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Effects of Environmental Factors and Compost Additives on *Bacillus sonorensis* 7-1v, a Cellulytic Strain Able to Degrade Rice Straw under Solid State Fermentation

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Abstract

In the present study cellulolytic bacteria were isolated from different sources for their abilities to degrade rice straw. Out of 49 isolates, isolate 7-1v exhibited maximum filter paperase, avicelase and CMCase activity of 26.9, 2.32 and 7.6 U/g-dry rice straw, respectively, therefore, it was selected for further studies. This isolate is Gram-positive, spore forming bacterium with rod-shaped cells. The isolate was identified as *Bacillus sonorensis* 7-1v based on biochemical test using VITEK 2 and 16S rRNA sequencing analysis. Rice straw degradation and cellulase production were investigated using different rice straw concentrations, pH values, and temperatures. Optimal conditions were found to be 50%, 5.0 and 55^{o}C respectively. Also, the effect of some compost additives on the activity of bacterial isolate and enzymes production was studied using eight commercial natural compost additives; rock phosphate, feldspar, dolomite, iron, manganese, zinc, gypsum and lime. Amongst those, feldspar has significantly enhanced cellulase production up to 117.4 (~3-fold) U/g-dry rice straw. Other chemical additives have slight increase or adverse effect on cellulase activity. The addition of organic or inorganic nitrogen to the rice straw mineral medium exhibited decreased cellulase production. Optimal inoculum size for enzyme production was 2 % (*v/v*) that achieved the highest production at 152.0 ±6.64 U/g-dry rice straw. Mixed-culture of two cellulytic isolates exhibited a compatible bacterial consortium for efficient degradation rice straw. These data suggest the availability of selected strain(s) for the composting process.

Keywords: *Bacillus sonorensis* 7-1v, rice straw degradation. cellulase production, solid state fermentation, compost additives, mixed culture.

Introduction

Lignocellulosic biomass is the most plentiful non-food material and one of the most inexhaustible renewable resources on the planet. Lignocelluloses are composed of cellulose, hemicellulose and lignin, in which the cellulose account for a large portion of this biomass **(Abdel-Rahman *et al*., 2011)**. Amongst those biomass, rice straw is one of the most abundant lignocellulosic crop residues in the world. Its annual production is about 731 million tons which is distributed in Africa, Asia, Europe and America **(Narra *et al*., 2012).** Rice straw is generally composed of 30–56% cellulose, 10–27% or more hemicelluloses, 3.0–30% lignin and 3.6–7.2% protein (**Sherief *et al*., 2010)**.

Cellulases, that catalyze the degradation cellulose, are multi-enzymatic complex proteins and require the synergistic action of three key enzymes; exoglucanase (EXG; EC 3.2.1.91), endoglucanase (EG; EC 3.2.1.4), and β-glucosidase (BG; EC 3.2.1.21) for the decomposition of cellulose to glucose **(Li *et al*., 2006)**. For many years, cellulose degrading bacteria

have been isolated and characterized from variety of natural sources including soil, organic matters, decayed plant materials, hot springs, feces of ruminants and composts **(Irfan *et al.*, 2012).** Cellulolytic microorganisms play an important role in natural biodegradation of agricultural wastes and can be applied in many beneficial applications.

Solid state fermentation, which holds microbial growth on moist solid substrates in the absence of free flowing water, has gained considerable attention due to the utilization of huge amounts of solid materials. The abundance of rice straw as an organic waste can be converted to fertilizer throughout the process of composting. Microorganisms use these materials and finally produce simple and useful compounds which are important for plant growth, and soil quality. Decomposition of organic matter to applicable compost depends of the ability of microflora to produce and excrete specific digestive enzymes**.** Bacteria constitute the largest number of microorganisms in the composting process than other microorganisms **(Khalil *et al*., 2001)**. **Strom, (1985)** reports that about 87% of the randomly selected colonies during the thermophilic phase of composting belong to the genus *Bacillus.*

Recently, much attention is given to the composting of organic materials into a useful product **(Giglotti *et al.*, 2005).** Because the conventional method of composting takes a long time to produce good compost, researchers suggested that additives are necessary for the rapid production of compost. This including microbial enhancement additive to catalyze microbial and enzymatic activities or inoculation of microbial additives such as cellulose decomposers (**Gaur *et al*., 1982).**

The objective of this study was to isolate and characterize cellulolytic bacterial isolate which capable of decomposing rice straw under solid state fermentation process for application in composting technology. Some nutritional and environmental condition controlling cellulase production and rice straw degradation were investigated to characterize the bacterial isolate. Also, studying the effect of some compost additives on the activity of bacterial isolate and enzyme production.

Materials and Methods

Isolation Sources

Thirty natural sources (including decomposed plant, cattle manure, chicken manure, compost, paper industry residues, and soil) were collected from different localities in Gharbia, Matrouh, Damietta, and Kafr El-Sheikh governorates, Egypt.

