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Wheat endophytic lactobacilli drive the microbial and biochemical features of sourdoughs

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ABSTRACT

The aim of this study was to assess whether wheat endophytic lactic acid bacteria (LAB) are able to dominate in sourdough ecosystem. To do that, a first experimental phase considered doughs produced under semi-sterile conditions and singly inoculated with different strains of endophytic LAB and *Lactobacillus sanfranciscensis* A4 isolated from sourdough. Notwithstanding the high frequency of *Lactobacillus plantarum* in the sourdoughs prepared in laboratory, only one of the starter strains, *L. plantarum* LB2, was detected after five days of back-slopping. Subsequently, the ability of this strain to dominate traditional sourdoughs was evaluated at bakery and laboratory level. Contamination of sourdoughs with *L. plantarum* LB2 caused an increased number of LAB and, accordingly, higher acidification, compared to the sourdoughs before this event. After six days of propagation, the wheat endophytic strain *L. plantarum* LB2 was retrieved as a component of the bacterial population, in all the sourdoughs and regardless of the place of propagation. In addition, the contamination event caused a modification of the lactic acid bacterium biota, which in turn influenced some sourdoughs biochemical features.

In conclusion, this study showed that wheat endophytic LAB could represent a potential reservoir for selecting robust strains to be used as sourdough starters.

1. Introduction

Lactic acid bacteria are the main responsible for the unique performances and valuable nutritional, sensory, shelf-life and texture traits of the sourdough baked goods (Gänzle, 2014; Gobbetti et al., 2016). Traditional sourdough (type Ib) is produced upon spontaneous fermentation of a dough consisting of flour, water and, eventually, additional ingredients (e.g., fruit, grape must, salt, sugar), followed by several back-slopping steps in which a part of the fermented dough is used as inoculum (De Vuyst et al., 2014; Ripari et al., 2016). In these types of sourdough, lactic acid bacteria may originate from flour, other dough ingredients and bakery environment. Lactic acid bacteria belonging to *Enterococcus* sp. and *Lactobacillus graminis* were first found in grains, bran and flour (Corsetti et al., 2007). Lactic acid bacteria contaminating flour may originate from milling, external layers of wheat plant organs (epiphytic) or may represent a part of the endophytic microbial community of wheat. The comparison of the polymorphic profiles of lactic acid bacteria isolated from different steps of wheat manipulation (threshing, milling, initial fermentation of dough) showed that, in some cases, they represented the same strain (Alfonzo et al., 2017). While some (especially *Leuconostoc citreum* and *Lactococcus garviae*) probably originated from ear or kernel, others (e.g., *Lactobacillus brevis* and *Lactobacillus plantarum*) possibly came from inner layers of plant organs. Endophytic lactic acid bacteria (*Lactobacillus, Lactococcus, Streptococcus, Enterococcus*) were detected since the early stages of durum wheat cycle (Minervini et al., 2015a). In addition, strains of *L. plantarum* isolated from the endophytic component of wheat were found during the whole life cycle and persisted in the milled flour and bran. As shown during sourdough production under semi-sterile conditions, some flour autochthonous bacteria (e.g., *Lactobacillus* sp., *Lactococcus* sp., *Pediococcus* sp., *Weissella* sp.) may dominate the sourdough ecosystem (Minervini et al., 2010; Siragusa et al., 2009).

Additional ingredients may be the source of lactic acid bacteria in *de novo* sourdoughs (Minervini et al., 2016; Ripari et al., 2016).

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The addition of plant materials to dough accelerated the formation of mature sourdoughs which contained a stable microbiota composed by *Lactobacillus sanfranciscensis*, *L. plantarum*, *L. graminis*, or *Lactobacillus rossiae*, and *Saccharomyces cerevisiae* (Ripari et al., 2016).

Bakery environment (the so-called "house microbiota") acted as a potential carrier of specific persistent strains of lactic acid bacteria in artisan sourdoughs (Scheirlinck et al., 2008). High throughput 16S rRNA amplicon sequencing performed on RNA extracted from bakery environmental samples showed that *L. sanfranciscensis* was consistently present on dough mixer and storage box contacting artisan sourdough (Minervini et al., 2015b). This suggests that species of lactic acid bacteria that are dominant in sourdough highly contaminate the house microbiota. Culture-dependent and -independent analyses performed on traditional sourdoughs propagated for 80 days at artisan or laboratory level showed that most of the laboratory-propagated sourdoughs differed from those propagated at artisan bakeries. In detail, most of the strains were identified only at either artisan or laboratory level (Minervini et al., 2012b). The continuous introduction of flour into bakery, as well as the daily propagation of traditional sourdough, would build up a house microbiota that may serve as an important inoculum for each fermentation (Scheirlinck et al., 2009).

