Comparative influence of medium composition on biomass growth, lactic acid and Exopolysaccharides Production by some Strains of Lactic Acid Bacteria

B Adebayo-Tayo, A Onilude

Abstract

To promote the overall quality of fermented dairy products (FDP) which depends on the types and characteristics of the starter organisms used in the production, the effect of medium composition on biomass growth, viscosity, exopolysaccharide and lactic acid production by EPS-producing lactic acid bacteria isolates was investigated. Medium composition had profound effect on the studied parameters. Among the five medium used for the cultivation of the isolates, maximum biomass growth and viscosity production was achieved in partially de-protenised whey medium (PDW) in which Lactococcus piscium (OLHF6) and Lactobacillus plantarum (LPN1) exhibit the highest biomass growth and viscosity with concentration of 1.58 and 1.561mPa.S. Lactobacillus plantarum (LPN1) (1.58mPa.S), L.casei (LCN1) (1.475 mPa.S), L.plantarum (LPN3) (1.48mPa.S), L.plantarum (LPY80 (1.48mPa.S) and Lactococcus piscium (OLHF6) (1.185mPa.S) was found to be a good starter for development of viscous medium in the respective media. Whey was the best medium for EPS production in which L. casei (LCN1)) was the best starter (198.69mg/l). Sweet whey medium, modified MRS medium, Whey and partially deproteinised whey medium was not favourable for lactic acid production by the isolates but Semi-define medium was the best for lactic acid development in which L.plantarum (LPW10) was found to be the best starter for lactic acid development (5.57g/l).

INTRODUCTION

The overall quality of fermented dairy products (FDP) depends on the types and characteristics of the starter organisms used in the production. The essential criteria for starter selection include acidification, aroma, flavour, stability and texture. Manufacturers can improve the quality of their FDP by substituting stabilizers with EPS-producing lactic acid bacteria (LAB) as starter. Lactic acid bacteria (LAB) are a group of gram-positive bacteria which produce lactic acid as the major end products of fermentation of carbohydrates (Axelsson, 1998). The growth of LAB is often accompanied by the production of polysaccharides, which are found outside the cell wall. These exopolysaccharides (EPS) may be found as capsule attached to the bacteria or they may be released to the environment as slime or both (Sutherland, 1977). Polysaccharides even though important to bacteria for adhesion, infection and protection may as well have commercial value. Some polysaccharides are known to have gelling properties that is; agar and gel rite (Davis et al., 1980; Lin and Casida, 1984). Others have emulsifying properties (Kaplan and Rosenberg, 1982; Sar and Rosenberg, 1983) and still others may be amalgans of certain important monosaccharides. In the dairy industry, the slime-forming LAB strains have traditionally been used in the production of fermented milk products, e.g. yoghurts, Finnish ‘villi’ and Scandinavian ‘langfil’. Preparation of fermented foods using lactic acid bacteria has been suggested to improve the acceptability of such foods (Hang and Jackson, 1967; Matsouka et al., 1968, Mital and Steinkraus, 1974). Futhermore, Pinthong et al., (1980) reported that lactic acid bacteria could also lead to products with sufficient acidity (low pH) for good keeping properties. The production of a reasonable level of acidity will also help to improve the flavor of the product. The use of EPS-producing LAB is an alternative way of improving the texture and stability of fermented dairy products.

This study was therefore aimed at investigating the effect of medium composition on some strains of lactic acid bacteria for selecting LAB strains with good biomass growth, high production of viscosity, lactic acid and exopolysaccharide.
MATERIALS AND METHODS

CULTURE COLLECTION

The pure cultures of the EPS-producing LAB isolates made up of 16 Lactobacillus spp., 3 Leuconostoc spp. and 1 Lactococcus sp. obtained from the various sources as reported in a previous work (Adebayo –Tayo and Onilude, 2008) were subcultured onto maintenance medium consisting of MRS broth with 12% (v/v) glycerol and incubated at 30°C until growth becomes visible. The stock cultures were stored at – 4°C for subsequent use and subcultured at 4 weeks interval.

INOCULUM PREPARATION

The working cultures were prepared by transferring 0.5ml of the stock frozen culture to 10ml of MRS broth and incubated for 16h at 30°C. The resulting culture was transferred (2% v/v) to modified exopolysaccharide selection medium (mESM) (van den Berg et al., 1993) containing 5% (w/v) skim milk (Oxoid); 0.35% yeast extracts (Oxoid); 0.35% peptone (Difco), 5% glucose (BDH) and incubated at 30°C for 16h. 10ml inocula of the 16h old culture containing 2.5x10^6 cfu/ml were used to inoculate larger volume of the formulated medium.

