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First Insight into the Technological Features of Lactic Acid Bacteria Isolated from Algerian Fermented Wheat Lemzeiet

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Abstract

Fermented cereals are part of the main traditional diets of many people in Africa, usually obtained from artisanal production. The intensification of their manufacturing, responding to the consumers demand, requires a better control to ensure their sanitary, nutritional, and taste qualities, hence, the need of selecting accurate and safe starter cultures. In the present study, 48 lactic acid bacteria (LAB) strains, previously isolated from Algerian fermented wheat *lemzeiet,* were analyzed for different technological properties. 14 LAB strains, belonging to *Pediococcus pentosaceus, Enterococcus faecium, Lactobacillus curvatus, Lactobacillus brevis,* and *Leuconostoc mesenteroides* species, decreased rapidly the pH of the flour extract broth close to 4 or below. 91% of strains showed extracellular protease activity, but only 12% were amylolytics. 18 LAB strains inhibited or postponed the growth of three fungal targets *Rhodotorula mucilaginosa* UBOCC-A-216004, *Penicillium verrucosum* UBOCC-A-109221, and *Aspergillus flavus* UBOCC-A-106028. The strains belonging to *Lactobacillus* spp., *Leuconostoc fallax*, *L. mesenteroides*, and *Weissella paramesenteroides* were the most antifungal ones. Multiplex PCR for biogenic amines' production did not reveal any of the genes involved in the production of putrescine, histamine, and tyramine for 17 of the 48 strains. The obtained results provided several candidates for use as starter culture in the future production of *lemzeiet*.

Introduction

Fermentation process was developed by ancient people to preserve food, and throughout human culinary history it has played a significant impact on people's eating habits. It has a key function in the development of the sensory and safety aspects of food products and its economic importance has drastically increased as a result of the industrialization of food bio-transformation and of the growing interest of consumers for functional foods [1–4].

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Africa with its age-long history of traditional fermentation practices could highly be considered as a place with a great variety of fermented foods [5]; however, their preparation remains a know-how specific to each region and they are often produced within households in villages or in artisanal industries using a spontaneous fermentation or backslopping [6–8]. Consequently, the dependence on undefined microbial consortium during fermentation could impact the repeatability and the stability of the final products [9]. In fact, in the Western world, the powerful economic systems and the significant scientific research have contributed to improve the quality and the processing conditions of fermented products, in particular, by starters' selections through the exploitation of the natural microbial diversity (i.e., positive microorganisms inherently present in the fermented alimentary matrix) [4].

Fermented cereals are a relevant example of this type of fermentation; generating varieties of sourdoughs, porridges, and beverages which could be variable in terms of quality and safety [10]. The scientific research outcomes demonstrate how the improvement of nutritional, safety, organoleptic, and functional quality of fermented cereal foods can be reached and made more reproducible by the thorough

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selection of autochthonous multifunctional microbial strains as starters. The abilities to realize fast acidification (i.e., to prevent the growth of pathogenic and spoilage flora), antimicrobial and bioactive compounds production, hydrolytic activities (i.e., ability to provide precursors for aromatic compounds via a series of biochemical reactions), and the nutritional proprieties, etc., are key functionalities and are considered as major criteria to prospect when looking for native multifunctional strains from indigenous cereal-fermented products [11–14]. Furthermore, microbial safety must be considered especially related to biogenic amine synthesis which can be toxic if their concentrations in cereal-fermented products are high [1, 15].

Fermented wheat is used to manufacture the Algerian couscous called *lemzeiet* [16]. A previous microbial study on *lemzeiet* showed that fermentation process allows the disappearance of fungi flora over time through the establishment of LAB characterizing the microbiota of this fermented wheat [17]. It revealed a high level of LAB species and strains diversity during the different stages of maturation with a marked effect of the fermentation process on the profile of volatile aroma compounds. In this study, 69 isolates were identified, belonging to 6 genera of 16 species and the clonal diversity of the predominant species, analyzed by Random amplified polymorphic DNA (RAPD) and Pulsed-field gel electrophoresis (PFGE) allowed the identification, from the 69 isolates, of 48 distinct clones. The latter could harbor various technological properties of interest worth being explored since most of functionalities are strain-dependent [18, 19].

