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Journal Name:	Journal of Scientific Research and Reports
Manuscript Number:	Ms_JSRR_113002
Title of the Manuscript:	FORMULATION OF CULTURE MEDIUM FOR THE ENUMERATION OF LACTIC STREPTOCOCCI AND LACTOBACILLI FROM FERMENTED MILK PRODUCTS
Type of the Article	

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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. Is the manuscript important for scientific community? (Please write few sentences on this manuscript) 2. Is the title of the article suitable? (If not please suggest an alternative title) 3. Is the abstract of the article comprehensive? 4. Are subsections and structure of the manuscript appropriate? 5. Do you think the manuscript is scientifically correct? 6. Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form. <u>(Apart from above mentioned 6 points, reviewers are free to provide additional suggestions/comments)</u>	1. Yes, but it should be precisely reviewed. A great problem with this manuscript is the long sentences which must be rephrased. Also, the section of M & M needs additions regarding the methods. 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes, but could be updated. -----	1.Ok, it will be taken care 2. Yes 3. Yes 4. Yes. 5. Yes 7. Yes updated and one more reference (number 32) of 2023 added in discussion and reference section: [32] Kim E, Lee SY, Gwak YS, Kim HJ, Kwak HS, Kim HY. Comparative Evaluation of Culture Media for Quantification of Lactic Acid Bacteria in Various Dairy Products. Microbiol. Biotechnol. Lett. 2023; 51(1): 10–17
Minor REVISION comments 1. Is language/English quality of the article suitable for scholarly communications?	Yes	Yes
Optional/General comments	<p>Please note that through the article: words highlighted with blue is used for deletion, while the red color is used for modifications and corrections.</p> <p>ABSTRACT</p> <p>Conclusion: The selective yeast glucose agar with 0.8 per cent and sodium benzoate of 1.2 per cent with 0.05 per cent BCP with purple colonies of lactic streptococci and yellow colonies of lactobacilli can be successfully used for the enumeration of mixed lactic flora in fermented milk products instead of M17 medium for lactic streptococci and MRS for lactobacilli separately. A too long sentence, please modify:</p> <p>Conclusion: The selective yeast glucose agar with 0.8 per cent and sodium benzoate of 1.2 per cent with 0.05 per cent BCP with purple colonies of lactic streptococci and yellow colonies of lactobacilli can be successfully used. This study verified the enumeration of mixed lactic flora in fermented milk products instead of M17 medium for lactic streptococci and MRS for lactobacilli, separately.</p> <p>INTRODUCTION</p> <p>Lines 18 & 19 Lactic acid bacteria are among the most important groups of microorganisms used in food</p>	Made all the corrections as suggested by the reviewer and shown in this as well as made yellow colour for showing the corrections in the manuscript <p>Conclusion: The selective yeast glucose agar with 0.8 per cent and sodium benzoate of 1.2 per cent with 0.05 per cent BCP with purple colonies of lactic streptococci and yellow colonies of lactobacilli can be successfully used. This study verified the enumeration of mixed lactic flora in fermented milk products instead of M17 medium for lactic streptococci and MRS for lactobacilli, separately.</p>

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	<p>fermentation. Lactic acid bacteria (LAB) are a group of Gram positive, non-spore forming cocci or rods, catalase negative and fastidious organism with high tolerance of low pH 5.5 to 5.8..... Please modify:</p> <p>Lactic acid bacteria (LAB) are among the most important groups of microorganisms used in food fermentation. LAB are a group of Gram positive, non-spore forming cocci or rods, catalase negative and fastidious organism with high tolerance of low pH 5.5 to 5.8^[1]. However, some members of the Lactobacillus genus can grow in pH varying between 4.5 and 6.5, whereas, some strains can grow in more lower pH values.