FERMENTATION MEDIUM

The growth media used were; (a) whey (whey only), (b) Semi-defined medium (SDM) with the following composition (grams/liter); dextrose, 20g; Tween-80, lml; Ammonium citrate; 2g; sodium acetate, 5g Mg50_4.7H_2O, 0.1mg; MnSO_4, 0.05mg; K_2HPO_4, 2mg; yeast extract, 5g and bacto casitone, 10g; pH 6.5; (c) modified MRS medium (mMRS); (d) Partially deprotenized whey (PDW) containing 1% peptone prepared by adjusting to 4.6 using 1N HCl, the pH of the Skim milk (from raw milk by centrifugation) and removing the precipitate by centrifugation. The whey recovered was adjusted to pH 6; (e) Sweet whey made up of Lactose, 50g; MgSO_4.7H_2O, 40.7mg; MnSO_4.7H_2O, 4.0mg; FeSO_4.7H_2O, 1.4mg; CaCl_2, 0.9mg; pH 6.2, H_2O 1 liter. 100ml of each medium was dispensed into 250ml Erlenmeyer flask and autoclave at 121°C for 15mins by autoclaving. the flask were inoculated with 10ml inocula of the 16h old culture containing 2.5x10^6 cfu/ml and incubated at 30°C for 48h. Samples were taken from each flask and analyzed for pH, lactic acid concentration, cell growth, viscosity and EPS production.

ISOLATION AND PARTIAL PURIFICATION OF EPS

Partial purification of exopolysaccharide was carried out according to the method described by Gancel and Novel (1994). Ten grams of culture medium were accurately weighed into a 50ml centrifuge tube and the content was heated in a boiling water bath for 10 min to inactivate enzymes potentially capable of polymer degradation (Cerning et al. 1988 and Cerning et al. 1992). Samples were cooled to room temperature. To precipitate protein, 250l of 80% (w/v) trichloroacetic acid was added and the samples was mixed well and stored at 40°C. Cells and precipitated proteins were removed by centrifuging for 20min at 6,000xg. The clear supernatant was collected and the EPS was precipitated at 4°C by the addition of 2 volumes of ethanol (100%). The resulting precipitate was collected after centrifugation (16,000xg for 15min) and re-dissolved in distilled water. The crude exopolysaccharide solution was decanted into dialysis tubing and dialyzed for 48h against four changes of distilled water. All assays were performed in duplicate. Uninoculated medium treated similarly served as control.

TOTAL SUGAR DETERMINATION

The total sugar concentration was determined by the phenol-sulfuric acid method using glucose as a standard (Chaplin, 1986). The results are expressed in milligrams of glucose per liter.

VISCOSITY DETERMINATION

This was measured as described by Schellhass and Morris (1985), using a Haake Rotovisco Rv20 coaxial cylinder viscometer with No. 1 sensor system (Haaker, Inc, Saddle Brook; NJ) at incubation temperatures of 25, 30 and 40°C. The shear rate increased from O to 1000/sec over a 3 min period.

MEASUREMENT OF GROWTH

Growth of the test organisms was determined spectrophotometrically by taking the Optical Density reading at 650nm after appropriate dilution of fermented samples earlier incubated at 30C for 48h (A.O.A.C. 1990).

QUANTITATIVE ESTIMATION OF LACTIC ACID

The production of lactic acid was determined by titrating 10ml of the homogenized sample against 0.25mol l^-1 NaOH using 1 ml of phenolphthalein indicator (0.5 % in 50% alcohol). The titratable acidity was calculated as
percentagelactic acid (v/v). Each milliliter of 1N NaOH is equivalent to 9.008mg of lactic acid. (A.O.A.C. 1990).

RESULT AND DISCUSSION

The effect of different medium on cell growth of twenty EPS-producing LAB strains is shown in Table 1.