The aim of our work was to evaluate and to analyze technological features of the 48 LAB strains isolated from *lemzeiet*, as part of the preceding study, in order to select specific culture starters with complementary functionalities, based on natural microbial diversity of fermented wheat, in order to be able ultimately to produce *lemzeiet*, on a larger scale, with desirable properties. For that purpose, we followed a step-by-step approach by screening the strains able to ensure a rapid acidification and those able to hydrolyze their substrate, those inhibiting toxigenic and spoilage fungi, and finally those not producing biogenic amines.

Materials and Methods

LAB Strains and Culture Conditions

Forty-eight LAB strains, isolated from *lemzeiet* and identified by molecular technics in a previous study [17], preserved in the Centre International des Resources Microbiennes - Bactéries d'Intéret Alimentaire (CIRM-BIA, INRA-Rennes, France, www6.inra.fr/cirm_eng/Food-Associated-Bacteria), were analyzed in this study (Table 1). Stored at −80 °C in MRS broth medium (Biokar Diagnostics) added to (1/1) with 30% (v/v) glycerol, they were cultured twice in the same broth at 30 °C for 24 h before each experiment.

Acidification Capacity

Kinetic acidifications of LAB were evaluated using sterile flour extract (SFE) broth [20] slightly modified as follows: 100 g of commercial wheat flour T110 (fat: 1, 3%; protein: 11%; fibers 7%, France) was suspended in 1 L of distilled water and agitated for 30 min at room temperature. The flour was then precipitated, by decantation, and the supernatant was sterilized by autoclaving at 121 °C for 20 min. The resulting SFE broth was used in subsequent experiments. Overnight, LAB cultures, grown in MRS broth (Biokar Diagnostics) at 30 °C, were harvested by centrifugation for 5 min at 5000 g and washed with sterile tryptone water 1% (w/v) (HiMedia Laboratories). Then, suspended in a fresh solution to an optical density at 600 nm of 1.00, corresponding to approximately 10^9 CFU per mL, as measured with a plate reader (Spectra Max M2; Molecular Devices Corp). The acidifying capacity of LAB (1% [v/v] in 20 mL of SFE incubated at 30 °C) was subsequently assayed using the Cinac system (Ysebaert, Frépillon, France) by measuring the pH every 2 h during 24 h. The kinetics obtained were subjected to an analysis of variance (ANOVA) using the XLSTAT software (Addinsoft, Paris, France), followed by a Tukey's post hoc test. Differences were considered statistically significant at $p \leq 0.05$. A principal component analysis (PCA) was performed on 48 LAB strain's kinetic acidification using the same software.

Screening for Extracellular Hydrolytic Enzymes

The ability of bacteria to excrete hydrolytic enzymes was studied by a substrate hydrolysis plate assay method. After a first culture at 30 °C for 24 h in MRS broth (Biokar Diagnostics); each strain suspension (10 µL) was spot-inoculated on the surface of the plates then incubated at 30 °C for 24 h to 72 h.

Proteolytic activity was monitored by screening hydrolysis zones around colonies growing on Plate Count Agar (Biokar Diagnostics) plates containing 10% (w/v) skim milk (HiMedia Laboratories) as described by Herranen et al. [21]. Lipase-producing LAB were screened on MRS agar (Biokar Diagnostics), supplemented with olive oil as the method described by Kouker and Jaeger [22]. Briefly, olive oil (2.5% v/v), fluorescent dye, and rhodamine B (Sigma-Aldrich, Germany) were added and the lipolytic activity was determined orange fluorescence emitted around spots after exposure of plates to UV light at 350 nm. Lipolytic activity was also determined by the research of a clear halo surrounding the

