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Journal Name:	<u>British Microbiology Research Journal</u>
Manuscript Number:	Ms_BMRJ_22959
Title of the Manuscript:	OVERPRODUCTION OF XYLANASE FROM MUTANTS OF <i>Bacillus subtilis</i> USING BARLEY HUSK AS THE PRIME CARBON SOURCE IN SUBMERGED FERMENTATION AFTER RANDOM MUTAGENESIS OF ETHYL METHANE SULFONATE (EMS) AND ACRIDINE ORANGE (AO)
Type of the Article	Original research Articles

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	1) Present study does not increase or contribute to the current scientific knowledge on xylanase production from <i>Bacillus subtilis</i>. 2) An extensive and careful editing is required for improvement of the English language throughout the manuscript. 3) Authors should follow journals reference style and therefore reference list in the manuscript should be modified according to the example given in author guidelines section of the BMRJ. 4) In submerged fermentations, it is a usual practice to keep the medium volume 1/5th of the vessel volume. Authors has used 250 mL medium in 500 mL flask i.e. 1/2 the volume of the flask, moreover, rate of agitation has been maintained 150 rpm. What is the rational of using high volume and slow speed for an aerobic bacterium like <i>B. subtilis</i>? 5) What is the reason behind using sterile distilled water for dilution of the bacterium instead of normal saline? (page no. 4, line no 116-117). Use of water can result in lowering the actual number of cells in inoculum thus causing methodological error. 6) Which of the methods described in Bailey et al (1992) has been used by authors, as this research article has not given any of its own method but compared methods used in 20 different laboratories? 7) Authors nowhere have presented enzyme activity with $\pm$ standard deviation value. 8) If Authors have checked for presence of cellulase, β-xylosidase and other carbohydrate degrading enzymes in the culture supernatant? Xylanase activity can just be a non-specific (additional) activity of a cellulase or any other carbohydrate degrading enzyme present in the culture supernatant. 9) Authors indicated that there was a direct relationship between secretion of extracellular xylanase and cell count of <i>B. subtilis</i> (page no. 9, line no. 315-316). It has also been shown that highest cell count was recorded in case of wild type <i>B. subtilis</i> (page no. 9, line no 301) as compared to mutant strains, then why the production was lower in case of wild type organism? 10) Incorrect use of terminology for indicating clear zone of hydrolysis	1) In the present study, we embarked on the following objectives to qualitatively confirm the activity of xylanase from the mutants of <i>B. subtilis</i> using plate hydrolysis assay and to elucidate overproduction of xylanase from the mutants of <i>B. subtilis</i> under submerged fermentation using the prime carbon source of barley husk compared with its parental wild type of <i>B. subtilis</i>. 2) The manuscript has been revised. 3) The reference has been revised 4) The whole fermentation was completed in 3 days, in fact, the maximum xylanase activity was achieved within 24 h. In order to proceed to scale up, the fermentation should be terminated when the maximum xylanase activity obtained. Therefore, microbial growth was not affected by the limitation of oxygen in the shake flask within the first 24 h. Moreover, 150 rpm was the optimised agitation speed for the growth of <i>B. subtilis</i>. 5) Haemocytometer was used to count the number of cells of wild type and mutants of <i>B. subtilis</i> as standard inoculums in the fermentation, respectively. 6) The xylanase activity was estimated according to Bailey et al. (1992) using 1% beechwood xylan in 0.05 M sodium phosphate buffer, pH 5.3 as the substrate to liberate xylose as the reducing sugar. The detection of xylose after xylanase activity was conducted using 3, 5-dinitrosalicylic acid (DNS). 7) Standard deviation values have been added into the manuscript accordingly. 8) According to Asano et al. 2005, xylanolytic activity was general quantitatively determined using 3,5-dinitrosalicylic acid (DNS). The assay was based on the enzymatic hydrolysis of xylan and the reaction of the liberated reducing sugar (Bailey et al., 1992). Xylose was used as a reference for reducing sugar. One unit of xylanase activity was defined as the quantity of enzyme that liberated reducing sugar of xylose at the rate of 1μmole/min (Reference: Asano K, Sriprang R, Gobsuk J, Eurwilaichitr L, Tanapongpipat S



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	because of xylanolytic activity of enzyme (authors have used ring zone of discoloration).	and Kirtikara K. Endo-1,4-β-xylanase B from <i>Aspergillus niger</i> BCC14405 isolated in Thailand: Purification, characterization and gene isolation. Journal of Biochemistry and Molecular Biology. 2005; 38(1): 17-23). Another study by Amita and Datta, 2005, xylanase (EC 3.2.1.8) activity was measured using 1% birchwood xylan (4-O-methylglucuronoxylan) solution as a substrate (Bailey et al. 1992). The release of reducing sugars in 10 min at 50°C, in 0.05 M citrate buffer, pH 5.3 was measured as xylose using the dinitrosalicylic acid method. One unit of enzyme activity (U) is defined as the amount of enzyme liberating 1μmol of xylose per min. On the other hand, cellulase activity was measured according to IUPAC recommendations by employing filter paper (Whatman No.1) as the substrate (Ghose 1994). The release of reducing sugar in 60 min at 50°C in 0.05 M citrate buffer, pH 4.8 was measured as glucose equivalent using dinitrosalicylic acid method. One unit of filter paper activity is measured as the amount of enzyme liberating 1 μmol of glucose per min. (Reference: Amita RS and Datta M. Xylanase production under solid-state fermentation and its characterization by an isolated strain of <i>Aspergillus foetidus</i> in India. World Journal of Microbiology and Biotechnology. 2005; 21: 233-243). 9) The increased production of xylanase in mutants of <i>B. subtilis</i> particularly MXB 4 with the highest maximum xylanase activity could owing to the ability of the mutant strain in gaining easier access of the hemicelluloses of xylan in barley husk [Azeri et al. 2010]. In essence, the results from the present study indicated the use of mutant strains was a constructive method for significant xylanase overproduction utilising lignocellulosic material of barley husk as the substrate. (Reference: Azeri C, Tamer U, Oskay M. Thermoactive cellulase-free xylanase production from alkaliphilic <i>Bacillus</i> strains using various agro-residues and their potential in biobleaching of kraft pulp. African Journal of Biotechnology. 2010; 9(1): 63-72). 10) Corrections have been performed.
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<u>Minor</u> REVISION comments	1) In table 1, column 1 should be adjusted as column 3 and labelled as Mutants generated instead of Mutant 2) Title of the table 1 should be changed to “mutagens concentrations used for generation of <i>Bacillus</i> mutants”	Corrections have been performed.
<u>Optional/General</u> comments		