Effect of different milk based media on lactic acid production and growth characteristics of bacteria isolates from vended soft cheese

Oluwadamilare Samuel Fawole & John Onolame Unuofin

1Institute of Child Health, College of Medicine, University of Benin, Benin, Nigeria
2Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa

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Lactic acid production has been traditionally practiced by locals worldwide for centuries. The discovery of its relevance in various industries, particularly the food industry, and in biopolymer production, has called for its economic production from suitable, cheap feedstocks like agroindustrial byproducts. This study, therefore, investigated the growth and lactic acid production patterns of some lactic acid bacteria (LAB) isolated from a named west African fermented food (Waraka) in different dairy media. The study was conducted using submerged fermentation, where whole milk, skim milk medium, cheese whey and non fat milk were assessed as suitable substrates, with respect to the varying temperature regimes (30, 37 and 42°C), over 48 h. The results revealed that (i) milk remains a vital source for lactic acid bacteria; (ii) medium components and environmental conditions are contributory in growth and lactic acid production of lactic acid bacteria in the different media; (iii) the active growth might not necessarily depict increased lactate production; (iv) the species of a genus might show similar population dynamics and physiological characteristics depending on the environment they exist; and (v) the dairy waste should be incorporated in large scale lactic acid production to improve the biomass yield.

Keywords: Cheese, Submerged fermentation, Waraka, Waste valorization

Materials and Methods

Sample
Waraka were purchased from Ojoo market, Iwo road and Bodija market in Ibadan, Nigeria. The different raw milk samples were collected from Kara at Moniya in Ibadan, Nigeria. The samples were collected in sterile ziploc bags and sample bottles and immediately transported to the laboratory for analysis.

Isolation of lactic acid bacteria
The sample (1 g of waraka) was introduced into sterile pestle and mortar, macerated, and 9 mL of
sterile distilled water was added and homogenized by thorough shaking; serial dilution was then carried out\textsuperscript{21}. Using pour plate method, 1.0 mL of the sample dilutions were aseptically pipetted into sterile prelabelled Petri plates, where molten sterile MRS agar medium was poured aseptically, gently swirled for even inoculum distribution and allowed to cast by cooling. Thereafter, the plates were inverted and maintained in an incubator at 35°C for 48 h, microaerophilically. Following incubation, the plates were observed for microbial growth and representative distinct colonies were subcultured by repeated conventional streaking to obtain pure culture which were preserved on MRS agar slant at 4°C for further analysis.

Identification of isolates

The isolates were identified based on conventional biochemical tests and by molecular methods, respectively\textsuperscript{22,23}. Their 16S rRNA sequences were submitted to the Genbank RNA database and the Basic Local Alignment Search Tool (BLAST) search algorithm was used to estimate their similarity.

Fermentation medium and conditions

Different milk based single substrates media viz. whole milk, cheese whey and non-fat milk and skim milk based medium (peptone supplemented) were inoculated with the identified bacterial isolates during a 48 h study at three different temperature regimes (30, 37 and 42°C). The operational volume for growth and lactic acid production studies was 200 mL.

Growth measurement

Growth of the choice isolates was estimated spectrophotometrical reading of Optical Density at 650 nm wavelength after appropriate dilution of aliquots obtained from the respective fermentations\textsuperscript{24}.

Quantitative estimation of lactic acid

The production of lactic acid was determined by titration with 0.25 mol/L NaOH using 1 mL of phenolphthalein indicator (0.5% in 50% alcohol). The titrable acidity was calculated as percentage lactic acid (%v/v). One milliliter of 1N NaOH is equivalent to 90.08 mg of lactic acid\textsuperscript{24}. The titrable acidity was calculated with the equation below:

\[
\text{% acid (wt/vol)} = \frac{N \times V_1 \times \text{Eq wt} \times 100}{V_2 \times 1000}
\]

where \(N\) = normality of titrant (NaOH), \(V_1\) = volume of titrant (NaOH), \(\text{Eq wt}\) = equivalent weight of acid (mg/mEq), \(V_2\) = volume of sample titrated (mL), and 1000 = factor relating mg to gram (mg/g).

Statistical analysis

The experimental data was analyzed using Analysis of Variance (ANOVA) to determine significant difference between the means and these were expressed as mean ± standard deviation (SD). The level of significance was set at \(P \leq 0.05\). The data were analysed using SPSS version 17.0.

Results and Discussion

Wara, a soft cheese prepared by spontaneous fermentation, is a highly perishable traditionally fermented food with a shelf life spanning 2-3 days at ambient conditions (30°C). Hence, it serves as a major source of bacterial diversity. Early stages of its fermentative production accommodates a diverse range of genera of microorganisms, which are reduced due to successional changes, such as low pH that occur as fermentation progresses. These conditions afford only acidophilic or acidotolerant organisms to thrive. LAB have been reported as prominent indicators of natural fermentation of African foods\textsuperscript{25}. In this study, all samples recorded a considerable amount of LAB isolates with the highest percentage occurring in samples purchased at Ojoo area of Ibadan, Nigeria (data not shown). Similar outcomes were recorded from samples purchased from local cheese vendors/hawkers in Abeokuta\textsuperscript{26}, Iwo\textsuperscript{27} and Okada\textsuperscript{28} in Nigeria, respectively. The selected isolates P9, P10, P11 and P13 were further identified to molecular level as Streptococcus macedonicus P9, Streptococcus infantarius P10, Enterococcus durans P11 and Enterococcus faecium P13 while their 16S rRNA gene sequences have been submitted to GenBank database and have been accorded the accession numbers KM504971, KM504972, KM504973 and KM504974, respectively. The proliferation of their cells was investigated in different milk based media viz.; whole milk, cheese whey, skim milk medium and non-fat milk at different incubation temperatures (30, 37 and 42°C).

