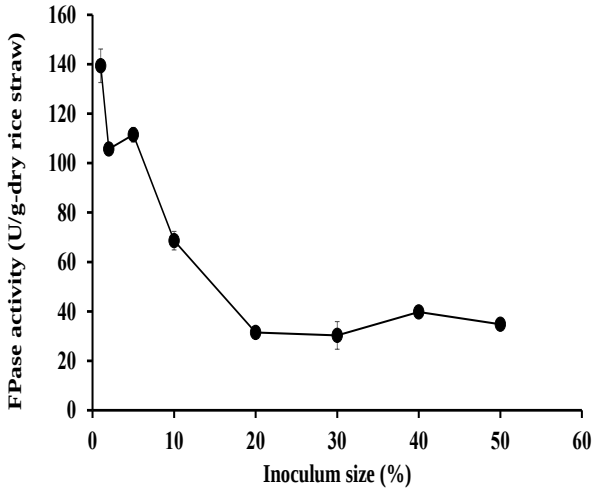

Fig. 5: Effect of inoculum size on cellulase activity using *Bacillus licheniformis* 1-1v.

Table (2): Effect of different nitrogen sources on cellulase production by *Bacillus licheniformis* 1-1v

Nitrogen source (%)	NH_4SO_4	NH_4NO_3	Urea
Control	63.2 ± 1.88	63.2 ± 1.88	63.2 ± 1.88
Without N (0 %)	62.4 ± 2.52	62.4 ± 2.52	62.4 ± 2.52
1 %	19.8 ± 4.38	10.5 ± 0.40	25.2 ± 0.06
2 %	14.5 ± 3.00	12.4 ± 0.43	23.3 ± 3.38
3 %	15.8 ± 1.00	11.9 ± 0.43	23.0 ± 2.13

DISCUSSION

In the present study, an attempt was made to isolate an efficient thermophtolerant cellulase-producing bacterial strain and to investigate its capability for utilization of rice straw under solid state fermentation condition for composting process. For this regard, isolation and screening of microorganisms from natural sources was performed using avicel or CMC containing media as a sole carbon source. Cellulase systems consist of endoglucanases, exoglucanase, and β-glucosidase and the synergistic effect of these enzymes complete the hydrolysis of cellulosic materials to simple sugars **(Lynd *et al*., 2002)**. Avicell (microcrystalline cellulose) and CMC (amorphous cellulose) are generally used as a substrate for the study of exoglucanases and endoglucanases, respectively **(Rastogi *et al*., 2010)**. Interestingly, various selected isolates exhibited both exo-, and endo-glucanase production on 2 % (*w/v*) avicell or CMC at 45 ºC and 50ºC indicating their potential for cellulose decomposition.

Since one purpose of this study is to efficiently utilize agricultural wastes for application in composting process, cellulase production was investigated under solid state fermentation using mineral medium containing 20 % (*w/v*) dried rice straw as substrate at 50ºC by the most potent isolates. The quantitative analysis of filter paperase (total cellulases), avicelase and CMCase production revealed the ability of selected isolates to utilize rice straw with the production of varied concentrations of exo- and endo-glucanase. Based on the cellulase concentrations, isolate 1-1v that obtained from cow manure was considered as the most potent amongst all bacterial isolates obtained in this study. FPase, avicelase and CMCase were 20.8, 2.42, 7.84 U/g-dry rice straw, respectively.

The selected isolate was characterized by bacterial identification kit of VITEK 2 system using BCL colorimetric card and 16S rDNA sequancing. The isolate showed the highest identity of 97% to that of reference strain *Bacillus licheniformis* strain DSM 13 16S ribosomal RNA gene (accession number NR-118996.1). Accordingly, the isolate was identified as *Bacillus licheniformis* 1-1v.

We have investigated the characteristics of the isolated strain at various environmental and chemical parameters in order to determine the best factors controlling the growth and enzymatic productivities. Because rice straw is one of the main agricultural wastes in Egypt, we therefore used rice straw as the main carbon source in this study. The enzyme activity increased with the increase of rice straw concentrations up to a value of 50% for *Bacillus licheniformis* 1-1v that gave almost double-fold production (41.3 U/g-dry rice straw) compared to fermentations at 20% (20.8 U/g-dry rice straw). We could not use higher than this concentration due to the decreased moisture content and inhomogeneity of fermentation medium.

Agricultural wastes have been also employed as substrates for the production of cellulases. **Gupta *et al.*, (2015)** reported that *Bacillus licheniformis* K-3 had efficiently utilized several agricultural residues including 1% rice straw, wheat bran, wheat straw, corn waste, soybean meal, almond hulls, and mustard cake as carbon source substrates for cellulase production. Also, *Bacillus* sp. NZ utilized a variety of agro-wastes as carbon sources while produced considerable enzyme endoglucanases productivity using wheat bran, wheat straw, filter paper, and sawdust **(Nizamudeen and Bajaj, 2009)**. **Goyal *et al*., (2014)** achieved the maximum carboxymethyl cellulase activity of 3.08 U/mL by *Bacillus sp.* 313SI using 1% (*w/v*) pretreated rice straw.