<table>
<thead>
<tr>
<th>ISOLATE</th>
<th>SW</th>
<th>MES</th>
<th>SDM</th>
<th>WHEY</th>
<th>PDW</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. plantarum (LPN6)</td>
<td>1.561</td>
<td>1.046</td>
<td>0.322</td>
<td>0.11</td>
<td>0.125</td>
</tr>
<tr>
<td>Lactococcus piscium (OLHF6)</td>
<td>0.294</td>
<td>0.282</td>
<td>0.322</td>
<td>0.11</td>
<td>0.125</td>
</tr>
<tr>
<td>Lactobacillus casei (LCN1)</td>
<td>0.132</td>
<td>0.099</td>
<td>0.099</td>
<td>0.217</td>
<td>0.217</td>
</tr>
<tr>
<td>L. lactis ssp. lactis</td>
<td>0.625</td>
<td>0.255</td>
<td>0.112</td>
<td>0.051</td>
<td>0.051</td>
</tr>
<tr>
<td>L. brevis (LBN1)</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
</tr>
</tbody>
</table>

The result of effect of medium composition on biomass growth by the 20 LAB isolates were in order SW>PDW>SDM>mMRS>WHEY. The biomass growth ranges from 0.0025 cfu/ml - 1.561 cfu/ml in which Lactococcus piscium (OLHF6) had the highest (1.561) in Sweet whey and Deproteinised whey respectively. In SDM the highest growth was recorded with Lactobacillus plantarum LPN6 (1.046). Generally whey, mMRS and SDM was not the best medium for biomass growth.

Among the 20 EPS producing LAB tested. Lactococcus piscium (OLHF6), Lactobacillus casei (LCN1) and Lactobacillus plantarum (LPWO10) could produce good biomass growth in SW and PDW medium (Table1). Growth medium used in this study had a profound influence on biomass production by the selected EPS producing LAB. The lowest cell biomass recorded when whey was used may be due to the fact that Whey and whey permeate have been said to lack sufficient low molecular weight nitrogen, which presents a challenge to the growth of many industrial microorganisms, so they often require supplementation (Amrane and Prigent, 1993). The ability of the isolates such as Lactobacillus plantarum to produce a very high cell biomass in whey may be because they require less complex nutritional requirement compared to other Lactobacillus species (Hammes et al., 1992).

Comparative effect of medium composition on EPS production from various LAB isolates was shown in Table 2. Medium composition had profound and significant effects on EPS production by the EPS producing LAB isolates. Comparatively, the result of the effect of medium composition on EPS - production by the 20 LAB isolates were in order Whey> SDM> mMRS>SW>PDW. The highest EPS was obtained from L. casei (LCN1) (198.69mg/l), L. plantarum (LPN6) (111.85 mg/l), Lact.lactis ssp plantarum (LPYS) (196.05) L.coprophilus (COFN1) (185.7), L.brevis (LBN1) (161.35mg/l) in the respective medium used.

There was variation in EPS production from cultures shown to be the same isolated species. For example the two Lactobacillus plantarum isolated from “nuu” produced EPS
yields from 80.85 – 189.0mg/l. Thus the amount of EPS production differs between species and varies within species of LAB which is dependent on growth medium. The maximum production of EPS was obtained when semi-defined medium was used for the cultivation of the EPS producing lactic acid bacteria strains. This agreed with the findings of Kimmel et al, (Kimmel et al. 1998) The SW and PDW medium did not support mass production of polymer. Association of low cell mass with greater polysaccharide production recorded in Whey has been explained by Sutherland (1972). It was reported that when cells were grown slowly, synthesis of cell wall polymer was also slow, making more isoprenoid phosphate available for EPS synthesis.

The higher EPS yields in mMRS during fermentation may be due to small amounts of nitrogen sources present in the media. This result agrees with the finding of Garcia-Garibay (1991) who reported the production of exopolysaccharides when the organism was grown in mMRS broth. mMRS, the usual medium for laboratory fermentation by LAB contains compounds (e.g. beef extract, peptone, yeast extract) that enhanced EPS synthesis.

The comparative effect of medium composition on lactic acid production by the isolates was shown in Table 3.

Table 3: Effect of medium composition on lactic acid production by the LAB isolates

<table>
<thead>
<tr>
<th>ISOLATE CODE</th>
<th>ISOLATE CODE</th>
<th>SW</th>
<th>MRS</th>
<th>SDM</th>
<th>WHEY</th>
<th>PDW</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. plantarum</td>
<td>LFWO10</td>
<td>3.89</td>
<td>4.13</td>
<td>4.49</td>
<td>2.34</td>
<td></td>
</tr>
<tr>
<td>L. lactis</td>
<td>LFWO12</td>
<td>4.30</td>
<td>4.19</td>
<td>4.75</td>
<td>2.42</td>
<td></td>
</tr>
<tr>
<td>L. casei</td>
<td>CFOW12</td>
<td>3.87</td>
<td>3.87</td>
<td>3.96</td>
<td>2.43</td>
<td></td>
</tr>
<tr>
<td>L. fermentum</td>
<td>LFFN3</td>
<td>2.70</td>
<td>2.70</td>
<td>2.70</td>
<td>2.70</td>
<td></td>
</tr>
</tbody>
</table>