</p> <p>Line 24 fermentation released by Lactic Acid Bacteria (LAB) due to their well-known status as GRAS Please don't repeat: fermentation released by LAB due to their well-known status as GRAS</p> <p>Line 29 The LAB could be mainly divided into two groups</p> <p>Lines 32 & 33 acidophilus produce lactic acid as the major or sole end product of glucose fermentation. Whereas, the heterofermentative group produce lactic acid, other acids, and even gases</p> <p>Line 36 single culture medium though selective M17 and MRS agar or general-purpose medium like yeast glucose add a dash</p> <p>Line 43 log(cfu)/mL and 4.14 log(cfu)/mL in cow and goat raw milk, respectively^[3]. add a comma before respectively.....</p> <p>Lines 95 & 96 nalidix acid (inhibit gram negative bacteria), ascorbic acid and yeast extract stimulate the growth of lactic streptococci and The pH indicator bromocresol purple enabled</p> <p>nalidix acid (inhibit Gram negative bacteria), ascorbic acid, and yeast extract stimulate the growth of lactic streptococci and the pH indicator bromocresol purple enabled</p> <p>Lines 106-110 count of more than 9 log^[8]. Yoghurt samples showed higher counts of lactic acid bacteria (LAB) that ranged from 1.0 × 10⁶ to 5.6 × 10⁷ cfu/mL and 2.2 × 10⁷ to 5.4 × 10⁸ cfu/ both at aerobic and anaerobic condition using MRS agar (pH 6.2-6.6) and Rogosa agar (pH 5.2- 5.6) for total LAB enumeration as well as lactobacilli isolation while YGLA (Yeast Glucose Lactic agar pH 7.0) for the isolation of streptococci incubated at 37 °C for 48-72 h both at aerobic and anaerobic conditions. Please avoid the long sentences, and rephrase.</p> <p>Line 115 bacteria^[20].(Nwamaioha <i>et al.</i>, 2017). Please check.</p> <p>And Line 120 respectively Nishino <i>et.al.</i> (2018)^[21]. Is it numbered or by the name?? respectively^[21].</p> <p>Line 128 & Line 140 retarded in this modified medium compared with their growth in MRS. The results thus suggested that</p>	<p>Lactic acid bacteria (LAB) are among the most important groups of microorganisms used in food fermentation. LAB are a group of Gram positive, non-spore forming cocci or rods, catalase negative and fastidious organisms with high tolerance to pH^[1]. However, some species of the Lactobacillus genus can grow in pH varying between 4.5 and 6.5, whereas, some strains can grow in more lower pH values.</p> <p>fermentation by LAB due to their well-known status as GRAS</p> <p>The LAB could be mainly divided into two group</p> <p>Where as the heterofermentative group</p> <p>general-purpose</p> <p>goat raw milk, respectively</p> <p>nalidix acid (inhibit Gram negative bacteria), ascorbic acid and yeast extract stimulate the growth of lactic streptococci and the pH indicator bromocresol purple</p> <p>than 9 log^[8]. Yoghurt samples showed higher counts of LAB</p> <p>Yoghurt samples showed higher counts of LAB that ranged from 1.0 × 10⁶ to 5.6 × 10⁷ cfu/mL and 2.2 ×</p>
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	<p>molds by leakage of PMF and thus ATP synthesis of fungi was affected^[23, 24]. Please add comma before and after thus. The results, thus, suggested that</p> <p>Please clarify the aim of the study in the end of Introduction.</p> <p>MATERIAL AND METHODS This section must be expanded with more methods for clarification. Line 152 were incubated at 30° C/48 h and 37 °C/48 h in anaerobic candle jar respectively. were incubated at 30°C/48 h and 37°C/48 h in anaerobic candle jar, respectively. Please add comma before respectively.</p> <p>Please add a part clarifying the statistical analysis used</p> <p>RESULTS AND DISCUSSION Lines 173-176 Fermented products such as market samples of curd, yoghurt and domestic curd samples were enumerated using Formulated (each ingredient was weighed and medium prepared -FM) and Readymade (powder reconstituted - RM) media like M17 for lactococci as well as <i>Streptococcus thermophilus</i> and MRS media for lactobacilli incubated in candle jar at 30°C and 37°C respectively for 48 h. A too long sentence, please modify:</p> <p>Fermented products such as market samples of curd, yoghurt, and domestic curd samples were enumerated. Treatments were performed using Formulated (each ingredient was weighed and medium prepared -FM) and Readymade (powder reconstituted - RM) media like M17 for lactococci as well as <i>Streptococcus thermophilus</i>, while MRS media was used for lactobacilli. All media were incubated in candle jar at 30°C and 37°C, respectively for 48 h.