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Research article

Novel approach for the use of dairy industry wastes for bacterial growth media production

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ABSTRACT

This work proposes a novel approach for the reuse and the recovery of dairy wastes valuable components. Thermal coagulation was performed for dairy effluents and the main responsible fraction for the organic matter content (protein and fat) was separated. Dairy curds were prepared for the formulation of bacterial growth media. Protein, sugar, fat and fatty acids contents have been assessed. Samples treated at 100 °C exhibited marked improvement in terms of protein (25–50%) recovery compared to those treated at 80 °C. Fatty acid analysis revealed the presence of unsaturated fatty acids (mainly oleic acid) that are essential to promote *Lactobacillus* growth. Previously isolated and identified bacterial strains from dairy wastes (*Lactobacillus paracasei, Lactobacillus plantarum, Lactococcus lactis and Lactobacillus brevis*) were investigated for their ability to grow on the formulated media. All the tested lactic acid bacteria exhibited greater bacterial growth on the formulated media supplemented with glucose only or with both glucose and yeast extract compared to the control media. By reference to the commercial growth medium, the productivity ratio of the supplemented bactofugate (B) and decreaming (D) formulated media exceeded 0.6 for *L. paracasei* culture. Whereas, the productivity ratio of the supplemented B medium was greater than 1 compared to the control medium for all the tested strains. As for the supplemented D medium, its productivity ratio was greater than 1 compared to the control medium for both *L. paracasei* and *L. plantarum* strains.

1. Introduction

Microorganisms are almost omnipresent, very diverse and indispensable to human survival. Preparation of suitable culture media is one of the prerequisites to study them. Whereas, the commercial culture media, presented as pure dehydrated preparations, are very expensive in local market of developing nations and in most cases are not available (Andualem and Gessesse, 2013). Several researches have focused the use of low-cost bio-products for the preparation of bacterial growth media (Andualem and Gessesse, 2013; Deivanayaki and Iruthayaraj, 2012; Tsakona et al., 2014). Major requirements of bacterial media are mainly carbon, nitrogen, sulphur, phosphorus and minerals (Andualem and Gessesse, 2013; Farhana et al., 2011). The nitrogen source is actually the most expensive component of bacterial growth substrates. Nitrogen could be obtained from plants (Annan-Prah et al., 2010), dairy proteins (Ummadi and Curic-Bawden, 2010) and meat wastes (Andualem and Gessesse, 2013). Since milk is a fairly nutritious growth medium for dairy strains (Ummadi and Curic-Bawden, 2010), caseins, whey proteins (Lucas et al., 2004), skim Mmariamtaha mariamtaha ilk, milk, whey protein isolates and concentrates and lactalbumin (Ummadi and Curic-Bawden, 2010) have been used to fulfill their nitrogen requirements. Comparing dairy peptones to meat or soy hydrolysates revealed that dairy hydrolysates are abundant in amino acids such as Glu, Ile, Leu, Val and Met (Ummadi and Curic-Bawden, 2010). Milk is also abundant in Proline (Pro). Such amino acid was found to stimulate the growth of several lactic acid bacteria (LAB) strains regardless of their ability to

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synthetize it (Smid and Konings, 1990). Furthermore, due to the regulatory requirements such as Genetically Modified Organism (GMO-free), Bovine Spongiform Encephalopathy-free (BSE-free) and Food and Drug Organization (FDA) regulations; the elimination of bovine origin components from the fermentation media composition is of a paramount importance (Hsieh et al., 1999; Ranganathan et al., 2010). Consequently, the raw materials for the production of dairy starter are usually limited to the dairy derived protein. On the other hand, it has been reported that considerable amounts of dairy proteins are actually wasted in dairy effluents (Kasmi, 2018). Recent investigation claimed bactofugate (B) and decreaming racking water (D) as the most polluting dairy effluents within the dairy industry due to their high organic matter content (Kasmi et al., 2016). Therefore, some efforts have been implemented in the aim to reuse those effluents for nutritive components (e.g. nitrogen, carbohydrates and minerals) recovery (Kasmi et al., 2016) or microorganism isolation and identification such as lactic acid bacteria (LAB) (Ghodhbane et al., 2016). LAB commonly known as probiotics, possess potential therapeutic properties and several technological features of interest mainly in food and pharmaceutical industries (Iyer and Versalovic, 2009). They are highly beneficial for human beings and are present abundantly in dairy products so their use should be promoted for good human health (Masood et al., 2011). It has been reported that physical-chemical treatments of dairy rejects allow the partial removal of the organic load through protein and fat precipitation by thermal coagulation, thermo-calcic coagulation or by using different chemical compounds such as aluminum sulphate, ferric chloride and ferrous sulphide (Kasmi et al., 2017b; Rusten et al., 1993). Previous investigations proposed to incorporate the separated dairy organic matter in animal feed (Scholten et al., 1999). To the best of our knowledge, this is the first time that the feasibility of recycling such polluting dairy wastes for growth medium production is presented. This work proposes a novel approach for the use of the dairy curds for bacterial growth media production. The formulation of a balanced growth medium for LAB using the recovered dairy valuable components is investigated and the productivity of the formulated media is discussed in comparison with both control and commercial growth media.