Isolation and Primary Screening of Potential Isolates

Cellulytic agar plates containing (g/L): $NaNO_3$, 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; agar 15.0 and cellulose substrate, 20 were used for isolation of bacterial isolates. Carboxymethyl cellulose (CMC) [Sigma Aldrich] or crystalline cellulose (avicel pH 101) [Alpha chemika] was used as sole carbon source. The pH value was adjusted at 7.0. The media were sterilized at 121°C for 20 min. Serially diluted isolation sources were spreaded onto agar plates and incubated for 72h at 50°C or 45°C. The obtained isolates were purified using same medium until single colonies were obtained.

To indicate the cellulase activity of isolates, diameters of clear zones around each colonies on avicelase and CMCase activity visualized by flooding the avicel- and CMC-agar plates with Gram's iodine solution. The appearance of clear zones around bacterial growth were investigated and taken as criteria for determining the exo- or endo- glucanase activities.

Quantitative Screening Tests

The most potent isolates selected from the previous test were assayed for the amount of avicelase, CMCase, and filter paperase (FPase) in rice straw broth media. Single colonies of the most potent isolates were inoculated into a preculture that consist of mineral salt medium containing 1% CMC and incubated for 24 h, before inoculation at 10% into the main fermentation media. Main fermentation broth medium was consisted of mineral salt supplemented with rice straw (2%, *w/v*) as a sole carbon source. pH was adjusted at 7.0. Sterilization was carried out by autoclaving for 20 min at 121°C. Each flask was inoculated with different microbial isolate and incubated at 50°C for 72h then avicelase, CMCase, and filter paperase activities were investigated. Cellulase activities were measured by calculating the reducing sugar concentration using dinitrosalcylic acid (DNS) method according to **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).**

Characterization and Identification of Isolate 7-1V

The bacterial isolates were identified by morphological, physiological and biochemical identification using a VITEK 2 compact system for bacterial identification and identified phylogenetically by 16S ribosomal RNA (16S rRNA) sequence analysis.

The morphological characteristics including shape, arrangement and Gram reaction of the purified bacterial isolate were demonstrated using Gram's stain method**.** Spore formation was investigated as described by **Schaeffer and Fulton's (1933).** Potassium hydroxide test was done as described by **Shushan *et al.*, (1981).** Catalase activity using 3% hydrogen peroxide as described by **(Benson, 2001).**

Environmental and Nutritional Factors Controlling Solid State Fermentation by Strain 7-1v.

Effect of Different Concentrations of Rice Straw

Different concentrations of rice straw *viz.*, 20, 30, 40, and 50 % (*w/v*) were supplemented into mineral salt media in 100 ml flasks. Media were inoculated by bacterial strain under investigation at 10 %. The flasks were then incubated at 50^{o}C for 72 hrs. At the end of incubation, celluloses were extracted by filtration after addition of 10 ml of distilled water. Filter paperase activities were determined (IU/g-dry rice straw) as described previously.

Effect of Different pH Values

Mineral medium contacting rice straw (50%, *w/v*) was prepared and inoculated at 10 % bacterial preculture in 100-ml flasks under solid state fermentation. Each flask contains 5 g of dried rice straw and 10 ml medium. The initial pH values 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^{o}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^{o}C for 10 min. The enzymatic activities were determined (IU/g-dry rice straw).

Effect of Different Incubation Temperatures

Rice straw (50%, *w/v*) mineral medium was prepared and inoculate at 10 % preculture in 100-ml flasks under solid state fermentation as described before. The flasks were incubated at different temperatures *viz*, 30, 35, 40, 50, 55 and 60°C for 72 hrs. Cellulases were then extracted and assayed as described before.

Effect of Compost Additives on cellulase(s) Production and Activity of isolate 7-1v

Influence of Rock Phosphate, Feldspar, and Dolomite

Rock phosphate (18% P_2O_5), Feldspar (12% K_2O), and dolomite were provided by Al-Ahram mining company for organic fertilizers, Cairo, Egypt. Different concentrations of these additives *viz.*, 0, 0.5, 0.75 and 1% were applied to the production medium. All optimal conditions was taken into consideration. At the end of incubation periods, cellulase activities were measured as previously mentioned.

Influence of Natural Powder of Iron, Zinc and Manganese Salt

Different concentrations of natural iron powder of, zinc and manganese salts *viz.*, 0, 0.025, 0.050 and 0.075% were supplemented to the production media. All optimal conditions were taken into consideration. At the end of incubation periods, cellulase(s) production were measured as previously mentioned.

Influence of Gypsum and Lime

Different concentrations of gypsum or lime at 0, 5, 7.5 and 10% were added to the fermentation media. The optimal conditions for fermentation were taken into consideration. Flasks were inoculated at 10 % and incubated for 72h, then enzymatic activities were measured.