Table 1 Properties of analyzed strains

Strain	Strain's code	Protease	Lipase		Amylase	Biogenic amines				Antifungal activity		
		Substrate				*hdc*	*agdi*	*odc*	*tdc*	*R. mucilaginosa UBOCC-A-216004*	*A. flavus UBOCC-A-106028*	*P. verrucosum UBOCC-A-109221*
		Casein	*Tween 80*	Olive oil	Starch							
P. pentosaceus CIRM-BIA1811	P111	+	–	–	–	–	–	–	–	–	–	+
P. pentosaceus CIRM-BIA1816	P149	+	–	–	–	–	+	–	–	–	–	–
P. pentosaceus CIRM-BIA2290	P85	+	–	–	–	+	–	–	–	–	–	–
P. pentosaceus CIRM-BIA2291	P96	+	–	–	–	–	+	–	–	–	–	–
P. pentosaceus CIRM-BIA1809	P98	+	–	–	–	–	–	–	–	–	–	–
P. pentosaceus CIRM-BIA2292	P92	+	–	–	–	–	–	–	–	–	–	–
P. pentosaceus CIRM-BIA2293	P100	+	–	–	+	–	–	–	–	–	–	–
P. pentosaceus CIRM-BIA2294	P103	+	–	–	–	–	–	–	–	–	–	±
P. pentosaceus CIRM-BIA1804	P76	++	–	–	–	+	–	–	–	–	–	±
P. pentosaceus CIRM-BIA1818	P160	++	–	–	–	–	+	–	–	–	–	±
P. pentosaceus CIRM-BIA1801	P68	++	–	–	–	–	+	–	–	–	–	–
P. pentosaceus CIRM-BIA1799	P50	++	–	–	–	+	–	–	–	–	–	±
P. acidilactici CIRM-BIA1803	P73	–	–	–	–	–	+	–	–	±	–	+
P. acidilactici CIRM-BIA1798	P49	++	–	–	–	+	–	–	–	–	–	–
L. brevis CIRM-BIA1812	L120	+	–	–	–	–	–	–	–	+	–	+
L. brevis CIRM-BIA2295	L164	–	–	–	–	–	+	–	–	+	–	+
L. coryniformis CIRM-BIA1814	L145	++	–	–	–	–	–	–	–	–	–	–
L. coryniformis CIRM-BIA1813	L141	++	–	–	–	–	–	–	–	–	–	–
L. rapi CIRM-BIA1800	L63	–	–	–	–	–	+	–	–	+	–	+
L. buchneri CIRM-BIA1795	L1	+	–	–	–	–	–	–	–	+	+	+
L. pentosus CIRM-BIA1815	L148	+	–	–	++	–	–	–	–	+	–	+
L. namurensis CIRM-BIA1819	L162	–	–	–	–	–	–	–	–	+	–	+
L. curvatus CIRM-BIA1806	L84	++	–	–	–	–	–	–	–	–	–	–
L. fallax CIRM-BIA1807	L91	++	–	–	–	–	+	–	–	+	+	+
L. mesenteroides CIRM-BIA1802	L70	+	–	–	+	–	+	–	–	+	–	+
W. paramesenteroides CIRM-BIA1810	W99	++	–	–	–	–	–	–	–	+	–	+
E. faecium CIRM-BIA1797	E20	+	–	–	–	+	–	–	–	–	–	–
E. faecium CIRM-BIA2296	E25	+	–	–	–	+	–	–	–	–	–	+
E. faecium CIRM-BIA1825	E190	+	–	–	+	+	–	–	–	–	–	–
E. faecium CIRM-BIA2297	E67	+	–	–	–	+	–	–	–	–	–	–
E. faecium CIRM-BIA2298	E66	+	–	–	–	+	–	–	–	–	–	–
E. faecium CIRM-BIA2299	E65	++	–	–	–	+	–	–	–	–	–	–
E. faecium CIRM-BIA2300	E80	+	–	–	–	–	–	–	–	–	–	–

Table 1 (continued)

Strain	Strain's code	Protease	Lipase		Amylase	Biogenic amines				Antifungal activity		
		Substrate				*hdc*	*agdi*	*odc*	*tdc*	*R. mucilaginosa UBOCC-A-216004*	*A. flavus UBOCC-A-106028*	*P. verrucosum UBOCC-A-109221*
		Casein	*Tween 80*	Olive oil	Starch							
E. faecium CIRM-BIA2301	E101	++	−	−	−	+	−	−	−	−	−	−
E. faecium CIRM-BIA2302	E192	+	−	−	−	−	−	−	−	−	−	−
E. faecium CIRM-BIA2303	E31	+	−	−	+	−	+	−	−	−	−	−
E. faecium CIRM-BIA2304	E154	+	−	−	−	+	−	−	−	−	−	−
E. faecium CIRM-BIA2305	E140	+	−	−	−	+	−	−	−	−	−	+
E. faecium CIRM-BIA1817	E159	+	−	−	−	−	−	−	−	−	−	+
E. faecium CIRM-BIA2306	E156	+	−	−	−	+	−	−	−	−	−	−
E. faecium CIRM-BIA2307	E147	+	−	−	−	+	−	−	−	−	−	−
E. faecium CIRM-BIA2312	E200	+	−	−	−	+	−	−	−	−	−	−
E. faecium CIRM-BIA2308	E157	+	−	−	−	−	−	−	−	−	−	−
E. faecium CIRM-BIA2309	E110	+	−	−	−	+	−	−	−	−	−	−
E. faecium CIRM-BIA2310	E150	+	−	−	−	+	−	−	−	−	−	−
E. faecium CIRM-BIA2311	E158	+	−	−	−	−	+	−	−	−	−	−
E. faecium CIRM-BIA1805	E82	+	−	−	−	−	−	−	−	−	−	−
E. hirae CIRM-BIA1808	E95	+	−	−	+	−	+	−	−	−	−	−