Medium composition had significant effect on lactic acid production by the isolates. In comparism with SDM, SW and mMRS, Whey and PDW medium was not favourable for lactic acid production by the isolates. In SDM, the highest (5.57g/l) lactic acid production was recorded from Lactobacillus plantarum (LPWO10), In SW, the highest (11.08g/l) was recorded from Leu.mesenteroides spp mesenteroides (UMMY4), in mMRS the highest (7.75g/l) was recorded from L.coryniformis spp coryniformis (LCOW1) while Partially deprotenised whey supported moderate production of lactic acid by the isolates, the highest (6.03g/l) was recorded from L. lactis spp hordiniae (OLHW4). It was observed that Whey was not the best medium for lactic acid development for the isolates though there was a significant different (P< 0.05) in acid production by the isolates in which the highest (2.70g/l) was recorded from L. fermentum (LFFN3). Leu.mesenteroides spp mesenteroides (UMMY4), L.coryniformis spp coryniformis (LCOW1), L. lactis spp hordiniae (OLHW4) and Lactobacillus plantarum (LPWO10) could serve as good starter for lactic acid production in the respective media (Table 3). Lactic acid bacteria (LAB) produce lactic acid as the major end products of fermentation of carbohydrates (Axelsson, 1998). This gives fermented product more shelf-
stable quality with characteristic aroma and flavours. The fermentative ability of raw milk by lactic acid bacteria improve the organic, nutritional and hygienic qualities and also extend its shelf life. All fermented dairy produced rely for their manufacture on the growth of relatively high population of Lactobacilli whose immediate function is to convert lactose to lactic acid (Fox, 1982).

The comparative effect of medium composition on viscosity development by the isolates was showed in Table 4; it was observed that medium composition had a significant effect on viscosity development by the isolates. The entire medium used enhanced viscosity development by the isolates. PDW, Whey, Sweet whey and SDM had a profound effect on development of viscous medium. Lactobacillus plantarum (LPN1) (1.58 mPa.S), L. casei (LCN1) (1.475 mPa.S), Lactobacillus plantarum (LPN3) (1.48 mPa.S), Lactobacillus plantarum (LPY8) (1.48 mPa.S) and Lactococcus piscium (OLHF6) (1.185 mPa.S) was found to be a good starter for development of viscous medium in the respective media. It was observed that there was no significant different in viscosity between some isolates cultured in a particular medium.

Lower viscosity development in Sweet whey, deproteinised whey and whey than Semi-defined medium, may probably be as a result of the removal of the peptide and peptones in the deproteinised whey medium and non-supplementation of such in the medium. Despite this result, however whey has been reportedly used for production of ethanol, lactic acid and citric acids, biomass proteins, and food yeas (Moulin and Galzy, 1984; Kennedy, 1985; Mann, 1987) Production of EPS by LAB in milk is an important factor in assuring the proper consistency and texture of fermented food (Ricciard and Clementi, 2000). Ability of these EPS producing LAB to produced viscous medium is an added advantage over stabilizer which can adversely affect the true taste, aroma and mouthfeel of fermented dairy products. An alternative way to improve texture and stabulity of fermented dairy is the use of lactic acid bacteria which are food–grade organisms and are generally recognised as safe status (GRAS). Their exopolysaccharides function as thickeners, stabilizers, emulsifiers, gelling and water-binding agents. It contributes to the specific rheology and texture of fermented milk products. These exopolysaccharides represent safe additives for novel food formulations and may have applications in non-food products [(Crescenzi, 1995, Giraffa, 1994).

Comparatively, medium composition had a significant influence on the final pH of the fermentation broth as showed in Table 5. The initial pH of the fermentation broth of Leu.mesenteroides spp mesenteroides (UMMY4), Lactobacillus plantarum (LPWO11), L. coryniformis spp coryniformis (LCOW1) and L. casei spp toleran (LCN6) in mMRS, SDM, WHEY and PDW respectively slowly decreased to 4.37, 3.57, 4.38 and 3.41. These EPS-producers can serve as good sterters for pH development during fermentation.
Conclusively, from the aforementioned results, growth, viscosity, pH, lactic acid development and exopolysaccharide production of LAB vary widely with respect to the LAB species and their nutritional status and most of the isolates can serve as good starter for the production of quality fermented foods. This result may provide a sustainable and means of adding value to fermentation of which will result in production of this promising industrial biomolecule and high quality fermented products.

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