Overall, the final microbiota composition of each sourdough is molded by selection, dispersal, drift, and speciation (Gänzle and Ripari, 2016; Nemergut et al., 2013; Vellend, 2010). Stochastic, temporal and deterministic drivers shape not only the composition of microbial communities but also, most relevantly, the functionality of the microbiota. The general paradigm is that generalist bacteria with redundant metabolic traits mainly assemble stochastically, whereas specialists for selected metabolisms are mainly determined by the environmental drivers (Wolfe and Dutton, 2015). The sourdough microbiota thus likely consists of both foundation functional features and unique functional traits related to microbes that occur or disappear because they are able or unable to thrive in this specific environment (Gänzle and Ripari, 2016). The aim of this study was to assess whether strains of wheat endophytic lactic acid bacteria are able to dominate in sourdough ecosystem. To do that, a first experimental phase considered *de novo* sourdoughs produced under semi-sterile conditions and singly inoculated with different strains of endophytic lactic acid bacteria and *L. sanfranciscensis* A4 isolated from sourdough. Subsequently, the ability of the persistent endophytic strain to face with traditional sourdoughs was evaluated at bakery and laboratory level.

2. Materials and methods

2.1. Microorganisms and culture conditions

Lactobacillus plantarum LA1, LB2, OLB3, OLD1, OLB4, OLC4, *Lactobacillus rossiae* OLC1, and *Enterococcus faecalis* LA2, isolated from the endophytic microbiota of durum wheat (spikes at the stage of physiological maturity) (Minervini et al., 2015a), and *Lactobacillus sanfranciscensis* A4 isolated from sourdough (De Angelis et al., 2002), were used in this study. Strains isolated from wheat were characterized by different fermentative profiles. Cell cultures were routinely propagated at 30° C for 24 h in Sour Dough Bacteria (SDB) broth (Kline and Sugihara, 1971). When used for sourdough fermentation, cells of lactic acid bacteria were incubated for 24 h, harvested by centrifugation (4528×*g*, for 5 min, at 4° C), washed twice with 50 mM potassium phosphate buffer, pH 7.0 and re-suspended in sterile tap water at cell density of about 7 log CFU/mL.

2.2. Sourdough production

Triticum durum flour was kindly supplied by L'Antico Molino Calemma (Altamura, BA, Italy). The gross composition was as follows: moisture, 12.0%; protein (N × 5.7), 12.5%; total carbohydrates, 73.5%; fat, 2.0%. For sourdough production, flour (62.5 g), sterile tap water (27.5 g) and cell suspension (10 mL), containing *L. sanfranciscensis* A4 and one of the above individual wheat strains (final cell number for A4 and each wheat strain in the dough of *ca.* 6 log CFU/g of dough), were kneaded by a continuous high-speed mixer (60 × *g*, dough mixing time 5 min) (Chopin & Co., Boulogne, Seine, France). A control dough, with no bacterial inoculum, was also produced under the same conditions (62.5 g of flour and 37.5 g of water). The value of dough yield (dough weight × 100/flour weight) was 160 for all doughs. The nine doughs (eight inoculated with *L. sanfranciscensis* A4 and individual wheat strains, and the control) were incubated in sterile plastic beakers at 30° C for 8 h. After fermentation, doughs were stored at 4° C for about 16 h and further used as starters, according to the back-slopping (or refreshment) protocol. In detail, each fermented dough was individually used to inoculate (20%, wt/wt) a mixture of flour (50 g) and sterile tap water (30 g). After inoculation, doughs were fermented at 30° C for 6 h. Between two consecutive fermentations, the doughs were stored at 4° C for about 18 h. Sourdoughs were obtained after five back-slopping steps. Each sourdough was produced three times monthly (each time corresponding to one biological replicate), at a between-each-time interval of nine days.

2.3. Sourdough intentional contamination and subsequent propagation

Three traditional sourdoughs were considered in this study, in order to assess the ability of the most robust wheat strain (*L. plantarum* LB2) to adapt to sourdough ecosystem. The sourdoughs were routinely propagated through daily back-slopping and used in three different artisan bakeries located in the south of Italy: AM (Altamura, Bari), CG (Castellana Grotte, Bari) and MT (Matera). Table 1 describes the ingredients and technology parameters used for back-slopping. The sourdoughs were intentionally contaminated by using cell suspension, containing about 8 log CFU/mL of *L. plantarum* LB2 (final cell number of *ca.* 7 log CFU/g) (Siragusa et al., 2009). After contamination, the sourdoughs were daily back-slopped at bakery (defined as "B7") and laboratory (defined as "L7") level. In parallel, traditional, non-contaminated sourdoughs were analyzed and propagated at bakery by the usual operators, using the same batches of flour and vessels. After six days of back-slopping, the sourdoughs, including the non-contaminated ones, were analyzed.

2.4. Lactic acid bacteria and yeasts enumeration

Ten grams of sourdough were homogenized with 90 mL of sterile saline solution. Lactic acid bacteria were counted by using modified MRS (mMRS, Oxoid, Basingstoke, Hampshire, UK), containing maltose 10 g/L, fresh yeast extract 50 mL/L (pH 5.6), and SDB agar media, supplemented with cycloheximide (0.1 g/L) (Minervini et al., 2012a). Plates were incubated under anaerobiosis (AnaeroGen and AnaeroJar, Oxoid) at 30° C for 48 h. The number of yeasts was estimated on Sabouraud Dextrose Agar (SDA) (Oxoid) supplemented with chloramphenicol (0.1 g/L) (Minervini et al., 2012a). Colonies were counted after incubation at 30° C for 48 h.