Isolation of lactic acid bacteria from Kindirmo and acidity of yoghurt

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Abstract

Milk and milk products are promising sources of energy to meet the global increase in food demand. Lactic acid bacteria are the key organisms in starter culture for milk fermentation. Lactobacillus bulgaricus and Streptococcus thermophilus from “kindirmo” have been isolated using the Man, Rogosa and Sharpe (MRS) a selective medium for lactic acid bacteria. Kindirmo is a kind of local yoghurt dominantly consumed in sub Saharan Africa and sold by Hausa-Fulani women in Nigeria. The starter culture was obtained from Fulani women hawking kindirmo in Federal University of Technology, Yola and fresh milk from local herdsman. The strains were identified according to their morphological, cultural, physicochemical and biochemical characteristics. Two isolates were able to grow on MRS medium. The isolate that was able to grow at 30°C, pH 5.4 and 6.3 was Lactobacillus bulgaricus, while the one identified as Streptococcus thermophilus does not show any growth at pH 5.4 and temperature of 42°C. The colonies revealed big sized, circular, irregular, creamy grey colonies indicative of Lactobacillus bulgaricus and Streptococcus thermophilus bacteria. There is need to exploit the potential in using the Fulani starter culture for increased yoghurt production using biotechnological techniques such as rational design using site-directed mutagenesis.

Key words: Kindirmo; Lactobacillus bulgaricus; mixed cultures; Streptococcus thermophilus; starter culture

INTRODUCTION

Livestock production contributed about 12.7% of the agricultural gross domestic product in Nigeria and the gap between supply and demand for dairy products is widening as a result of increase in population and urbanization (Yahuza, 2001). About 90% of the dairy cattle in Nigeria belong to Fulani agropastoralists and Fulani women strictly controlling the processing and marketing of their milk (Umoh and Oranusi, 2001).

Lactic acid bacteria belong to a group of food fermenting bacteria that produce lactic acid (C3H6O3) as their major metabolic product during sugar fermentation. Typically, Lactic acid bacteria are Gram positive, catalase negative, non-spore forming and devoid of cytochromes. Mostly grown at temperatures 5 to 45°C and acid tolerant able to survive at pH 4.4. The optimum growth is at pH 5.5-6.5 (Bergey, 1934; Buchanan and Gibbons, 1974; Reddy et al., 2008). Yoghurt is the most widely available milk products not only in Africa but in the Western world today; where its popularity derives more from its flavour and versatility than from its keeping qualities (Adams and Moss, 1995; Fashakin and Unokiwedi, 1993). The shelf-life of milk and quality depend on measures such as heating of raw milk to kill pathogens and spoilage organisms, use of controlled fermentation, starter cultures and short incubation period with a fast drop in pH (Umoh and Oranusi, 2001).

Lactic acid bacteria (probiotics) are exclusively consumed as fermented dairy products such as yoghurt, cheese, freeze-dried cultures or other dairy products (Reddy, et al., 2008; Roberfroid, 2000). It was observed that lactic acid bacteria lower carbohydrate content and the pH of food they ferment due to lactic acid production. This acidification process is the most desirable effects of lactic acid bacteria. The drop in pH, to as low as 4.0 is low enough to inhibit the growth of most other microorganisms including the most common human pathogens, thus allowing these foods prolonged shelf-life. The acidity also changes the texture of the foods due to
Table 1. Identification of Lactobacillus bulgaricus and Streptococcus thermophilus Isolates from “Kindirmo”

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>L. bulgaricus</th>
<th>S. thermophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on MRS agar (pH 6.3)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth on MRS agar (pH 5.4)</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>Temperature of incubation</td>
<td>30°C</td>
<td>42°C</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Grams reaction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>rods</td>
<td>cocci</td>
</tr>
<tr>
<td>Colony size</td>
<td>Small</td>
<td>big</td>
</tr>
<tr>
<td>Colony shape</td>
<td>circular, irregular</td>
<td>circular, irregular</td>
</tr>
<tr>
<td>Colony color</td>
<td>creamy grey</td>
<td>creamy white</td>
</tr>
</tbody>
</table>

Key: + means positive; - means negative

precipitation of some proteins, and the biochemical conversions involved in growth enhancing flavour.

Peter and Ann (1992) described the technology of cultured milk production as being based upon the conversion of milk sugar (lactose) to either lactic acid alone or in combination with other products. Milk can sour naturally but is better to introduce appropriate bacteria as a starter culture in the form of small quantities of previously cultured product or commercially prepared cultures.