hdc histidine decarboxylase, *agdi* agmatine deiminase, *odc* ornithine decarboxylase, *tdc* tyrosine decarboxylase

(+), positive for enzyme production, biogenic amines genes detection, or antifungal activity (no growth of target); (−), negative for enzyme production, biogenic amines genes detection or antifungal activity; (++), high proteolytic; (±), postponed growth of fungi

spots at the surface of the MRS plates containing Tween 80 (1% v/v) (Labogros, France).

The amylolytic activity trials were performed in 96 plates containing the API50 CHLMedium broth (bioMerieux, France) supplemented with soluble starch (1% w/v) as the sole carbon source. The acids produced during fermentation decrease the pH of the medium and yield a shift in color from purple to yellow (API 50 CHL Medium contains a bromocresol purple as pH indicator), showing a previous starch hydrolysis.

Antifungal Activities

The antifungal activity of LAB was determined against three targets, *Rhodotorula mucilaginosa* UBOCC-A-216004, *Penicillium verrucosum* UBOCC-A-109221, and *Aspergillus flavus* UBOCC-A-106028, obtained from the Université de Bretagne Occidentale Culture Collection (UBOCC, Plouzané, France, www.univ-brest.fr/ubocc). The assays were performed on the wheat flour hydrolysate agar (WFH, pH 5.6) as described by Le Lay et al. [23]. To sum up, after a first culture at 30 °C for 24 h in MRS broth (Biokar Diagnostics) of LAB, 10 µL of culture was added in each well of a 24-well plate; then 1 mL of WFH agar was poured. After incubation at 30 °C for 48 h, 50 spores (molds) or cells (yeast) of each target fungus were inoculated on the agar surface respecting defined positions on the plate. Plates were then incubated at 25 °C, and fungal growth was evaluated visually after 7 and 15 days and compared with negative controls.

Production of Biogenic Amines

In order to assure the safety of the strains of interest, the genetic ability to synthetize biogenic amines was investigated by focusing on four major genes; *agdi*/*odc, hdc*, and *tdc*, involved in the production of putrescine, histamine, and tyramine, respectively. Those genes were searched by a multiplex PCR, which was carried out according to the method described by Coton et al. [18]. The primers used were HDC3/HDC4 to amplify a fragment of the histidine decarboxylase gene, AgD1/AgD2 for a fragment of the gene encoding agmatine deiminase, ODC1/ODC2 to amplify ornithine decarboxylase gene, and finally TD2/TD5 to amplify a fragment of the tyrosine decarboxylase gene. In parallel, an internal control was added in the reaction (primers BSF8 and BSR1451 targeting the gene encoding 16S rRNA) to validate each PCR.

Total DNA was extracted by DNeasy Blood tissue kit (Qiagen ref 69504; Hilden, Germany) using manufacturer protocol, for Gram positive, slightly modified as described by Parayre et al. [24].The PCR mix (25 µL) consists of Taq buffer with $MgCl_2$ (Q-Biogene/EPTQA100), dNTPs (2 mM/µL, Q-Biogene/NTPMX050), Taq DNA polymerase (2.5 U/µL), and 1 µL of the extracted DNA (25 ng/µL). Primers concentrations were 0.8 mM for ODC1, ODC2, AgD1, and AgD2; 0.2 mM for TD2 and TD5; 0.12 mM for HDC3/HDC4; and 0.05 mM for BSF8 and BSR1541. DNA amplification was carried out in a C1000TM thermal cycler (Bio-Rad) using the following conditions: 95 °C 5 min, 35 cycles of 95 °C 1 min, 52 °C 1 min, 72 °C 1 min 30 with a final extension at 72 °C for 5 min. The amplified products were resolved by electrophoresis (100 V, 1 h) on 0.8% agarose gel (w/v) in 0.5 × TBE buffer. The gels were stained in GelRed (3 × in 0.1 M NaCl solution) (FluoProbes®, Interchim, France) for 15–30 min and the images were obtained by means of GBox equipment (Syngene, Cambridge, UK).