</p> <p>Lines 176-180 The viable count of lactococci on formulated M17 medium in fermented milk samples ranged from 7.53 to 8.88 log₁₀cfu/g whereas highest count was found in yoghurt of about 8.88 log₁₀cfu/g while market curd sample hadlow count of 7.53 log₁₀cfu/g. Please modify: The viable count of lactococci on formulated M17 medium in fermented milk samples ranged from 7.53 to 8.88 log₁₀cfu/g (Table 1). The highest count was found in yoghurt of about 8.88 log₁₀cfu/g, while market curd sample hadlow count of 7.53 log₁₀cfu/g.</p> <p>Line 183 one market curd sample (Table 1). Viable count</p> <p>Please delete this part and modify it to add under Table 1: ➤ CD – Critical difference</p>	<p>10⁷ to 5.4 × 10⁸ cfu/ both at aerobic and anaerobic condition using MRS agar (pH 6.2-6.6) and Rogosa agar (pH 5.2- 5.6), respectively for total LAB enumeration as well as lactobacilli isolation. YGLA (Yeast Glucose Lactic agar pH 7.0) was used for the isolation of streptococci incubated at 37 °C for 48-72 h both at aerobic and anaerobic conditions (Split into 2 sentences)</p> <p>Reference name removed</p> <p>Reference name removed</p> <p>The concentration of 0.1-0.3 per cent inhibited yeast and molds which the studies presumed due to leakage of PMF, thus, ATP synthesis of fungi was affected^[23, 24]</p> <p>The focus of the present study was to formulate a single medium for lactic streptococci and lactobacilli in fermented milk products along with curbing the growth of contaminants such as spore formers and fungi (Added the aim of the study)</p> <p>Material and Methods – Expanded with subheadings</p> <p>Added the statistical analysis as one paragraph</p> <p>Fermented milk products such as market samples of curd, yoghurt and domestic curd samples were enumerated. Treatments were performed using Formulated media (FM) and Readymade media (RM) like M17 for lactococci as well as <i>Streptococcus</i></p>
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	<p>➤ For lactococci M17 medium (formulated and readymade media) was used with incubation at 30°C /48 h and lactobacilli using MRS medium in candle jar at 37°C/48 h</p> <p>➤ Same superscripts in the column indicate non-significance while different superscripts indicate significance difference</p> <p>• CD, Critical difference; all the values are average of three trials; same superscripts in the column indicate non-significance; different superscripts indicate significance difference; lactococci M17 medium (formulated and readymade media) was used and incubated at 30°C/48h, and lactobacilli using MRS medium in candle jar at 37°C/48 h.</p> <p>Lines 198-202 Among formulated and readymade media used for lactococci (M17 agar) and lactobacilli (MRS agar) from fermented milk products,readymade media gave better recovery of viable cells of lactic acid bacteria, which might be due to use of pure forms of ingredients in the dehydrated media compared to formulatedones where each ingredient is weighed which was time consuming. Readymade media are easy to prepare as it was just reconstitution and warming and do not require any pH adjustment which was not true with The underlined sentence is too long, please modify, and avoid repeating of the word which</p> <p>Please go through the article to separate some words such as:</p> <p>Line 198 Among formulated and readymade mediaused</p> <p>Line 200 might be due to use of pure forms of ingredients in the dehydrated media compared to formulated ones, formulated ones</p> <p>Line 201 where each ingredient is weighed which was time consuming. Readymade media areeasy to prepare ..., are easy to prepare</p> <p>Line 215 Market yogurt showed more acidity of 1.10 while domestic curd sample had loweracidity of 0.68, lower acidity</p> <p>Line 236 yeasts that appearedas surface colonies on both the media., appeared as</p> <p>Lines 215 & 216 Market yogurt showed more acidity of 1.10 while domestic curd sample had loweracidity of 0.68 per cent, with range of 0.68 to 1.10 per cent lactic acid in fermented milk products (Table 2). Please add her.