Influence of Nitrogen Sources

Different nitrogen sources (sodium nitrate, ammonium sulphate, ammonium nitrate and urea) were investigated. Nitrogen sources were incorporated individually into the production medium at the concentration of 1, 2 and 3% (*w/v).* All previous optimal condition were taken into consideration. At the end of incubation periods, enzymatic activities were measured as previously mentioned.

Effect of Different Inocula Sizes

Different inocula sizes from pre-culture medium of *Bacillus sonorensis* 7-1v were applied *viz*. 1, 2, 5, 10, 20, 30, 40 and 50 % (*v/v*) to the production medium.

Each ml of the inoculation medium contain 36×10^6 CFU. All other optimal conditions were taken into consideration. At the end of incubation periods, the activity of cellulase enzyme were measured as previously mentioned.

To study the effect of mixed culture, bacterial precultures were firstly prepared by mixing *Bacillus licheniformis* 1-1v and *Bacillus sonorensis* 7-1v at 1:1 and 1:2, respectively before inoculation to the main production medium. Using these mixtures, different inocula sizes were inoculated at the main production media, viz. 1, 2, 5, 10 and 20 % (*v/v*).

Results

Isolation and Screening of Cellulase Producing Bacteria

Forty nine morphologically distinct isolates were obtained and purified. Of these, 38 isolates were obtained from avicel medium (Exoglucanase producers) and 11 isolates were obtained on carboxymenthy cellulose (Endoglucanase producers). The highest cellulase-(either exo- or endo cellulase) producing isolates (13 isolate) were tested against both substrates (2% avicell and 2% CMC) to evaluate their abilities for different cellulases production at 50°C. Isolate 7.1v exhibited the highest cellulases activities by showing the biggest clear zone using both substrates at 4.97 cm for avicelase and 4.6 cm for CMCase as shown in **Fig. 1.** Therefore isolate 7.1v was considered as the most potent isolate.

Further investigation was carried out by the most potent 13 isolates using mineral salt broth media supplemented with rice straw (2%) at initial pH 7.0, 50°C for 72h for analysis of cellulase(s) production. Exogluganse (avicelase), endoglucanase (CMCase), and total cellulase (Filter paperase, FPase) activities [IU/g-dry rice straw] were measured at the end of incubation period as shown in **Table 1.** All isolates showed capability of rice straw decomposition. However, isolate 7-1 v exhibited the highest productivity among other isolates for cellulase production of FPase, avicelase and CMCase at 26.9, 2.32, and 7.6 U/g-dry rice straw, respectively. Therefore it was chosen for further experiments.

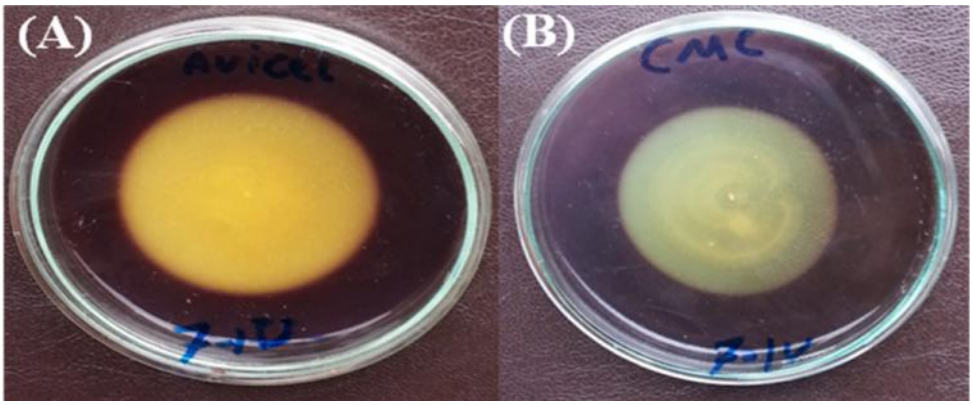

Fig. 1: (A) Avicelase and (B) CMCase activity on avicell and CMC-agar plates by isolate 7-1V. The clear zones around the isolate growth indicate the enzyme activities.

Table (1): Filter paperase, avicelase, and CMCase production from mineral medium supplemented with rice straw (2%, *w/v*) by the best 13 bacterial isolates at 50°C for 72h.

Isolate code	Cellulase activity (U/g-dry rice straw)		
	Filter paperase activity	Avicelase	CMCase
1-2v	17.8	2.20	7.00
7-1v	26.9	2.32	7.60
2-3c	9.56	2.16	7.02
6-1c	15.9	2.28	6.88
2-1v	20.1	2.22	7.80
6-2c	20.2	2.06	6.20
4-3v	15.0	2.24	6.36
4-3c	18.3	2.24	6.92
1-1c	10.9	1.98	6.76
6-1v	12.3	2.22	6.36
18b	17.8	2.22	7.26
11b	9.56	2.18	7.26
15b	9.56	2.16	5.12

Identification of the most potent bacterial isolate 7-1V:

Isolate 7-1v is Gram-positive and spore forming bacterium with rod-shaped cells, catalase positive and showed negative potassium hydroxide test. The isolate was characterized by bacterial identification kit of VITEK 2 system using BCL colorimetric card (**Table 2).** Interestingly, this isolate showed β-glucosidase activity that can complete the hydrolysis of cellobiose in cellulosic materials. Moreover, it showed β-xylosidase activities which indicate the ability to degrade xylan derived sugars of lignocellulosic part.

