



SDI Review Form 1.6

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

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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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	- There are too many language and grammatical errors in the manuscript. A reviewer cannot correct all of them. Just a few examples of sentences that are hard to understand— - Page 2 Line 57: Enzymes are the biocatalysts function with crucial roles---- - Page 2 line 61: ----they are highly biodegradable and economically feasible that commonly produced in large- - Page 3 Line 85: In accordance, many studies have also been evaluated using barley --- - Page 6 line 199: Based on the results, all mutants of <i>B. subtilis</i> ATCC 6633 were detected with the ability to produce xylanase. - Page 10 lines 344-346: ----- the current inducement of this study is also investigated the deployment of xylan-rich low-cost agricultural residue such as barley husk. - Page 10 lines 346: The finding obtained from this study is homogenous to the published reports from <i>Bacillus</i> species in attempt---- - Page 10 lines 349: Sugumaran et al. [4] exhibited 7.5 U/mL of xylanase activity from <i>B. subtilis</i>----- Materials and Methods: - Under 2.3, page 4 line 126: To begin, 20 µL of the five mutant cultures were aseptically pipetted on the xylan agar, respectively. 20 µL suspension spread on 3” petri dish is rather small volume. - Under 2.4, page 5, line 148: ----- using agricultural extract of barley husk as the sole carbon. Compare with page 4, line 141-- barley husk. Question: Was it agricultural extract of barley husk or barley husk that was used? - Under 2.10, Statistical analysis: Why use ANOVA? You should compare individual putative mutant with the wild type and not with each other. T-test will do. - You have stated on page 12, lines 423-424 that “As such, we concluded that there was significant difference in the mean values for the production of xylanase between the mutants and wild type of <i>Bacillus subtilis</i> ATCC 6633 using barley husk as the carbon source under submerged fermentation.”	- Corrections have been done. Materials and Methods: 20 µL of the five mutant cultures were aseptically pipetted on the xylan agar, respectively. Using sterile hockey stick, the mutant cultures were spread uniformly on the agar. The agar plates were then incubated at 37°C until the mutant cultures developed and covered the thin layer on the surface of the agar after 2 to 3 days of incubation. - Correction has been done. - We intentionally want to compare the xylanase production between the wild type and mutants and among each other. Based on the results, we observed that there were variations in the sum of square (SS) in xylanase production, producing values of 0.098316 and 0.010815 between and within the groups of wild type and mutants of <i>B. subtilis</i> in the present study. As such, we concluded that there was significant difference in the mean values for the production of xylanase between the mutants and wild type of <i>Bacillus subtilis</i> ATCC 6633 using barley husk as the carbon source under submerged fermentation. - All sequences of the mutants are detected and identified, refer to the in press journal titled: Detection and analysis of the random mutagenesis site(s) of xylanase gene from mutants of <i>Bacillus subtilis</i> subsp. spizizenii ATCC 6633. Results and Discussion: - In the present study, the data of the xylanase production by wild type and mutants of <i>B. subtilis</i> ATCC 6633 in the submerged fermentation in this study was statistically evaluated using single factor analysis of variance (ANOVA) at a significance level of $p < 0.05$. Based on the results, we observed that there were variations in the sum



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	Question: Which of MXB1, MXB2, MXB3, MXB4 and MXB5 is actually a mutant and which is not? Results and Discussion: - Under 3.2, page 9: The maximum cell counts (cells/mL) were attained at 24 h of fermentation. Under 3.3, page 11: Maximum xylanase activity/production (U/mL). No difference between WT and the putative mutants. - Page 11 line 409: The authors have claimed a 1.36-fold increase of xylanase activity of MXB4 over the wild type. They divided $1.76/1.36 = 1.3$. But the actual increment should be, $1.76-1.36 = 0.4$. And $0.4/1.36 = 0.294$-fold increase or 29.4% increase, which is $< 100\%$ increase. - The increase of “mutant” value compared to the wild type is very small when compared with “xylanolytic activity of 439.5 ± 2.84 U/mL using sugarcane bagasse, Irfan et al. [45] on page 10, lines 357-360. - Under 3.4, page 13: Extracellular protein production. Page 13 line 490: The maximum level of extracellular protein (mg/mL) was attained after 24 h of fermentation. But the maximum cell counts obtained on page 9 after 24 hours (see above), are different from the values presented on pages 13 and 14. - Page 14 line 495: The authors have claimed a 1.49-fold increment in protein secretion of MXB2 and MXB4 over the wild type. They divided $0.82/0.55 = 1.49$. But the actual increment should be, $0.82-0.55 = 0.27$. And $0.27/0.55 = 0.49$-fold increase or 49.1% increase $< 100\%$ increase.	of square (SS) in xylanase production, producing values of 0.098316 and 0.010815 between and within the groups of wild type and mutants of <i>B. subtilis</i> in the present study. Furthermore, there were also differences in the mean square (MS) of xylanase production between and within the groups of wild type and mutants with the values of 0.098315 and 0.002704, respectively. MS was quantified by dividing the SS with the degree of freedom (df). In addition, since the F-statistics value of 36.363187 is larger than F-critical value of 7.708647 and p value of 0.003812 is less than the alpha value of 0.05, we rejected the null hypothesis. As such, we concluded that there was significant difference in the mean values for the production of xylanase between the mutants and wild type of <i>Bacillus subtilis</i> ATCC 6633 using barley husk as the carbon source under submerged fermentation. - Correction has been done. - Different species of <i>Aspergillus</i> produce different amounts of xylanase activity. - Correction has been done. - Correction has been done.
Minor REVISION comments	The conclusion is too long. Only the main findings should be captured.	Correction has been done.
Optional/General comments	There are too many grammatical errors in the manuscript.	Correction has been done.