Fulani women have been using their starter cultures for years with little or no attempt in identifying the fermentation factors. There was also no attempt to characterize and improve the starter culture for possible increase in product. Similarly, there is only little information on the isolation and characteristics of the kindirmo available to the researcher. Yet, scientific communities all over the world are committed in search of lactic acid bacteria with desirable physiological properties (Steinkraus, 2002). The objectives of this work were to isolate the lactic acid bacteria indigenous to Fulani kindirmo, characterise it and determine the physicochemical properties of yoghurt produced using the isolates.

MATERIALS AND METHODS

“Kindirmo” starter culture was obtained from Fulani women hawking “kindirmo” in Modibbo Adama University of Technology, Yola. The fresh milk employed for the study was obtained from Mallam Bakari (Fulani herdsman) at Bajabure, Girei Local Government Area, Adamawa State, Nigeria.

Isolation of Lactic Acid Bacteria

Lactic acid bacterial strains were isolated from “kindirmo” culture (Dilli et al., 2010) on a selective medium for lactic acid bacteria (De Man et al., 1960). The strains were further characterized using microscopic observation according to their cell morphology, colony size shape and color. While physicochemical and biochemical characteristics were determined using standard methods. Stock cultures were maintained at 4°C on MRS medium for further use.

Colony morphology was observed on MRS agar plate and cell morphology was performed according to Ridge (1982). For each culture Gram staining was performed. Catalase test was performed according to Schierl and Blazevic (1981). A drop of 3% hydrogen peroxide was placed on a glass slide. A sterile tooth pick was used to remove a speck of the test strain and immersed in hydrogen peroxide solution. The production of gas bubbles from the surface indicated a positive reaction while a negative test did not show bubbling or frothing. Milk fermentation and measurement of titratable acidity were done as earlier described by Harrigan (1998) and Tserovska et al. (2002).

RESULTS AND DISCUSSION

The result for identification of lactic acid bacteria (LAB) isolated (Table 1) shows that two isolates were able to grow on MRS agar at pH 6.3 (30°C and 42°C) and at 30°C, pH 5.4. Streptococcus thermophilus strain did not show growth on the same media at pH 5.4 when grown at 42°C. The organisms are Gram positive and showed negative catalase activity. Microscopic examination of the isolates revealed rods and cocci like cells that showed no catalase activity and are Gram positive.

Growth of isolate on MRS agar, the selective media for lactic acid bacteria(De Man, et al., 1960 ) at pH 6.3, incubation temperature of 42°C and pH 5.4, incubation temperature of 30°C revealed big sized, circular, irregular, creamy grey colonies indicative of Lactobacillus bulgaricus and Streptococcus thermophilus bacteria respectively. The level of acidity produced as a result of incubation of milk inoculated with Lactobacillus bulgaricus and Streptococcus thermophilus for 0 to 24 h period
(Figure 1) indicates that the acidity of kindirmo produced by these lactics increases with time. (Buchanan and Gibbons 1974; Leisner et al., 1999; Togo et al., 2002; Tserovska, et al., 2002; Varnam, 2002). reported on acidity of fermented milk sold in Zaria. The acidity produced by Streptococcus thermophilus is slightly greater than the one produced by Lactobacillus bulgaricus under the same conditions.

These lactics (Lactobacillus bulgaricus and Streptococcus thermophilus) might possess potential benefit when used as mixed cultures for kindirmo production. This may lead to accumulation of glycine and histidine that could produce synergistic effect on acid lactic acid production. Lactic acid is an important product of milk fermentation which occupy a prime position in industries have wide applications in food, pharmaceutical, leather, textile industries and chemical feedstock for many other chemicals (Bautista et al., 1966; John et al., 2009; Reddy, et al., 2008). Biotechnological improvement of kindirmo isolates through manipulation of fermentation gene will serve as a promising potential for production of lactic acid from milk.

CONCLUSION

The present study indicates that kindirmo starter culture contains Lactobacillus bulgaricus and Streptococcus thermophilus lactics that are usually responsible for fermentation of milk. The acidity of the yoghurt by combination of these isolates might produce a better product. There is a need therefore for the improvement of these organisms and their subsequent exploitation for industrial production of “kindirmo”, cheese and other dairy products.

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REFERENCES