During the microbial metabolism of substrates and/or their conversion to valuable or environmental friendly products and the associated proliferation of biomass, the interaction of biochemical and physiological processes generate intermediate products which are characteristic of the different phases of microbial growth. One of such phases is the tropophase which marshals exclusively the production of substances crucial to microbial growth; organic acids, nucleotides and amino acids. Whole milk and skin
milk medium jointly supported the growth of the all the isolates with their growth rate increasing with increasing temperature (Table 1). This is characteristic of thermophilic bacteria which originate from traditional fermented products in subtropical countries\(^\text{29}\). This could be due to the fact that milk is one of the natural habitats of lactic acid bacteria\(^\text{30}\) presumably because of its contents of amino acids, dipeptides, tripeptides and oligopeptides in high amounts\(^\text{31}\). Furthermore, its casein content, which switches on the proteolytic system, is a crucial factor to the metabolism and growth of LAB\(^\text{32,33}\). The skim milk medium presented better LAB growth irrespective of strain compared to non-fat milk medium due to the inclusion of peptone which serves as the limiting factor. Furthermore, peptone could be observed as a more readily metabolized nitrogen source compared to other components and could possibly trigger a second exponential growth phase within the isolates\(^\text{31}\). Similarly, whey could not support rapid proliferation due to lack of sufficient low molecular weight nitrogen which is the vehicle behind the growth of many industrial microorganisms\(^\text{34}\). In addition, peptides derived from whey proteins have been reported to have antibacterial activity and have been accorded much discuss due to their high activity against a wide range of Gram-positive and Gram-negative bacteria\(^\text{35}\).

Lactic acid fermentation has been accorded good credence due to its close synergy with food related applications. This study, therefore, investigated its production from four different media at 30°C. Whole milk media and non-fat milk media vigorously supported the increase in lactic acid production of all species between 24 and 36 h, respectively, represented by a display of an exponential increase (Fig. 1). This highlights the importance of the fermentability of raw milk by LAB to enhance its organoleptics and most importantly, prolong its shelf life. The non-fat milk, notwithstanding its poor support of biomass proliferation, contributed to the generation of high amounts of lactic acid. This, however, is not in congruence with the universal understanding that increase in biomass is synchronous with acidification activity; an indication that organic acid production is affected by media composition\(^\text{36}\).

It was also observed that all Streptococcus species showed consistent production of lactic acid in all the tested media over 36 h suggesting their active involvement in probiotic and preservative formulations. Conversely, the Enterococcus species showed a somewhat similar production pattern in all

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**Table 1 — Growth (OD650) of LAB in different milk-based media at different temperature regimes**

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Whole Milk</th>
<th>Cheese Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30°C</td>
<td>37°C</td>
</tr>
<tr>
<td><em>S. macedonicus</em></td>
<td>1.586±0.0003(^a)</td>
<td>1.856±0.0006(^d)</td>
</tr>
<tr>
<td><em>S. infantarius</em></td>
<td>1.746±0.0004(^d)</td>
<td>1.560±0.0006(^b)</td>
</tr>
<tr>
<td><em>E. durans</em></td>
<td>1.729±0.0006(^c)</td>
<td>1.758±0.0003(^a)</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>1.648±0.0001(^b)</td>
<td>1.510±0.0007(^b)</td>
</tr>
</tbody>
</table>

[Value of means of replicate readings ± SD. Mean value with different alphabets in superscript along the column are significantly different (P ≤ 0.005)]

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![Fig. 1 — Lactic acid production by LAB from different milk-based media; (A) whole milk; (B) cheese whey; (C) skim milk; and (D) non-fat milk.](image-url)
the media tested; they elicited a relatively high lactic acid activity at 48 h in all the media tested except the cheese whey medium. This is characteristic of the bottlenecks encountered during the batch production of lactic acid by cheese whey fermentation\textsuperscript{37}.

Conclusion

It was observed during the course of investigation that whole milk, due to its abundance of nutrients supported the growth of all isolates tested, yielding absorbance (OD\textsubscript{650}) ranging from cca 1.56 to 1.87. Also, growth and lactic acid production varied with regards to species and environmental factors. However, species of a genus showed seemingly similar growth trends, hence establishing the basis of their phylogenetic relationship. Notwithstanding the poor but considerable support of whey, further strategies should be developed to encourage its employment in large scale production of lactic acid, as it is the most abundant component of milk discarded as waste. This would alleviate the serious pollution problems it causes for the surrounding environment.

Conflict of Interest

Authors report no potential conflict of interest.

References

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