Results

Acidifying Ability

From the 48 LAB strains tested, 37 (77%) lowered significantly the pH of SFE broth in the first 8 h of fermentation (Fig. S1). However, only 15 strains (25%) reached a pH close to 4 or below for longer periods of incubation; 3/21 strains of *Enterococcus faecium* (E80, E31, E82), 8/12 strains of *Pediococcus pentosaceus* (P111, P85, P96, P92, P103, P76, P68, P50), 1/2 strains of *Lactobacillus brevis* (L120), *Lactobacillus curvatus* (L84), *Leuconostoc mesenteroides* (L70), and *Weissella paramesenteroides* (W99).

A PCA was performed to analyze the main differences in acidifying profiles of all strains (Fig. 1). PC1, describing 98% of the variability, was negatively associated with strains having low or no acidifying activity and positively correlated with strains with high or average acidifying ability (high Δ pH and low final pH). It separated the 48 LAB strains, according to their acidifying rate, into three distinct groups. The first represents the highest acidifiers, the second constitutes the average acidifiers and the third corresponds to the lowest acidifiers. Indeed, group one comprises 12 strains among which eight were pediococci (67%), while group two with 24 strains was dominated by enterococci (58%). Besides, PC2 accounting for 2% of the variability was negatively related to three strains with slow acidification pace but quite capable of reaching low pH tardily (*Lactobacillus brevis*; L120, *L. curvatus*; L84, and *W. paramesenteroides*; W99).

Hydrolytic Enzymes

In protease assay, 44 strains (91%) showed a clear area of casein hydrolysis on skim milk agar whose 12 with large zones around the cultures were as follows: *P. pentosaceus* (P76, P160, P68, P50), *Pediococcus acidilactici* P49,

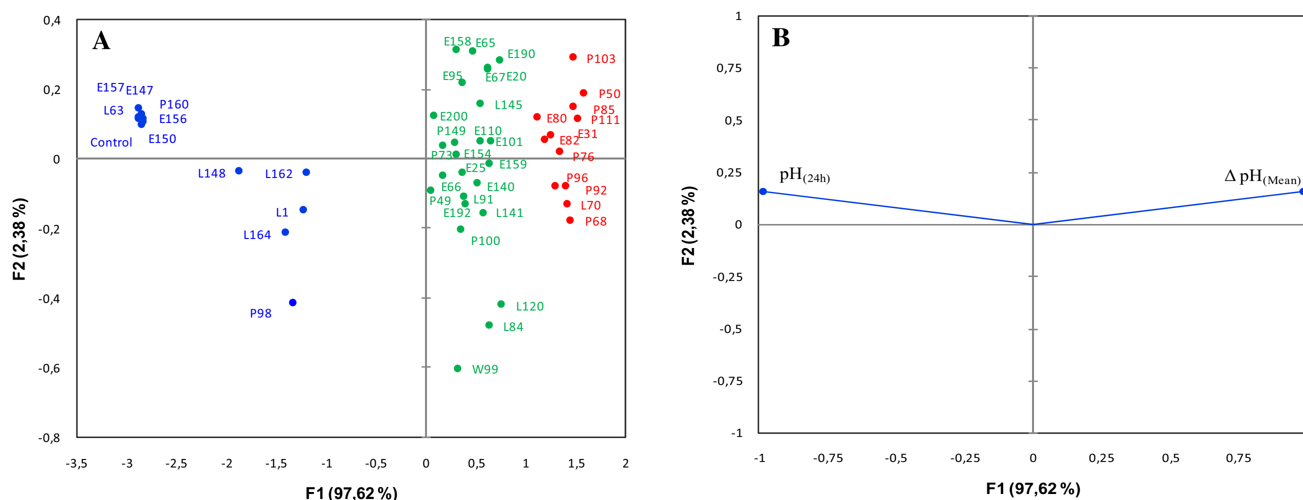

Fig. 1 Individuals' factor map (**a**) and variables' factor map (**b**) of principal component analysis on 48 LAB strains' rate acidification of sterile SFE (means of Δ pH) and the final pH attended after 24 h. They are grouped by acidifying ability; lowest acidifiers in blue, average acidifiers in green, and highest acidifiers in red. The non-inoculated SFE is represented by Control. The names of LAB strains are simplified according to Table 1 (Color figure online)