Table (2): Biochemical characteristics of isolate 7-1V using Biomerieux VITEK 2 system on BCL card.

	Test	Mnemonic	Strain 7-1v		Test	Mnemonic	Strain 7-1v
o	Beta-Xylosidase	BXYL	+	o	D-Mannitol	D MAN	+
o	L-Lysine Arylamidase	Lys.A	-	o	D-Mannose	D.MNE	+
o	L- Asparate Arylamidase	AspA	-	o	D-Melezitose	D.MLZ	-
o	Leucine Arylamidase	Leu.A	+	o	N-Acetyl-D-Glucosamine	NAG	-
o	Phenylalanine Arylamidase	Phe A	+	o	Palatinose	PLE	+
o	L-Proline-Arylamidase	Pro A	-	o	L-Rhaminose	IRHA	(-)
o	Bata-Galactosidase	BGAL	+	o	Beta- glucosidase	BGLU	+
o	L- Arylamidase	Pyr.A	+	o	Beta-Mannosidase	BMAN	+
o	Alpha-Galactosidase	AGAL	+	o	Phosphoryle Choline	PHC	-
o	Alanine Arvlamidase	Ala.A	+	o	Pvruvate	PVATE	+
o	Tyrosine Arylamidase	Tyr A	+	o	Alpha-Glucosidase	AGLU	+
o	Beta-N-Acetyle-Glucosaminidase	BNAG	-	o	D-Tagatose	D TAG	+
o	Ala-phe-Pro-Arylamidase	APPA	-	o	D-Trehalose	D TRE	+
o	Cyclodextrin	CDEX	+	o	Inulin	INU	+
o	D-Galactose	D.GAL	(-)	o	D-Glucose	D.GLU	+
o	Glycogen	GLYG	+	o	D-Ribose	D.RIB	+
o	myo-Inositole	INO	+	o	Putrescine Assimilation	PSCNa	-
o	Methyl-α-D-Glucopyranoside acidification	MdG	+	o	Growth in 6.5% NaCl	NaCl 6.5%	+
o	Ellman	ELLM	+	o	Kanamycin Resistance	KAN	+
o	Methyl-D-Xyloside	MdX	-	o	Oleandomycin Resistance	OLD	+
o	Alpha-Manosidase	AMAN	-	o	Esculin hydrolysis	ESC	-
o	Maltotriose	MTE	+	o	Tetrazolium Red	TTZ	+
o	Glycine Arylamidase	Gly A	-	o	Polymixin-B Resistance	POLYB R	+

The16S rRNA gene sequence and phylogenetic tree analysis were confirmed about the 98% identity of the strain as *Bacillus sonorensis* strain NBRC 101234 (accession number NR-113993.1) available in the NCBI (http://www.ncbi.nlm.nih.gov). The phylogenetic tree of isolate 7-1v is shown in **Fig.2.** Accordingly, we concluded that the isolate 7-1v was identified as *Bacillus sonorensis* 7-1v.

