

## Review Form 1.6

Journal Name:	<a href="#">Asian Food Science Journal</a>
Manuscript Number:	Ms_AFSJ_73876
Title of the Manuscript:	Isolation, characterization and acid/alkaline tolerance of lactic acid bacteria present in fermented rye, wheat, oat and barley
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:

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**PART 1: Review Comments**

	<b>Reviewer's comment</b>	<b>Author's comment</b> (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)
<b>Compulsory</b> REVISION comments	<p><b>Recommendation</b></p> <p>Major Revision</p> <p><b>Overview and general recommendation</b></p> <p>The manuscript is focused on evaluating the survivability of lactic acid bacteria (LAB) from fermented cereals in simulated gastrointestinal tract conditions. Primarily, the authors isolated 13 LAB strains from fermented rye, wheat, oat and barley grains. The isolates were identified as <i>Lactobacillus plantarum</i>, <i>L. brevis</i> 1, <i>L. collinoides</i> and <i>Leuconostoc citreum</i> based on their fermentation patterns. All tested microorganisms were capable of surviving in simulated gastric and pancreatic juice. The authors suggest that consumption of fermented cereal products might facilitate intake of potentially probiotic LAB.</p> <p>Overall, the data regarding the ability of cereals-derived LAB to survive gastrointestinal transit might be valuable for the industrial and healthcare application. Yet, the methodology and the data analysis are confusing and should be reviewed and verified by the authors. Moreover, the interpretation of the results in the abstract is very different from the interpretation, given in the text. Both interpretations do not fit the graphical representation of the data (Figure 1). The authors do not address the increase in the viable CFU counts after the incubation in acidic or alkaline conditions (which are considered detrimental for the tested bacteria). Furthermore, there are notable differences in viable CFU counts at the end of the exposure to acid conditions (80a) and in the beginning of exposure to alkaline conditions (80b). Samples for 80a and 80b should be obtained almost simultaneously, therefore the differences in LAB counts should be explained. These observations suggest that the methodology or data analysis might be inadequate or biased by technical mistakes.</p> <p>I would suggest the authors to measure pH of the simulated gastric and pancreatic juice after autoclaving. Measuring pH of the medium after the addition of NaOH and pancreatic juice should be performed (since acidic simulated gastric juice may partially neutralize NaOH). Also, the tested LAB strains might form biofilms, which may distort CFU counts. Therefore, studying the samples using microscopy might be of interest. To this end, biofilm formation by the studied bacteria could explain (at least in part) their persistence in the simulated conditions of gastrointestinal tract.</p>	
<b>Minor</b> REVISION comments		
<b>Optional/General</b> comments	<p><b>Specific comments:</b></p> <p><b>Abstract:</b></p> <p>"Isolated LAB strains were cultured with MRS broth and were characterized using API 50 CH kit (Biomérieux, France)". It might be helpful to add that the fermentation patterns</p>	

