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# Monitoring of wheat lactic acid bacteria from the field until the first step of dough fermentation

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ABSTRACT

The present work was carried out to retrieve the origin of lactic acid bacteria (LAB) in sourdough. To this purpose, wheat LAB were monitored from ear harvest until the first step of fermentation for sourdough development. The influence of the geographical area and variety on LAB species/strain composition was also determined. The ears of four *Triticum durum* varieties (Duilio, Iride, Saragolla and Simeto) were collected from several fields located within the Palermo province (Sicily, Italy) and microbiologically investigated. In order to trace the transfer of LAB during the consecutive steps of manipulation, ears were transformed aseptically and, after threshing, milling and fermentation, samples of kernels, semolinas and doughs, respectively, were analysed. LAB were not found to dominate the microbial communities of the raw materials. In general, kernels harboured lower levels of microorganisms than ears and ears than semolinas. Several samples showing no development of LAB colonies acidified the enrichment broth suggesting the presence of LAB below the detection limit. After fermentation, LAB loads increased consistently for all doughs, reaching levels of 7.0–7.5 Log CFU/g on M17. The values of pH (5.0) and TTA (5.6 mL NaOH/10 g of dough) indicated the occurrence of the acidification process for several doughs. LAB were phenotypically and genotypically differentiated by randomly amplified polymorphic DNA (RAPD)-PCR into eight groups including 51 strains belonging to the species *Lactobacillus brevis*, *Lactobacillus coryniformis*, *Lactobacillus plantarum*, *Lactococcus lactis*, *Lactococcus garvieae*, *Enterococcus casseliflavus*, *Enterococcus faecium*, *Leuconostoc citreum*, and *Pediococcus pentosaceus*. Lactobacilli constituted a minority the LAB community, while lactococci represented more than 50% of strains. Lower LAB complexity was found on kernels, while a richer biodiversity was observed in semolinas and fermented doughs. For broader microbiota characterisation in doughs before fermentation, the 16S rRNA gene fragment profiling was conducted on the unfermented doughs using MiSeq Illumina. LAB group was represented by *Enterococcus*, *Lactococcus* and members of *Leuconostocaceae* family whose relative abundances differed according to both geographical area and variety of wheat. The culture-independent approach confirmed that pediococci and lactobacilli constituted low abundance members of the semolina LAB microbiota and that although some strains may pass from wheat ear to fermented doughs, most are likely to come from other sources.



## 1. Introduction

Foods derived from cereals are popular worldwide (http://www.fao.org/worldfoodsituation/csdb/en/) and differ depending on the local culture and traditions (De Vuyst and Neysens, 2005; Jenson, 1998; Spicher, 1999). Wheat is the crop plant that best represents the history of mankind and has been a staple for the populations that inhabited the today's Europe since the Stone Age (Gifford and Baer-Sinnot, 2007). Wheat has been also a major agent of landscape

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change (Head and Atchison, 2016). Within *Triticum* genus, the two species predominantly cultivated are *Triticum aestivum* and *Triticum durum* (Toderi, 1989), commonly known as tender wheat and durum wheat, respectively. Durum wheat is a key component of diet, economy, and culture in Italy (Flagella, 2006) where it is used mainly to produce pasta, even though in some Southern regions (especially Apulia and Sicily) it is also used for the production of several bread typologies (Corsetti et al., 2001; Quaglia, 1988). The raw materials, the microbiota developing during the fermentation process and the technological parameters affect the characteristics of the final breads (De Vuyst et al., 2014).

Bread production is within the most ancient of human biotechnological activities (Pomeranz, 1987) and, probably, represents one of the first examples of a food obtained from raw materials transformed through fermentation. This process has been carried out with sourdough for millennia (Manetta, 2016). The introduction of baker's yeast accelerated the leavening process to the detriment of the organoleptic and nutritional characteristics of the final products (Gobbetti and Corsetti, 2010). However, thanks to its numerous advantages over the baker's yeast, the sourdough technology has not been abandoned (Venturi et al., 2012), especially for traditional and typical breads (Corsetti and Settanni, 2007).

Basically, sourdough originates from a mixture of flour and water that undergoes a fermentation carried out by indigenous lactic acid bacteria (LAB) and yeasts present in flour (De Vuyst and Vancanneyt, 2007; Vogel et al., 1999). LAB are responsible for the acidification of dough, but partly contribute to volume increase of dough (Gobbetti et al., 1995). The leavening action is exerted only by the obligate heterofermentative species and is basically due to the $CO_2$ produced through 6-phosphogluconate/phosphoketolase pathway (Endo and Dicks, 2014). Since raw materials are not subjected to thermal treatments before fermentation, they are contaminated by microorganisms, which is one of the causes for eventual change of sourdough microbial community (Alfonzo et al., in press; Harth et al., 2016).