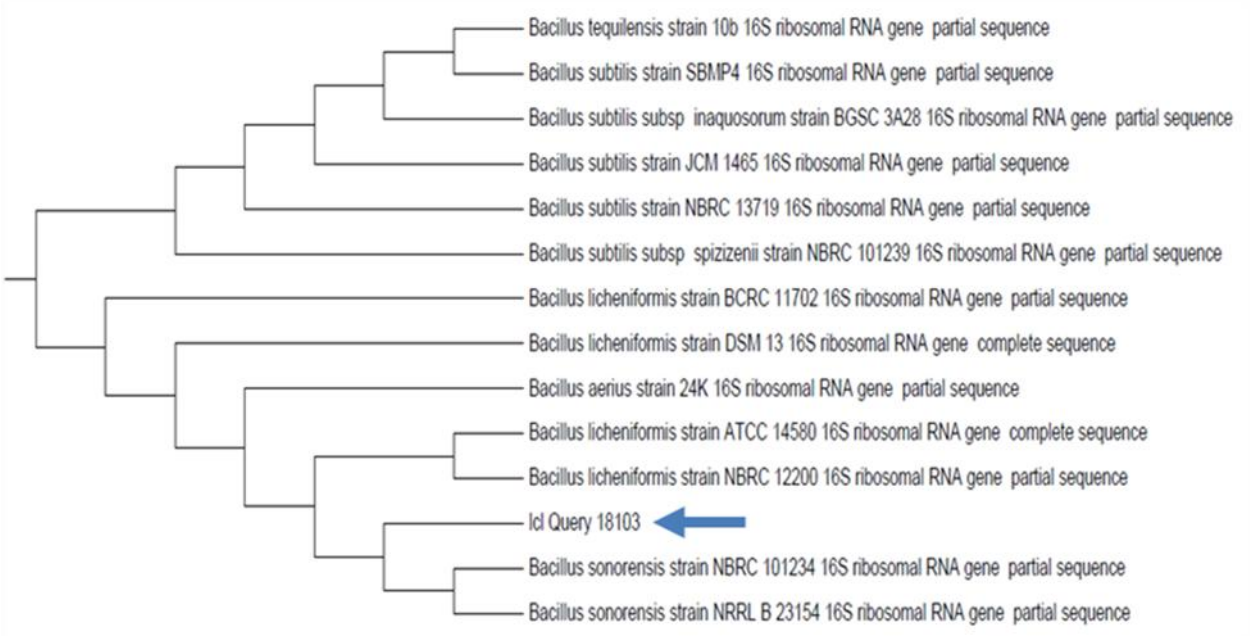

Fig. 2: Phylogenetic tree of isolate 7-1v based on 16 S rRNA sequance. The arrow refer to the isolate 7-1v

Effect of Different Concentrations of Rice Straw, pH Value and Temperatures

Characterization of *Bacillus sonorensis*1-1v for degradation of rice straw under solid state fermentation and its availability for straw composting were investigated. Cellulase production was increased with an increase of rice straw concentration achieving the highest cellulase production at 50% (*w/v*) with production of 31.2 U/g-dry rice straw (**Fig. 3A).** The optimum pH were in slightly acidic range at 5.0 with 35.0 U/g-dry rice straw. Interestingly, even at alkaline pH values the strain produced a considerable amount (U/g-dry rice straw) cellulase as shown in **Fig 3B.** The strain showed cellulase production and high activities at a wide range of temperatures (**Fig. 3 C),** with the optimal temperature at 55^{o}C. At this value, 37.8 U/g-dry rice straw of cellulase was obtained.

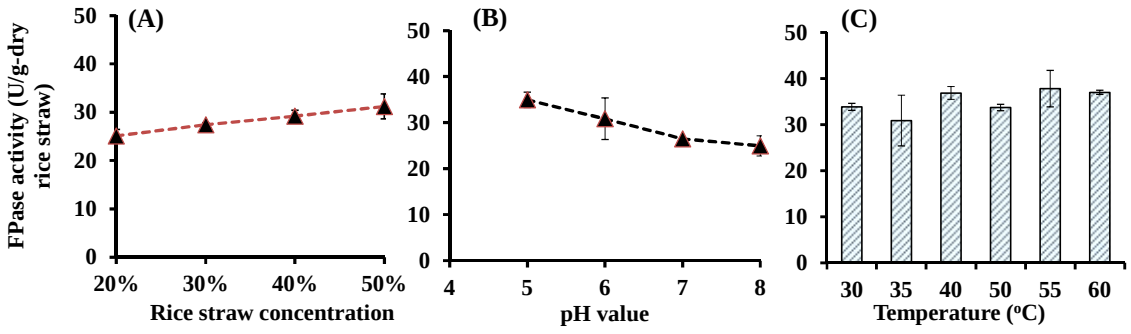

Fig. 3: Effect of different rice straw concentrations (A), initial pH values (B), and incubation temperatures (C) on cellulase production by *Bacillus sonorensis* 7-1v.

Effect of Some Compost Additives on Cellulase Production

The effect of rock phosphate, dolomite or feldspar supplementation to the rice straw during solid state fermentation by *Bacillus sonorensis* 7-1v were summarized in Table 3. Cellulase production was decreased with supplementation of rock phosphate that ranged 27.1–34.6 U/g-dry rice straw compared to control(38.3 U/g-dry rice straw). On the other hand, supplementation of dolomite had stimulated cellulase production at 0.75% (*w/v*) with 50.9 U/g-dry rice straw compared to control. Interestingly, the addition of feldspar has significantly enhanced cellulase production. The highest production was obtained by supplementation of 0.75 % (*w/v*) of feld spare with cellulase productivity of 117.4 U/g-dry rice straw.

Table (3): Influence of different rock phosphate, feldspar, and dolomite on cellulase production by *Bacillus sonorensis* 7-1v using mineral salt medium supplemented with 50% (*w/v*) rice straw.

