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Effect of dominant specie of lactic acid bacteria from tomato on natural microflora development in tomato purée

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Abstract

The dominant lactic acid bacteria specie from tomatoes surface and its effect, as competitive microflora, on tomato purée during storage at 30 °C was investigated. Four genera were found *Leuconostoc mesenteroides* ssp. *mesenteroides* being the dominant group. *Leuc. mesenteroides* ssp. *mesenteroides* Tsc when inoculated on tomato purée, pH 4.1, grew approximately 2 log cycles in 48 h, inhibiting natural bacterial development and delaying the growth of yeasts. The faster organic acids production by inoculated microorganism contributed to the diminution in the natural microflora cells number. This microorganism could be considered to help to control the contaminates proliferation on tomato purée during storage at abusive temperature.

Keywords: Tomato; Lactic acid bacteria; *Leuconostoc mesenteroides*; Competitive bacteria; Metabolism

1. Introduction

Vegetables and vegetable-based food are normal parts of the human diet and are consumed daily in large quantities in most civilizations. The natural microflora of vegetables includes bacteria, yeasts, and molds representing many genera. The microflora can vary considerably depending on the type of vegetable, environmental considerations, seasonality, and harvesting conditions. In tomato nearly half of the total dry matter consists of the reducing sugars, glucose and fructose, about 10% is organic acid and about 1% is skin and seeds, with the remainder being alcohol, insoluble solids (cellulose, pectins, hemicellulose and proteins), minerals (mainly potassium), pigments, vitamins and lipids. Glutamic acid is the principal amino acid found in tomato (Hayes, Smith, & Morris, 1998). The organic acids content of tomato is responsible for a pH between 4.0 and 4.6 (Gutheil, Price, & Swanson, 1980). The low pH and the nature of the organic acid molecule per se select the growth of acid tolerant microorganisms, such as fungi (Splittstoesser, 1987) and lactic acid bacteria (LAB) (Brackett, 1988). Traditionally, fruits and vegetables have been regarded as microbiologically safer than other unprocessed foods such as meat, milk, eggs, poultry and seafood. Extensive handling and temperature abuse of vegetable provide opportunities for microbial contamination and growth of pathogens (Wiessinger, Chantarapanont, & Beuchat, 2000). For example, salmonellosis has been attributed to fresh vegetables and in particular to consumption of tomatoes (Hedberg, MacDonald, & Olsterholm, 1994; Wood, Hedberg, & White, 1991). Gombas (1989) reported that the use of a biological control system could be efficient for decreasing the microbiological spoilage of food.

The objective of this work was to determine the dominant specie of LAB from tomatoes surface and further to investigate the effect of its growth and metabolism on

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autochthonous microflora evolution on tomato purée during storage at temperature abusive.

2. Materials and methods

2.1. Microorganism

Leuconostoc mesenteroides ssp. *mesenteroides* Tsc was isolated from tomato. The strain was stored at −20 °C in MRS medium (De Man, Rogosa, & Sharpe, 1960) supplemented with glycerol (30%, v/v).

2.2. Samples

All tomatoes (Rio Grande type) were obtained from Farms of the Tucumán State, Argentina. The products were all of agreeable sensory quality.

2.3. Processing, enumeration and isolation of LAB from surface of tomatoes

For isolation of LAB, skins of tomatoes were washed three times with sterile distilled water. Each water washing was collected under sterile conditions. Successive decimal dilutions were carried out with sterile peptone water 0.1% (w/v). From each dilution 0.1 ml volumes were plated in duplicate on MRS agar (De Man et al., 1960) acidified to pH 5.0 and supplemented with 1.3 μg/ml of Pimaricin (Sigma) (MRS-P) to inhibit yeasts growth. Aerobic mesophilic bacteria were determined on plate count agar (PCA, Oxoid, Basingstoke, UK). Agar plates were incubated anaerobically, MRS-P plates, (BBL GasPak Anaerobic System) and aerobically (PCA) at 30 °C for 7 d before enumeration. The PCA agar plates were flooded after enumeration with 3% H_2O_2 in order to observe for presence of catalase positive colonies.