	<p>of the tested strains were characterized API 50 CH kit.</p> <p>“There was no significant difference in the LAB cell count between 0<sub>a</sub> and 120<sub>b</sub> minutes (p &gt; 0.05)”. This statement contradicts to the Figure 1.</p> <p><b>Introduction:</b></p> <p>“These beneficial bacteria are known as probiotic microorganisms [12] and <u>usually</u> belong to <i>Lactobacillus</i> and <i>Bifidobacillus</i> species [11]”. Bacteria in the colon belong not only to <i>Lactobacillus</i> and <i>Bifidobacillus</i> species, gut microbiota are very diverse and include many different taxa. However, microorganisms, used as probiotic ones, frequently belong to those genera.</p> <p>Is “<i>Bifidobacillus</i>” a synonym of <i>Bifidobacterium</i>?</p> <p>“The organisms that are involved in fermentation of cereals include bacterial species such as <i>Lactobacillus</i>, <i>Streptococcus</i> and <i>Bacillus</i>, yeast species of <i>Saccharomyces</i> and mould such as <i>Cladosporium</i> and <i>Penicillium</i> species”. Could you please indicate which products of fermentation of cereals you mean. Are those products treated thermally before consumption (Processing of cereal foods is often completed with a final boiling or baking stage [Ashaolu, T.J.; Reale, A. A Holistic Review on Euro-Asian Lactic Acid Bacteria Fermented Cereals and Vegetables. <i>Microorganisms</i> <b>2020</b>, <i>8</i>, 1176. <a href="https://doi.org/10.3390/microorganisms8081176">https://doi.org/10.3390/microorganisms8081176</a>]). In this case, the heat treatment might eliminate the live LAB present in the fermented product. Please elaborate.</p> <p><b>Methods</b></p> <p>“Rye, wheat and barley grains were washed three times with tap water, milled using a smoothie maker (Kenwood – model number: 0WSB26001) and were fermented for 72h”. Please add more information regarding the conditions of the fermentation (e.g. temperature, what volume of water was added to the milled grains, type of vessel in which fermentation took place).</p> <p>“One hundred micro liters of each serial dilution was streaked on MRS (deMan Rogasa Sharpe) agar (Technical No. 3 Oxoid, Basinstoke, UK) plates. “ Did you mean Rogosa?</p> <p>“The pH of each fermented cereal sample was adjusted to 2.0 using 1Mol HCl (hydrochloric acid). Fifty milliliters of each fermented cereal sample was dispensed into 100cm<sup>3</sup> medical flasks in batches and were autoclaved for 15 minutes at 121<sup>0</sup>C and pressure of 15 psi (pounds per square inch).” Did you measure pH of the fermented samples after autoclaving to confirm that it did not change?</p> <p>Each solution was mixed thoroughly using a votex mixer (Stuart, Staffordshire, UK) and was incubated at 37<sup>0</sup>C for 80mins [22]. This was followed by increase in the pH of the fermented cereal medium to 8.0 using 1Mol of NaOH (sodium hydroxide). Ten milliliters of pancreatic juice was then added to each cereal medium and was incubated at 37<sup>0</sup>C for 120mins [23]. Did you measure pH after NaOH addition to make sure that it had been adjusted to 8?</p>	
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	<p><b>Results and discussion</b></p> <p>The high LAB cell count obtained in these fermented cereal grains may be due to their high nutrient contents, especially carbohydrates and vitamins, which promote the growth and activity of LAB in the presence of moisture and other environmental conditions such as <b>oxygen</b> and temperature [22]. LAB are considered as microaerophilic since they do not utilise oxygen. Please explain what oxygen requirements you mean.</p> <p>Using API 50 CH characterization test, two LAB strains isolated from barley were characterized as <i>Lactobacillus plantarum</i>1 while others were characterized as <i>L. collinoides</i> and <i>Leuconostoc citreum</i>. Could you please briefly describe how the bacteria were identified based on their carbohydrate fermentation pattern (since I could not find the relevant information in reference 17). Did you also perform molecular identification? Could you please explain what you mean by <i>Lactobacillus plantarum</i>1. What does index 1 mean? Is that subspecies?</p> <p>“All isolated LAB strains had different API 50 CH identification profile. Interestingly, none of the six <i>Lactobacillus plantarum</i>1 strains and five strains of <i>L. brevis</i> 1 isolated in this study shared the same fermentation pattern.” It might be worth specifying what were the differences in fermentation patterns, since metabolic products of LAB might have beneficial health effects [Filannino, Pasquale; Di Cagno, Raffaella; Gobbetti, Marco (2018). Metabolic and functional paths of lactic acid bacteria in plant foods: get out of the labyrinth. <i>Current Opinion in Biotechnology</i>, 49(), 64–72. doi:10.1016/j.copbio.2017.07.016].</p> <p>“All the isolated LAB strains from the four selected cereals survived in the simulated gastric juice at pH of 2.0 (before and after incubation at 0<sub>a</sub> min and 80<sub>a</sub> mins) and after addition of simulated pancreatic juice at pH of 8.0 (before and after incubation at 0<sub>b</sub> mins and 120 mins respectively). There was significant (p = 0.017) increase in the colony counts of all the strains at all times”.</p> <p>Contradicts to the abstract “There was no significant difference in the LAB cell count between 0<sub>a</sub> and 120<sub>b</sub> minutes (p &gt; 0.05)” and to the Figure 1 (according to the Figure 1, there was no increase in viable CFU counts at all tested time points (for instance, B1, B2, O1, R3, etc). Could you please explain why there was notable increase in viable CFU counts at 80<sub>a</sub>, 80<sub>b</sub> and 200 minutes?</p> <p>Figure 1:</p> <p>Please indicate significant changes in CFU counts on the graph (for example, by letter subscripts). It might be helpful to change the units of Y axis from 10<sup>7</sup> CFU/ ml to CFU/ml or log CFU/ml</p> <p>“The lowest viable cell count of 2.0 *10<sup>8</sup> cfu/ml obtained in R<sub>3</sub> at 0<sub>b</sub> minutes before incubation in alkaline medium (at pH of 8.0) containing simulated pancreatic juice is higher than 1 * 10<sup>7</sup> cfu/ml, which is the minimum viable count that any bacteria destined to confer a beneficial effect in the GIT is expected to have at the time of consumption [26]”. In some sentences you refer to the time point before incubation in alkaline medium</p>	
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	<p>as 0b, and in other sentences - as 80b. It might be desirable to use the same abbreviation (ether 0b or 80b) throughout the paper. Why do you compare the recommended CFU count at the time of consumption with the lowest viable CFU count at the time point 80b, but not 200 minutes?</p> <p>Did you evaluate potential probiotic properties of these strains and/or their capacity to form biofilms?</p>	
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**PART 2:**

	<b>Reviewer's comment</b>	<b>Author's comment</b> <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>
<b>Are there ethical issues in this manuscript?</b>	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	

**Reviewer Details:**

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