Concentration (%, *w/v*)	FPase activity (U/g-dry rice straw)		
	Rock phosphate	Feldspar	Dolomite
Control (0)	38.3 ±2.40	38.3 ±2.40	38.3 ± 2.40
0.5	34.60 ±3.50	102.8 ±4.88	32.1 ± 2.63
0.75	27.1 ±0.390	117.4 ±0.31	50.9 ± 1.80
1.0	29.46 ±0.68	82.7 ±1.88	37.7 ± 1.94

The effect of Fe, Zn, or Mg supplementation to the rice straw during solid state fermentation by *Bacillus sonorensis* 7-1v were summerized in Table 4. Cellulase production was enhanced by application of different concentrations of Fe, Zn, or Mg as compared to control. High cellulase productivities ranged 50.6–93.0, 56.9–90.6, and 96.9–120.3 U/g-dry rice straw were obtained by supplementation of Fe, Zn, or Mn, respectively. The highest enhancement of cellulase production was achieved by application of 0.05 %, (*w/v*) of Mn that reached up to 120.3 U/g-dry rice straw compared to control experiment at 38.3 U/g-dry rice straw.

Table (4): Influence of magnetite (Fe), zinc powder, and manganese on cellulase production by *Bacillus sonorensis* 7-1v.

Concentration (%, *w/v*)	FPase activity (U/g-dry rice straw)		
	Magnetite (Fe)	Zn	Mn
Control (0)	38.3 ± 2.40	38.3 ± 2.40	38.3 ± 2.40
0.025	50.6 ± 0.00	90.6 ± 7.95	119.1 ±2.22
0.050	93.0 ± 2.50	77.8 ± 5.57	120.3±7.17
0.075	77.2 ± 2.50	56.9 ± 2.53	96.9 ±4.51

The effect of gypsum and lime supplementation to the rice straw during solid state fermentation by *Bacillus sonorensis* 7-1v were shown in Table 5. Addition of gypsum did not apparently exert a significant effect on cellulase production. Cellulase production was ranged 36.4–38.6 U/g-dry rice straw compared to 38.3 U/g-dry rice straw. On the other hand, addition of lime resulted in decreased cellulase production. About 11.8–26.1 U/g-dry rice straw was produced by lime supplementation compare to control at 38.3 U/g-dry rice straw.

Table (5): Effect of gypsum and lime on cellulase production by *Bacillus sonorensis* 7-1v.

Concentration (%, *w/v*)	FPase activity (U/g-dry rice straw)	
	Gypsum ($CaSO_4$, %)	Lime ($CaCO_3$, %)
Control (0)	38.3 ± 2.40	38.3 ± 2.40
5.0	39.4 ± 0.201	26.1 ± 1.50
7.5	36.4 ± 0.40	25.4 ± 2.22
10	38.6 ± 1.56	11.8 ± 2.94

Effect of Different Nitrogen Sources on Cellulase Production

Supplementation of nitrogen sources have resulted in a significant decrease in cellulase production as compared to control experiment as shown in **Fig. 4.** Cellulases production were ranged 14.0–23.5, 8.7–17.0, and 3.5–11.3 U/g-dry rice straw by supplementation of ammonium sulphate, ammonium nitrate, or urea at (1-3%, *w/v*), respectively

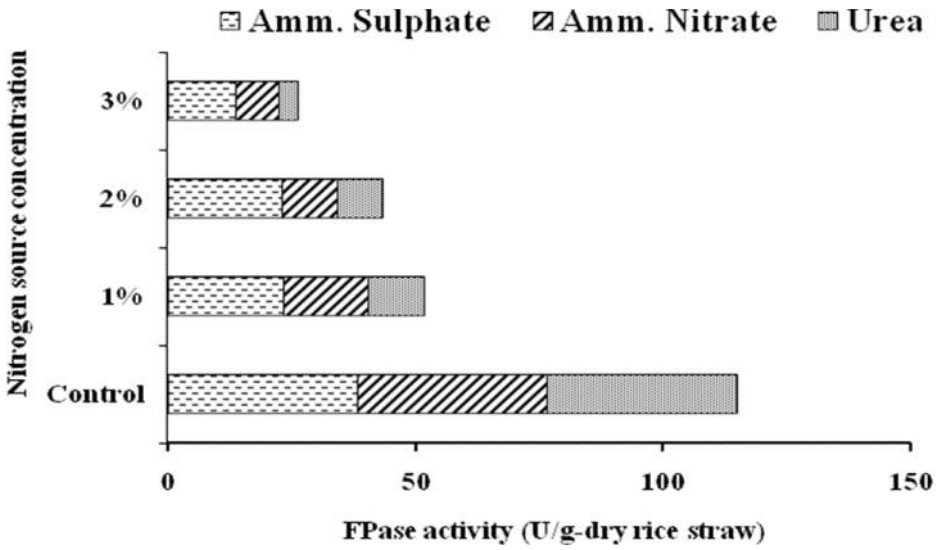

Fig. 4: Effect of different nitrogen sources on cellulase production by *Bacillus sonorensis* 7-1v.

