



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_15199
Title of the Manuscript:	<i>Clostridium thermocellum</i> cel5L – Cloning and Characterization of a New, Thermostable GH5 Cellulase
Type of the Article	Original Research 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 <i>(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)</i>
<u>Compulsory</u> REVISION comments	Title L3 Please change the strain designation from "cel5L" to "Cel5L" Title L3-L5 Emend the manuscript title. The work presented did not correspond to the title itself. Abstract 1- Please check the word spacing such as "thatferments" and "Clostridium thermocellumCel5L". 2- Please spell the abbreviation in full such as CMC - carboxymethylcellulose and CBM-carbohydrate binding module. 3 - It's not surprising for glycoside hydrolase (GH) enzymes to lack of CBM. In some cases, CBM couldn't be detected from the sequence itself but can be determine from the enzyme crystal structure or homology modelling. 4 - The phylogenetic analysis conducted is not conclusive as the enzyme can be either cellulase or endoglucanase. The BLASTp result indicated that the enzyme shared 62-100% similarity with endoglucanases from various sources. 5- Please re-write the abstract. Introduction L19-L28 Please shorten this part. Textbook knowledge can be	cel5L is not a strain but an enzyme. The reviewer seems to be completely unclear on what the designation refers to. Manuscript is about the enzyme, Cel5L, clearly reflected in the title. Other two reviewers had no problem with this. Spacing and abbreviations are corrected. Added "annotated" to carbohydrate binding module. Phylogeny is based solely on sequence homology similarity and is function agnostic. Again, not a problem for the other two reviewers. Rewritten to reflect changes Deleted L19-28.



SDI Review Form 1.6

	omitted. L28-L38 Please remove excessive citation and include recent, related-publication. I would like to suggest following recent article as it closely related to this work: • Thomas L, Joseph A, Gottumukkala LD (2014) Xylanase and cellulase systems of <i>Clostridium</i> sp.: an insighton molecular approaches for strain improvement. Bioresour Technol. 158:343-350 L38 Please remove excessive citation of CAZy website. L44 The authors failed to clarify what strain used in this work. Is strain Cel5L different from strain ATCC 27405^T? If yes, please include the 16S rRNA percentage similarity here. If not, change the strain designation to ATCC 27405^T throughout the manuscript. L56-57 Genomic analysis of the bacteria or bioinformatics analysis of the enzyme? Please clarify. Introduction in the revised manuscript should include: • Description of GH groups in CAZy • Description of GH groups in relation with cellulose (or endoglucanase) • Description of CBM • EC number of cellulose (or endoglucanase) • Substrate of cellulose (or endoglucanase) • List a few of celluloses (or endoglucanases) from <i>Clostridium</i> sp.	The introduction answers the question “Why is cloning, expression and characterization of another C. thermocellum enzyme important?” The citations are needed to build the understanding of the C. thermocellum problem. Removed. Again, the reviewer seems unclear on the fact that this is about a protein, Cel5L, not a strain. I have changed L47 genomic to bioinformatic to make this clearer. Clarified Descriptions of GH, CBM, substrates, and list of cellulases added as requested.
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	Material and methods L60-L79 Shorten this part. Only mention materials that are very crucial to the work. L80-L95 The authors failed to describe in detail regarding: • Cultivation of strain ATCC 27405^T • Expression of the recombinant enzyme from <i>E.coli</i> L96-L129 Please clarify the definition of enzyme unit for each enzymatic assays. Example: • L111-L112 - Is it one unit of enzyme activity equal to one micromoles of what type of reducing sugar per minute per milligram of protein under what time and what temperature? L113-L116 Separate this part into section "Effects of temperature and pH on enzyme activity". State the range of temperature and pH tested. Why β-glucan concentration used in temperature and pH experiments is different? L132-L133 4-20% acylamide?? Report the exact concentration for acrylamide used in this work. L133 The material and method section for revised manuscript should include: • Detailed description of the enzyme purification steps	Sources of carbohydrates are important as differences in quality exist between suppliers and influence results. Didn't grow the <i>C. thermocellum</i>, clarified this in text. Added growth of <i>E. coli</i> section The section clearly states "Micromoles of sugars formed were determined using a glucose standard curve, and unit activity calculated as micromoles of reducing sugar per minute per milligram of protein. " A micromole is a unit on its own, not like milligrams of sugar as glucose. Added the temperature to the definition. Correction of beta-glucan concentration made and ranges added. 4-20% gradient gels are a common lab reagent We used Bio-Rad 4-20% Mini-PROTEAN® TGX™ Gel, 10 well, 30 μl 456-1093
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	L145-L149 Simplified this part to increase the readability Results and discussion Check the abbreviation used in this section. Do not spell the abbreviation in full if it have been described earlier. L163-L164 This is rather ambiguous. Omit. L175-L188 Move to "Materials and methods" section and re-arrange to increase clarity. L188-L197 Figure 2 did not show any purified enzyme. Report as partially purified enzyme and emend the manuscript title. L203-206 Include the data in the revised manuscript. Change "purified Cel5L" to "partially purified Cel5L". L210-L215 The statement is rather ambiguous. Citation?? L228-L230 Citation to support the argument?? L236-L242 Citation to support the argument?? L251-L265 Describe in detailed this part in Material and methods section for the revised manuscript.	Done Done Done Again reviewer didn't read the manuscript carefully. It clearly stated "The final enzyme purity was greater than 95% for Cel5L (Figure 2), as well as for all other enzymes used in the comparisons." Clarified, these are reported results. No argument, reported results No argument, reported results This is present in the original manuscript L123-L129. I assume the reviewer missed it.
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	L256-L259 Rather ambiguous. Citation for the argument?? L263-L265 Citation? "Non-traditional binding domain"????? Re-write to increase clarity. L279-L281 The increment in glucose production is not necessarily from the presence of the CBM. Re-write this part. L299-L308 Omit or move to Material and methods. L308-L315 Omit the abbreviation and spell the full name in the phylogenetic tree. L322-L324 Citation??? L324-L327 Citation??? L329 - Figure 7 Explain the "dock"?? Not clear. L333-L335 Not clear. Re-write. L352-L358 Not relevant to this work. Omit. Conclusion Emend according to the revised manuscript.	Citation added and argument clarified Clarified and referenced. Clarified This is the Legend supplied as part of the MEGA5 tree construction to accompany the tree. Abbreviations are standard genomic abbreviations used by UniProt and others Full names won't fit in the tree. Reference added References added. Changed dock to dockerin in figure Section rewritten to clarify. Conclusion now agrees with results presented.
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	References Please check the references in detail. Example: L383 - "table of contents"???? L396 - volume, pages??	References corrected and updated.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		