Wheat flours (from *T. aestivum*) and semolinas (from *T. durum*) are naturally contaminated by LAB (Alfonzo et al., 2013). These bacteria have at least a double origin: LAB might be endophytic components of wheat plants (Minervini et al., 2015) or derive from the outer layers of kernels (Berghofer et al., 2003; Gobbetti et al., in press). In the latter case, LAB should derive from the environment. Thus, the LAB present on the awns, palea, lemma, glumes and rachis might be transferred to the kernels during threshing and from the kernels to the flour/semolina during milling. Wheat kernels have been found to host several LAB species that do not typically dominate the mature sourdoughs (Corsetti et al., 2007a), but are present in these ecosystems at subdominant levels (Corsetti et al., 2007b). Kernel LAB were found to play a defining role during the first step of sourdough production, mainly inhibiting the indigenous undesired microorganisms by lowering the pH, thus preparing the environment for the establishment of the typical sourdough (e.g. *Lactobacillus*) species (Corsetti et al., 2007b). The strong competitiveness of kernel LAB was directly related to their bacteriocin production (Corsetti et al., 2008). Several LAB are applied in the process of bakery products also for their antifungal activities (Valerio et al., 2016).

Based on these considerations, the aim of the present study was to follow the route of LAB from the field, where wheat is cultivated, to bakery, where the resulting semolina is processed, in order to clarify the origin of the LAB populations found in sourdough. The specific objectives of our study were (i) to characterise the LAB populations on ears and kernels, and in semolinas and fermented doughs by classical culture-dependent methods, (ii) to assess the microbial diversity of unfermented doughs by culture-independent 16S rRNA gene sequencing, and (iii) to evaluate the influence of the geographical area and *T. durum* variety on the bacterial populations before the first fermentation step of traditional sourdough production.

## 2. Materials and methods

### 2.1. Wheat fields, threshing, milling and collection of samples

In this study, four *T. durum* varieties (Duilio, Iride, Saragolla, and Simeto) cultivated in adjacent experimental fields (20 m × 500 m) of five farms located in four geographical areas within the Palermo province (Sicily, Italy) were microbiologically investigated. The sites for the wheat cultivation were as follows: Campofiorito (37°76′N, 13°25′E, elevation 483 m), Ciminna (37°86′N, 13°52′E, elevation 329 m), Contessa Entellina (two sites: A, 37°75′N, 13°15′E, elevation 378 m; B, 37°76′N, 13°15′, elevation 320 m), and Valledolmo (37°71′N, 13°79′E, elevation 619 m). Once the plants reached the physiological maturity stage, the ears with approximately 10 cm of culm were manually randomly collected with disposable gloves and dissecting scissors, transferred into paper bags (two aliquots of 100 and 300 ears) and immediately transported at ambient temperature to the laboratory of Agricultural Microbiology – University of Palermo, where they were kept in a refrigerated (7 °C) chamber. The smaller aliquots of ears were used for the microbiological analyses, while the larger ones for threshing.

Threshing was carried out aseptically under a laminar flow hood. The external teguments were removed manually. To exclude any contamination of the kernels with the microorganisms hosted in the inner layers of wheat, all damaged kernels were also removed. The kernels were then mixed and 100 g were transferred into sterile plastic bags for the microbiological analyses, while the rest, put in sterile bags, was used for milling without conditioning. Both aliquots were kept refrigerated before processing.

Milling was performed at ambient temperature with a Retsch centrifugal Mill ZM1 (Haan, Germany) equipped with a 1 mm grid. In order to perform the process in aseptic conditions, the mill was put under the laminar flow hood, the grid and the rotor were cleaned with ethanol and subjected to 30 min UV treatment before milling each sample of kernels. The resulting whole-meal semolinas were put in sterile plastic bags and kept refrigerated.

### 2.2. Dough production, monitoring of acidification and sampling

The doughs were prepared by mixing manually 125 g of each semolina with 75 mL of sterile tap water to a dough yield (weight of dough/weight of flour × 100) of 160. Dough production (in duplicate for each semolina) was performed aseptically under a laminar flow hood in sterile glass beakers using a stainless steel spoon for mixing. The doughs were fermented for 21 h at 30 °C. The acidification process was followed every 2 h for the first 8 h and at 21 h by pH, determined electrometrically with the pH meter BASIC 20+ (Crison Instrument S.A., Barcelona, Spain), and total titratable acidity (TTA), determined by titration (expressed as mL of NaOH/10 g of dough) (Lonner et al., 1986). All determinations were carried out in duplicate.

### 2.3. Microbiological analyses

The ears were analysed after removal of culms by dissecting scissors. Samples of ears (five ears, approximately 15–20 g) and kernels (15 g) were separately transferred into 500 mL sterile glass flasks and added with Ringer's solution (Sigma-Aldrich, Milan, Italy) till reaching a ratio 1:10 (sample:diluent) (Hartnett et al.,