Effect of Different Inocula Sizes on Cellulase Production

The study was carried out with keeping all other conditions at their optimum levels (temp, pH, rice straw) and without supplementation of nitrogen sources. The optimal inoculum size for the maximum cellulose productivity was 2% (*v/v*) at which 152.0 ±6.64 U/g-dry rice straw was obtained. Lower or higher inocula sizes have resulted in lower cellulase production as shown in **Fig. 5 A.**

Interestingly, mixed preculture inocula induced higher cellulase production than single bacterial culture. In addition, lower inocula sizes (1-5 %, *v/v*) enhanced cellulase production than that of higher inocula. Moreover, higher cellulase production was obtained using mixed preculture at 1:1 than obtained using mixed culture at 2:1 that ranged 66.7-219.0 and 22.6-191 U/g-dry rice straw, respectively. Maximum cellulase production was achieved by inoculation of 1% of mixed *Bacillus licheniformis* 1-1v and *Bacillus sonorensis* 7-1v at 1:1, since the cellulase activity reached up to 219.0 ±7.58 U/g-dry rice straw **(Fig. 5 B).**

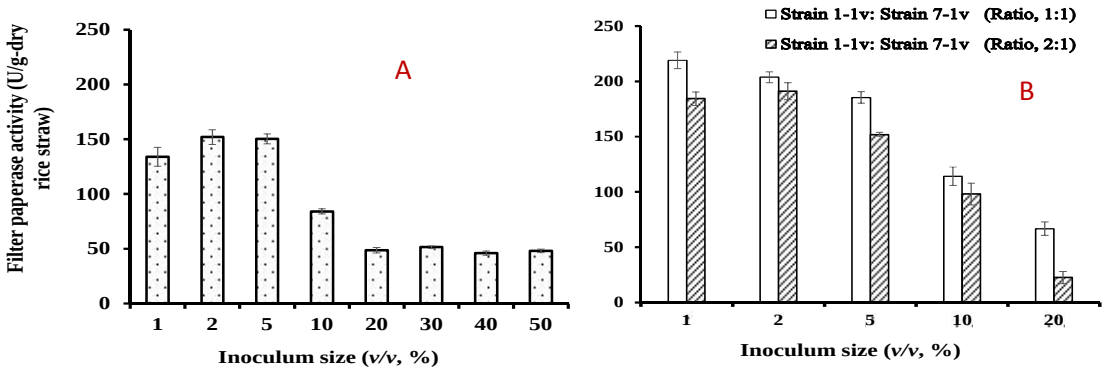

Fig. 5: Effect of different inocula sizes on cellulase production by (A) *Bacillus sonorensis* 7-1v and (B) Mixed culture of *Bacillus licheniformis* 1-1v and *Bacillus sonorensis* 7-1v at various ratios (1:1 or 2:1).

Discussion

Rice straw has shown to be a promising agricultural by-product in the bioconversion of biomass to value-added products such as compost. Efficient hydrolysis of cellulose, a main constituent of rice straw, require cellulytic bacteria that produce exo-, endo glucanases and β-glucosidase. The aim of this study was to isolate potential cellulolytic bacterial strain for efficient degradation of rice straw under solid state fermentation and to characterize this isolate for its feasibility to be applicable in composting technology. For this goal, we obtained 49 bacterial isolates from different environmental samples using enrichment cultures containing commercial cellulose substrates, crystalline cellulose (avicel pH 101) and carboxymethyl cellulose (CMC) for obtaining exo-glucanase and endoglucanase producers, respectively **(Dashtban *et al.*, 2010)**. The best 13 cellulase producing isolates were selected and further investigated for their cellulase production using rice straw containing media. Interestingly, all strains exhibited both exo-, and endo-glucanase production. The highest cellulases activities were recorded for isolates 7.1v. This isolate was then characterized by bacterial identification kit of VITEK 2 system using BCL colorimetric card and showed 93% similarly to *B. licheniformis*. On the other hand,16S rRNA sequence analysis indicated 98% homology to *Bacillus sonorensis* strain NBRC 101234 (accession number NR-113993.1). Based on these data and the phylogenetic tree (**Fig. 2**), we identified the isolate as *Bacillus sonorensis* 7-1v.

Many researchers indicated that environmental and nutritional factors have a great influence enzyme production using different strains. Due to strain variation, it is therefore necessary to determine the effect of these factors on the enzymes activity by the selected isolate. For characterization of *Bacillus sonorensis* 7-1v to determine the factors controlling the enzyme productivity and rice straw degradation, we conducted several experiments including effect of substrate concentration, pH, temperature, additives and inoculum sizes.

Cellulase activity increased with the increase of rice straw concentration up to 50% (*w/v*) with cellulase productivity of 31.2 U/g-dry rice straw. This indicate that cellulase production by solid state fermentation is 24 % higher than obtained by submerged fermentation using 2% rice straw. **Jo *et al.*, (2008)** reported the highest cellulase production by *Bacillus amyloliquefacince* at 5% rice hull. **Goyal *et al.*, (2014)** reported the maximum CMCase activity of 3.08 U/mL by *Bacillus sp.* 313SI using 1% (w/v) pretreated rice straw.