</p> <p>Lines 233-236 7.80, 7.50 and 8.00 in market curd, yogurt and domestic curd samples respectively. Reduction in counts was noticed in both M17 and MRS agar after incorporation of calcium propionate and sodium benzoate at 1.5 per cent each and 2 percent each, respectively helped in control of both aerobic spore formers and yeasts that appearedas surface colonies on both the media. A too long sentence, please modify:</p>	<p>thermophilus while MRS media was used for lactobacilli. The poured plates of lactococci and Streptococcus thermophilus as well as lactobacilli were incubated in candle jar at 30°C and 37°C, respectively for 48 h.</p> <p>The viable count of lactococci on formulated M17 medium in fermented milk samples ranged from 7.53 to 8.88 log₁₀cfu/g (Table 1). The highest count was found in yogurt of about 8.88 log₁₀cfu/g while market curd sample had low count of 7.53 log₁₀cfu/g</p> <p>One of the collected market yogurt sample showed high lactic acid of 1.02 per cent while one sample of domestic curd had lower acidity noticed of 0.68 per cent (Table 1)</p> <p>• CD, Critical difference; all the values are average of three trials; same superscripts in the column indicate non-significance; different superscripts indicate significance difference.</p> <p>The Readymade media used for pour plating gave better recovery of viable cells of lactic acid bacteria, from fermented milk samples due to use of pure forms of ingredients. The preparation of readymade media was easy as it was only reconstitution, no pH regulation followed by sterilization. Formulated media preparation on the other hand was time consuming as each ingredient to be weighed, dissolved in water, pH adjustment and then sterilization. Apart from that, recovery rate in formulated media was lower.</p> <p>Taken care to separate the words</p> <p>Corrected</p>
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	<p>Reduction in counts was noticed in both M17 and MRS agar after incorporation of calcium propionate and sodium benzoate at 1.5 percent, and 2 percent, respectively. Such treatment helped in control of both aerobic spore formers and yeasts that appeared as surface colonies on both the media.</p> <p>CONCLUSION</p> <p>Line 434 purpose medium yeast glucose agar (YGA) to selective and differential medium for bo?? the microflora</p> <p>Lines 437-441 The viable lactic streptococci formed purple colonies while yellow colonies of lactobacilli as a measure of differentiation based on acid production in case of mixed lactic flora in fermented milk products containing both lactic cocci and lactobacilli, the inhibitors calcium propionate and sodium benzoate inhibited the most common microbial contaminants of fermented milk products, aerobic spore formers and yeasts, respectively. A very long sentence, please modify.</p>	<p>corrected</p> <p>Market yogurt showed more acidity of 1.10 while domestic curd sample had lower acidity of 0.68 per cent, with range of 0.68 to 1.10 per cent lactic acid (Table 2) in fermented milk products</p> <p>Reduction in counts was noticed in both M17 and MRS agar after incorporation of calcium propionate and sodium benzoate at 1.5 percent, and 2 percent, respectively. Such treatment helped in control of both aerobic spore formers and yeasts that appeared as surface colonies on both the media</p> <p>, purpose medium yeast glucose agar (YGA) to selective and differential medium for both the microflora</p> <p>One sentence split into two The viable lactic cocci formed purple colonies while yellow colonies of lactobacilli as a measure of differentiation based on acid production in case of mixed lactic flora in fermented milk products. The inhibitors calcium propionate and sodium benzoate incorporated in the YGA inhibited the most common microbial contaminants in fermented milk products, aerobic spore formers and yeasts, respectively.</p>
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PART 2:

	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)
Are there ethical issues in this manuscript?	(If yes, Kindly please write down the ethical issues here in details)	No ethical issues involved in this study