2. Materials and methods

2.1. Samples collection

Bactofugate (B) and decreaming racking water (D) samples were collected from a regional dairy industry (Centrale Laitière du Cap Bon, Soliman, Nabeul governorate). The plant produces approximately 1,696,744 hL of drinking milk and three tons of butter per year (MedTest, 2012). According to the industry statistics, an average of 1200 L of washing water is generated daily from Decreaming machines. An equal volume resulting from Bactofugation as separated milk is also recorded per day (Belouarda, 2012). Samples were collected during the high lactation period (March–May) in plastic sterile containers of 20 L and stored at 4–6 °C.

2.2. Samples treatment

The thermal coagulation was performed in glass vessels containing 1 L of each sample. Samples were emerged in a controlled temperature heating bath under continuous stirring (100 rpm). The heat treatment was performed at different temperature ranging from 20 to 100 °C. Clotting time is indicated as time from the sample exposure to the desired treatment temperature to the formation of the first visible floccules (Kasmi et al., 2016). Thereafter, samples were decanted in conic devices until a steady pellet settling.

2.3. Dairy curds recuperation and preparation

After decantation, supernatants were poured off and the dairy curds (settleable solids) were recuperated, crumbled (if necessary) and spread on stainless steel plates to be dried at 60 °C until the mass is stabilized. Then, to produce a quality bacterial culture medium, the dried dairy curds were finely powdered using a balls mill (Retsch RS 200, 700 rpm) and the moisture was removed by incubation at 60 °C for 24 h (Andualem and Gessesse, 2013).

2.4. Growth media formulation

2.4.1. Media solubility

An amount (m_0) of the powdered dry curds (P) is gradually introduced into a glass bottle with a volume of 1 L of distilled water until saturation. The bottle is then placed on a heating plate with stirring until it boils. After centrifugation at 4000 rpm for 15 min, the precipitate is recovered after filtration, dried at 105 °C and weighed (m_1). Sterilization is then carried out under the same conditions of preparation of commercial growth media in the laboratory. The quantity of the product solubilized by 1 L is expressed in (g) and is determined as follow: m_s (g) = m_0-m_1; m_s is an average of three replicates. To improve the medium clarity, it is proposed to add sodium carbonate (Na_2CO_3). The latter is a dispersing agent which limits the agglutination of particles (Borja et al., 2015; Kujore, 2009). The solubility index (SI) is determined as follow:

$$SI(\%) = \frac{m_s}{m_0} \times 100$$

These tests result in an optimum soluble mass (m_p) of the product (P) and an optimal dose of Na_2CO_3 (d_{Na2CO3}). The pH of the growth media were determined using multi-parameters device Consort C860 and adjusted to 6.2 if necessary using H_2SO_4 (0.1 M) or NaOH (0.1 M) solutions.

2.4.2. Supplementation of the formulated growth media

A part from agar supplementation (1.5%) which is required for the production of an agar growth medium (Biolife, 2012), different formulated media were prepared with both 2% glucose and 0.5% yeast extract supplementation, with only 2% glucose supplementation, and without any supplement in 1 L of distilled water. The media were autoclaved (121 °C, 20 min). Then, they were poured on petri dishes and left to jellify. Commercial growth media Man, Rogosa and Sharpe (MRS agar with Tween 80, Biolife, Italiana) and control growth media were prepared for comparison purpose.

2.4.3. Stability test

To investigate the stability of the formulated growth media, different temperatures were tested. The prepared agar media were incubated at room temperature, 37 °C and 4–6 °C for 5 days. The incubated media were inspected daily to detect any bacterial growth.

2.5. Isolation and identification of microorganisms from dairy wastes

Dairy wastes including B and D raw samples next to the wasted fermented dairy products (Kasmi et al., 2017b) have been used for lactic acid bacteria isolation. Ten milliliters of each sample were enriched on MRS broth medium (Biolife, Italia) and incubated at 37 °C for 48 h. Seeding was carried out subsequently on agar MRS