In this study, the optimum pH value for cellulase production was 6.0. Interestingly, the enzyme production didn't significantly affected within the tested pH range (5.0-8.0). Since the pH value might be varied during composting process, these results indicate the potentiality of the isolated strain for rice

straw degradation even under the varied pH value during composting as a result of microbial fermentation products. Our results were related to that by **Karim *et al.*, (2015)** who reported maximum enzyme production by *Bacillus licheniformis KIBGE-IB2* at pH 6.0 when growing in submerged fermentation medium.

Temperature is an important factor that influences the enzyme production and activity **(Immanuel *et al.*, 2006).** In the present work the optimum incubation temperature for maximum cellulase activity by *Bacillus licheniformis* 1-1v was 50ºC. In addition, even at lower or higher temperatures, the strain exhibited considerable cellulase production and consequently rice straw decomposition. These data support the utilization of this strain for composting industry due to the expected high activity of the strain at different stages of composting process. These results were more related to several authors. **Acharya and Chaudhary, (2012)** reported that 50-55ºC was the optimal temperature for cellulase production by *Bacillus licheniformis* MVS1 *and Bacillus sp*. **Annamalai *et al.*, (2014)** have reported that 50ºC was the optimal temperature for cellulase production by *Bacillus carboniphilus* CAS 3.

In order to enhance composting, several additives might be applied during compost pile preparation. Therefore, the effectiveness of several commercial natural additives (including rock phosphate, feldspar, dolomite, iron, manganese, zinc, gypsum and lime) on cellulases productivities by *Bacillus licheniformis* 1-1v were investigated. The concentration of these additives were adjusted to be in the recommended ranges in composting processes.

Amongst those, feldspar (a natural source for potassium at 0.75 %, *w/v*) has significantly enhanced cellulase production where the productivity was increased up to 131.1 (~2-fold) U/g-dry rice straw compared to 63.2 U/g-dry rice straw. Other chemical additives have adverse effect on cellulase activity. **Hubbert *et al.*, (1958)** found that the potassium of fermentation medium is essential for *in vitro* cellulose digestion, although sodium is not essential. The result also agree with **Mohee, (2007),** who reported that the addition of dolomite to cellulase fermentation medium didn't not improve cellulase productivity due to the increase organic carbon. Dolomite also results in rise in pH value as it contain magnesium and calcium and they act as a base **(Vogtmann *et al*., 1993)**.

In the present study, addition of gypsum have no effect on cellulase productivity while addition of lime resulted in a decrease of cellulase production. This might be the change of pH vales that affect microbial growth and cellulase activity **(Bajwa and Josan, 1989; Gabhane *et al.*, 2012).**

The inhibition of cellulase activities by the most of natural supplemented chemicals in this study might be attributed to the presence of microbial inhibitors as a part in their composition. From the above results, supplementation of feldspar is highly recommended during composting process using our strain due to the great enhancement of enzymatic production and substrate degradation.

The effect of organic or inorganic nitrogen supplementations (ammonium sulphate, ammonium nitrate, or urea) on the cellulase productivity revealed a decreased cellulase production compared to control experiment or without N–supplementations. It is noteworthy to mention that control medium contains yeast extract as a nitrogen source. In addition, rice straw contains about 0.5–0.8 (%, dry matter) of nitrogen **(Dobermann and Fairhurst, 2002). Dobermann and Fairhurst, (2002)** reported that, about 40 percent of the nitrogen, 30–35 % of the phosphorus, 80–85 percent of the potassium, and 40–50 percent of the sulfur taken up by rice remains in vegetative plant parts at crop maturity. This might explain that the endogenous nitrogen content of rice straw without additional nitrogen supplementation is appropriate for *Bacillus* growth and cellulase production.

Inoculum size also affects the maximum cellulase enzyme production. The optimum inoculum size was determined by assaying the enzyme activity were 1 % that achieved cellulase productivity at 139.4 U/g-dry rice straw that was about ~7 fold increase compared to data before conducting optimization experiments (20.8 U/g-dry rice straw). Higher inocula sizes exhibited lower cellulase production. Inoculum size (2–3%) was found to be optimum for cellulases produced by *B. subtilis* CY5 and *B. circulans* TP_3 in solid state fermentation **(Ray *et al.*, 2007).**

In conclusion, the above results indicate the potentiality and suitability of *Bacillus licheniformis* 1-1v for application in composting industry based on its brilliant characteristics in term of its high cellulytic activities at a wide range of pH and temperatures, without additional nitrogen source requirements, and its high activity at low inoculum size in the presence of feldspare. These data also indicate that this isolate might achieve high degradation level of cellulose component of rice straw, reduce composting time, and enhance the quality of the compost. Further studies on the applications of this strain in composting process are under progress **(Abdel-Rahman *et al*., 2016).**

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GRAPHICAL ABSTRACT:

HIGHLIGHTS:

- Thirty nine thermotolerant cellulase producing bacteria were isolated.
- Isolate 1-1v showed the highest activities at 50ºC using rice straw as a sole carbon source.
- It was identified as *Bacillus licheniformis* 1-1v using VITEK 2 system, and 16S rRNA sequence analysis.
- 1-1v showed high cellulase activity at a wide range of temperatures and pH values.
- The optimal conditions for cellulase production were 50 % (*w/v*) rice straw concentration, pH 6.0, and 50ºC.
- Feldspar (one of compost additives) has significantly enhanced cellulase production.
- The highest cellulase production obtained after optimization was 139.4 ±6.76 U/g-dry rice straw that is ~7 fold higher than that obtained before optimization.

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