The pH value of the fermentation plays a critical role in the performance of the microbial cell and its enzymatic activities **(Bacha *et al.*, 2015).** The optimum pH value for cellulase production by *Bacillus sonorensis* 7-1v were 5.0 with production of cellulase at 35.0 U/g-dry rice straw. Interestingly, the enzyme production was high within the tested pH range of 5.0-8.0. These results indicate the capability of the selected isolate for the decomposition of rice straw under the varied pH due to the formation of several products during composting process by the endogenous microbes.

The effect of temperature on the isolated stain was also investigated with the optimal incubation temperature at 55^{o}C. In addition, even at lower or higher temperatures, the strain exhibited considerable cellulase production and consequently rice straw decomposition. *Bacillus sonorensis* 7-1v produced cellulase at 30.9-37.8 U/g-dry rice straw at temperature ranged 30-60^{o}C. The composting process go through different phases including mesophilic phase (20–40^{o}C) then thermophilic phase (40–60^{o}C), then second mesophilic phase **(Liu *et al.*, 2011)**. Therefore, our data support the availability of this strain for composting technology due to its high activity over a wide range of temperatures.

For further characterization of strain 7-1v, the influence of different compost additives on cellulases activities by *Bacillus sonorensis* 7-1v were investigated. Rock phosphate, Feldspar, and dolomite were applied at different concentrations up to 1%, *w/v*. Rock phosphate have decreased the activity of the isolate. The addition of dolomite, a natural sources for magnesium, had slightly stimulated cellulase, however, feldspar (a natural source of potassium) addition at 0.75 %, *w/v* achieved ~3-fold enhancement in cellulase production compared to control (without Feldspar supplementation). This suggests the important role of potassium and magnesium to increase and stabilize the enzymes production **(Paliwal *et al.*, 1994)**.

Supplementation of magnetite (iron), zinc powder, or manganese salt improved cellulose production by *Bacillus sonorensis* 7-1v. About ~3-fold of cellulase amount was obtained by addition of Mn at 0.05%. Metal ions apparently protect the enzymes against thermal denaturation and play an important role to

continue the active conformation of the enzymes at high temperatures **(Paliwal *et al*., 1994).**

The effect of gypsum ($CaSO_4$) and lime ($CaCO_3$) on cellulase production was also investigated at 0-10%. Gypsum had no effect on cellulase production while lime slightly decreased the production. **Gabhane *et al*., (2012)** had not found a significant influence on the growth of microbes and cellulase activity using various compost additives including lime, and phosphogypsum. Our date suggest the feldsper supplementation is highly recommended during the application of this strain in composting process of rice straw due to its great enhancement of enzymatic production and substrate degradation.

The effect of organic or inorganic nitrogen supplementations showed decreased cellulase production compared to control experiment. It is noteworthy to mention that control medium contain yeast extract as a nitrogen source beside the protein content of rice straw of about 3.6-7.2% (**Sherief *et al*., 2010)**. Yeast extract is an organic source of amino acids, proteins and vitamins. It contains abundant nitrogen compounds as well as many growth factors.

Inoculum size also affects the maximum activity and cellulase enzyme production. The balance between the accessible nutrient and biomass would achieve an optimal enzyme production **(Singh and Kaur, 2012).** Bacterial co-cultures are applicable to improve hydrolysis of cellulose and enhance product utilization to obtain increase desirable fermentation products. We have recently reported a potential cellulase producing strain, *Bacillus licheniformis* 1-1v, able to degrade rice straw at 50^{o}C (**Abdel-Rahman *et al*., 2015**). When bacterial consortium of *Bacillus licheniformis* 1-1v and *Bacillus sonorensis* 7-1v (1:1) was used as inoculum, a significant increase in cellulase production was obtained even at low inoculum size compared to the single bacterial culture. A maximum cellulase production of 219 U/g-dry rice straw was achieved by inoculatin of 1% of co-culture at 1:1. This might due to the broader substrate specificity by both isolates rather than single isolate. Furthers studies on composting of rice straw using inoculum of bacterial consortium contains these strains (1-1v and 7-1v) showed a significant decrease in composting duration compared to either single strain or control treatment without bacterial inoculation **(Abdel-Rahman *et al*., 2016).**

Khelil *et al*., (2015) reported high level of cellulase production using waste newspaper by mixed culture of *Bacillus* sp. R2 and *Bacillus cereus* 11778 rather than single bacterial culture. On the other hand, **Rastogi *et al*., (2010)** reported equal levels of cellulase and CMCase activities using mixed cultures of *Bacillus* sp. DU- SELR13 and *Geobacillus*sp. WSUCF1 or pure single cultures.

In conclusion at the present study, a compatible lignocellulolytic bacterial consortium (co-culture of *Bacillus licheniformis* 1-1v and *Bacillus sonorensis* 7-1v) potential for rapid and efficient decomposition of rice straw was successfully developed.

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