Colonies by random selection were picked up from MRS-P, purified by sub-culturing in MRS broth at 30 °C and further characterized. A small number of isolates were also obtained from PCA medium.

2.4. Phenotypical characterization of selected isolates

The isolates were characterized for Gram and catalase reaction, cell shape, cytochrome-oxidase activity, spore formation, production of ammonia from arginine (Devriese, Pot, & Collins, 1993) and fermentative catabolism of glucose. Gas and D- or L-lactic acid isomers production from glucose metabolism were determined in Gibson medium (Gibson & Abdel-Malek, 1945) and by using an enzymatic method, Boehringer Kit (Mannheim, Germany), respectively.

Ability of growth, under microaerophilic conditions in BBL GasPak jars in which the content of oxygen was reduced by use of a lighted candle, was determined on MRS agar plates incubated at 15, 30, 37 and 45 °C. Ability of growth at different NaCl concentrations (2, 4, 6 and 8% w/v) and pH values (4, 5, 5.5 and 6.5) were also investigated. Production of dextran from sucrose (5%) was determined on agar medium. Studies of fermentation of carbohydrates and related compounds were carried out in MRS broth without glucose and containing bromocresol purple (0.04 g/l) as a pH indicator, inverted Durham tube and supplemented with 1% of following carbohydrates: lactose, sucrose, xylose, arabinose, sorbitol, fructose, galactose, mannose, cellobiose, raffinose, melezitose and melobiose and by using API 50 CH galleries (BioMérieux, Marcy-l'Etoile, France).

2.5. Fluorescence in situ hybridisation (FISH)

This method uses fluorescent oligonucleotide probes, homologous to 16S rDNA of each species or genus. The 16S rRNA sequences used in this study were obtained from EMBL and GenBank databases by Blasco, Ferrer, and Pardo (2003). The eubacterial Eub 338 probe 5′ end-labelled with fluorescein by MWG biotech was used as positive control and Non 338 5′-fluorescein labeled, complementary to 338, as the negative control for non-specific binding (Amann et al., 1990). LU2 oligonucleotide probe 5′ end-labelled with fluorescein was specific for *Leuc.* FISH experiments were performed according to the method described by Blasco et al. (2003).

Fluorescence was detected with a Leica DMRB microscope fitted for epifluorescence microscopy with a 100-W mercury lamp high-pressure bulb and Leitz filter blocks A (UV light exciter BP 340–380 nm, beamsplitter RKP 400 nm, emitter LP 430 nm), I3 (blue light exciter BP 450–490 nm, beamsplitter RKP 510 nm, emitter LP 520 nm), and N2.1 (light exciter BP 515–560 nm, beamsplitter RKP 580 nm, emitter LP 580 nm). Color photomicrographs were obtained using Kodak Gold 800 ASA color print film. The exposure times used were 0.10–0.30 s for phase-contrast photomicrographs and 10–120 s for epifluorescence photomicrographs.

2.6. Preparation of tomato purée, growth conditions and culture procedures

Fresh tomatoes were washed with water, peeled, blanched in saturated steam for 2 min and immediately cooled in water at 20 °C. The tomatoes were then processed with a Brown Minipimer to provide purée. Tomatoes purée (100 g) with a pH 4.1 were aseptically dispensed into sterile glass flasks with caps. The time lapse between the preparations of tomatoes purées and microbial inoculation was approximately 15 min.

Cells of *Leuc. mesenteroides* ssp. *mesenteroides* Tsc grown in MRS broth were harvested at the end of exponential growth phase (8 h) by centrifugation, washed twice and resuspended in sterile distilled water to $OD_{560\,nm} = 2.0$. The cellular suspension was used to inoculate the tomato purée medium with ca. 10^6 cfu/g. The inoculum was thoroughly distributed in tomato purée by vigorously mixing with a