



SDI Review Form 1.6

Journal Name:	<u>Biotechnology Journal International</u>
Manuscript Number:	Ms_BJI_35030
Title of the Manuscript:	Production of raw starch degrading amylase by <i>Bacillus subtilis</i> TLO3 and its application in bioethanol production using starch-rich flours
Type of the Article	

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.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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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	<p>Using amylase produced from <i>Bacillus subtilis</i> to treat raw starch to produce bio-ethanol is attractive. The process of saccharification for raw starch biomass pre-treatment in low temperature faces many problems, one is that rare high efficient amylase could be used. The study focused on using an amylase hyperproducer strain <i>Bacillus subtilis</i> TLO3 to improve saccharification efficiency for raw starch to ferment ethanol fermentation, the ideas are meaningful, however more valuable information should be provided.</p> <p>Major concerns are:</p> <p>1) The results of optimized amylase production from <i>Bacillus subtilis</i> TLO3 could not be convinced. The authors should provide the data related to the activity or the productivity of <i>Bacillus subtilis</i> TLO3 produced amylase.</p> <p>2) As we know, the products from amylase reaction could be oligo-glucose, maltose, and glucose. In this study, the authors try to show the newly certified <i>Bacillus subtilis</i> TLO3 which could produce highly functional amylase. What kinds of reducing sugar are produced by the amylase should be clarified.</p> <p>3) in abstract, they said " The</p>	<p>I would like to thank the reviewer for these valuable remarks.</p> <ol style="list-style-type: none"> 1. The data about amylase production optimization are provided in Table1. 2. The end product of <i>B. subtilis</i> TLO3 amylase hydrolysis of starch is dextrines, maltose and glucose, since we already know by the mean of genome sequencing and annotation that this strain possesses an α-amylase , a glucoamylase and pullulanase (https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP021169.1) 3. These results are in Page 7 line 162-163 4. It is true, the ethanol yield is quiet low, but it is because we choose the fermentation period of 24h; due to the interest of producing ethanol within a short time for industrial interest. 5. There are no ethical issues. 6. Properties of the alpha-amylase were investigated, but they are not shown because it is the subject of another paper.



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	<p>fermentation process monitoring showed a continuous decrease in the total sugars, concurrently with an increase in ethanol production that reached(2%) for wheat flour and (2.4%) for corn flour after 24 h. ". I never saw these data in the MS.</p> <p>4) I understand that the authors wanted to show the advantages of using the amylase, which produced by Bacillus subtilis TLO3. However, ethanol concentrations using the yeast S. cereviseae and reducing sugar based media were too low and would not much attract the interests from the potential readers.</p> <p>Please clarify the ethical issue if any. Yes. It is high anticipated to see the properties of amylase from the newly isolated Bacillus subtilis TLO3.</p>	
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		