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# Application of *Lactobacillus plantarum* Lb9 as starter culture in caper berry fermentation

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ABSTRACT

Fermentation of caper berries is a traditional process relying on the indigenous microbiota. In this study, the application of *Lactobacillus plantarum* Lb9 isolated from spontaneous caper berry fermentation as starter culture was evaluated by means of culture-dependent and independent methods. The results obtained here indicated that *L. plantarum* Lb9 was able to carry on an accelerated lactic acid fermentation of caper berries, dominating the background microbiota present in the raw material until the end of fermentation even when fermentation broth was added with high salt concentration. Persistence of *L. plantarum* Lb9 in the starter-added fermentation was confirmed by RAPD-PCR of isolates from viable cell counting during the fermentation. TTGE analysis of the microbial community throughout fermentation process also indicated that lactobacilli were the predominant microbiota, and thus the microbial composition during the controlled fermentation was more homogeneous compared with spontaneous fermentations. Furthermore, the sensorial quality of the starter-added fermented capers was similar to the spontaneous fermented products. In conclusion, *L. plantarum* Lb9 was an adequate starter culture strain for caper berry fermentation prevailing over other bacteria present in the raw material. This fermentation ensured both the quality and safety of the end product.



## 1. Introduction

Caper berries are the fruits of *Capparis* species (mainly *Capparis spinosa* L.), a Mediterranean shrub cultivated for its buds and fruits. It is a plant that has medicinal and aromatic properties and is mainly cultivated for its flower buds (or caper) and fruits (or caper berries). As a spontaneous plant, caper has a large natural distribution (Tlili et al., 2011). The important sources are Turkey, Morocco, Spain, Greece, France and Italy (especially Sicily and the Aeolian island of Salina and the Mediterranean island of Pantelleria) (Alkire, 2000). In recent years, *Capparis* spp. with its processed buds (brined or fermented) has gained great importance in the food industry (Inocenio, Rivera, Obon, Alcaraz, & Barrena, 2006).

Caper fruits are fermented in a traditional way, often in small enterprise or at home (Luna & Perez, 1985). During summer the fruits are collected and immersed in tap water, where the fermentation takes place for approximately one week. Usually, fermentation vessels are left on sunny terraces where temperatures may reach 40–45 °C. After this, fermented caper berries are placed in brine containing 10% NaCl (wt/vol). During the water storage period, a lactic acid fermentation takes place resulting in a fast acidification (Pérez Pulido et al., 2005). Caper berries made by this traditional process have a bright color, firm texture, and a unique distinctive bitter flavor (salty, acidic, piquant, with pungent aroma notes) which can be in part attributed to isothiocyanates formed after hydrolysis of glucocapperin, the main glucosinolate of capers (Fahey, Zalcmann, & Talalay, 2001; Sanjust, Mocci, Zucca, & Rescigno, 2008). Attempts to ferment caper berries in salted brines have also been described, resulting in slower fermentation. According to Özcan (1999), the best length of fermentation with respect to product color, flavor, acidity, pH, and LAB activity in brine was 20–25 days and the most suitable caper berries fermentation for LAB activity was 5%–10% NaCl. In this case, the quality of fermented caper berries was maintained when stored in fresh brine with a concentration of 15% NaCl at equilibrium.

One of the shortcomings of caper berry fermentation is the current lack of specific starter cultures. Since the traditional process relies on spontaneous fermentation and is carried out in water (without a salt selective pressure to drive a lactic acid fermentation), there is a risk of stuck fermentations. As the fermented

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product cannot be pasteurized without loosing its characteristic flavor and taste and development of unpleasant cooked taste, some producers now add chemical preservatives and the product cannot be claimed to be naturally preserved. Furthermore, production of fermented caper berries has traditionally been carried out by small-medium enterprises in small-volume fermentation vats. With the increasing demand of fermented caper berries, the development of large-scale fermentations would greatly benefit from the availability of suitable starter cultures. Therefore, it would be interesting to develop starter cultures having metabolic activities specifically adapted to use the nutrients available from the substrate and able to achieve a fast acidification and to avoid the problem of stuck fermentations. The use of a starter culture producing antimicrobial activity would also provide other beneficial effects such as a selective advantage of the starter against competitors in the fermentation (achieving a more homogeneous fermentation), decreasing the risk of spoilage and contributing to product stability (Ruiz-Barba, Cathcart, Warner, & Jiménez-Díaz, 1994).

Studies carried out in our laboratory on the fermentation of caper berries suggested a complex microbial community of lactic acid bacteria (LAB) responsible for the fermentation, in which *Lactobacillus plantarum* was the most predominant species during the entire fermentation period (Pérez Pulido et al., 2005). Those studies allowed us to get a better knowledge about the microbial dynamic that take place throughout the fermentation process of caper fruits, as well as the functional characteristics of strains isolated from the final product (Pérez Pulido et al., 2005). Fermented capers were a valuable source of LAB strains with interesting functional properties (Pérez Pulido et al., 2006, 2007). Among all LAB isolated in this study, *L. plantarum* Lb9 was able to degrade nondigestible α-galactoside sugars and exhibited useful technological properties such as phytase activity, bile salt hydrolase activity, galactosidase and glucosidase activities besides its antimicrobial activity against *Listeria monocytogenes* (Pérez Pulido, 2004). Thus, strain Lb9 seemed a potential candidate as a starter for caper berry fermentation. Accordingly, the present study aimed to evaluate the autochthonous *L. plantarum* Lb9 strain for possible application as starter culture to improve the microbiological quality of the process, to produce consistently homogeneous product with desirable and controlled properties and to reduce the risk of spoilage and stuck fermentations.

## 2. Materials and methods

### 2.1. Preparation of starter culture

*L. plantarum* Lb9 strain was isolated from fermented caper berries in our laboratory by Pérez Pulido, 2004. The strain was inoculated into Man Rogosa Sharpe (MRS) broth and grown at 30 °C for 24 h. Then cultures were harvested by centrifugation at 7500 rpm for 5 min, washed with sterile saline solution (0.9%) and the cell pellets were resuspended in sterile saline solution to produce *L. plantarum* Lb9 suspensions of $10^9$ CFU/ml to be inoculated as starter culture in caper berry fermentation.

### 2.2. Fermentation procedures

The samples analyzed in this study were prepared with caper berries (1.5–2.5 cm diameter) harvested from a private plantation. All experiments were done in duplicate. About 900 g of fruits placed in 2 L vats filled with tap water (1 L) were inoculated or not with *L. plantarum* Lb9 at $10^7$ CFU/ml and allowed to ferment at ambient temperature for 6 days. Then concentrated NaCl was added to the vats to achieve a final salt concentration of 10% (wt/vol). Sampling was performed from two vats under aseptic conditions at 24 h intervals (one sample per vat per time point) throughout 6 days of fermentation and after 24 h and 48 h of storage in brine (days 7 and 8).

### 2.3. Microbiological analysis

Serial dilutions of brine samples were used for microbial enumerations with the following media: Tryptone Soya Agar (TSA) (Scharlab) for estimation of total aerobic mesophilic bacteria and McConkey agar (Scharlab) for enterobacteria. All enumerations were done by plate counting. Aliquots (0.1 ml) of appropriate dilutions were spread plated in triplicate. Counts were obtained after 48 h of incubation at 30 °C. Results were calculated as the means of three determinations.

For LAB enumeration and isolation, caper brine samples were serially diluted in sterile saline solution and plated in triplicate on MRS agar (Scharlab, Barcelona) supplemented with 0.4 g/l sodium azide (Sigma, Madrid). Viable cell counts were performed after 5 days of incubation at 30 °C. For isolation of lactic acid bacteria, 20% of the colonies (i.e., 10–25 colonies per sample) from the highest dilutions were randomly selected and purified (Harrigan, 1998). Pure cultures were checked for catalase activity and Gram reaction, and microscopically examined prior to storage in MRS broth supplemented with 20% glycerol at −80 °C.

### 2.4. Sensory evaluation

A sensory evaluation of caper berry samples was performed at the end of fermentation (8 days) by a trained panel consisting of ten persons according to ISO 13299:2003. Briefly, a panel of 10 judges (6 females and 4 males, aged between 21 and 40 years) was trained in preliminary sessions, using different samples of commercial fermented capers, in order to develop a common vocabulary for the description of the sensory attributes of fermented capers and to familiarize themselves with scales and procedures. The sensory attributes taken into account included descriptors frequently used by local caper fermenting enterprises: abnormal fermentation, salty, bitter, acid, hardness, fibrousness, and crispness. Sensory data were subjected to Kruskal–Wallis non parametric analysis of variance (Quinn & Keough, 2002) using Minitab release 14.1 (Minitab Ltd., Coventry, UK). The predetermined acceptable level of probability was 5% for all comparisons ($P < 0.05$).

### 2.5. Genotypic characterization of lactic acid bacteria

#### 2.5.1. DNA extraction

DNA isolation from all LAB strains was carried out from the pellet of overnight cultures by using the Illustra bacteria genomicPrep Mini Spin Kit (GE Healthcare) according to manufacturer's instructions. DNA concentration and quality were assessed by measuring optical density at 260 and 280 nm using a nanodrop 2000 spectrophotometer (Thermo Scientific).

#### 2.5.2. RAPD-PCR fingerprinting

RAPD-PCR analysis was performed as described previously (Ben Omar et al., 2004) using the primer M13 (5′-GAG GGT GGC GGT TCT-3′). DNA was amplified by using the following conditions: 94 °C for 1 min; 40 °C for 20 s, ramp to 72 °C at 0.6 °C/s for 2 min; 72 °C for 2 min. The gels were stained with ethidium bromide and photographed on a UV transilluminator. Photo positives were scanned into a computer and subsequently analyzed using the Bionumerics software, version 2.5 (Applied Maths, Kortrijk, Belgium). RAPD-PCR patterns were grouped by means of cluster analysis with the Pearson product moment correlation coefficient and the unweighted pair group method using arithmetic averages (UPGMA).