Lactobacillus coryniformis (L145, L141), *L. curvatus* (L84), *Leuconostoc fallax* (L91), *W. paramesenteroides* (W99), and *E. faecium* (E65, E101). For the amylolytic activities, only six strains (12%) presented ability to degrade starch: *P. pentosaceus* (P100), *L. pentosus* (L148), *L. mesenteroides* (L70), *E. faecium* (E190, E31), and *Enterococcus hirae* (E95). *L. pentosus* (L148) showed the highest amylolytic activity compared to the other strains characterized by a moderate shift in color. However, no strain has demonstrated a lipolytic activity on the two types of the tested media. All the results are presented in Table 1.

Antifungal Activities

The antifungal activity of 48 LAB strains was tested against three selected fungal targets. Strains exhibited varied antifungal activity spectra according to species (Table 1 and Fig. 2). *L. buchneri* (L1) and *L. fallax* (L91) were the only ones capable to inhibit all tested fungi (i.e., no growth of the indicator fungi after 7 and 15 days). It is also to be noted that strains of six species (*L. mesenteroides*, *W. paramesenteroides, L. brevis*, *L. pentosus*, *L. namurensis*, *L. brevis*) showed a notable inhibitory action

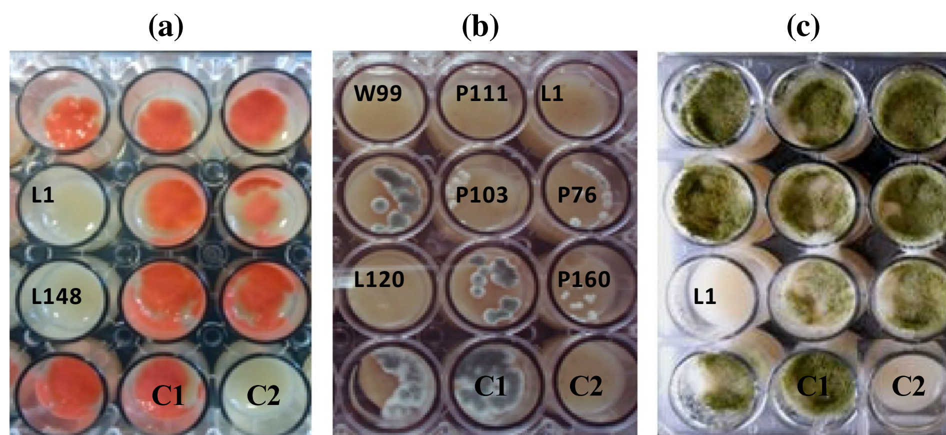

Fig. 2 In vitro screening on WFH agar of LAB's antifungal activities against: **a** *R. mucilaginosa UBOCC-A-216004,* **b** *P. verrucosum UBOCC-A-109221,* and **c** *A. flavus UBOCC-A-106028.* L1; *L. buchneri* CIRM-BIA 1795, L148; *L. pentosus* CIRM-BIA, W99; *W. paramesenteroides* CIRM-BIA 1810, P111; *P. pentosaceus* CIRM-BIA 1811, L120; *L. brevis* CIRM-BIA 1812, P103; *P. pentosaceus* CIRM-BIA2294, P76; *P. pentosaceus* CIRM-BIA 1804, P160; *P. pentosaceus* CIRM-BIA 1818, C1; negative control for intact growth of the indicator fungi inoculated on the WFH agar without LAB in well, C2; control for no growth on the WFH agar incubated without LAB and indicator fungi in well. The remaining wells represent examples of LAB strains without activities against the target fungi

on *P. verrucosum* UBOCC-A-109221 and *R. mucilaginosa* UBOCC-A-216004. Some strains of *pediococci* can postpone the growth of *P. verrucosum* UBOCC-A-19221 and *R. mucilaginosa* UBOCC-A-216004 (i.e., slow growth of the indicator fungi, with small colonies not widespread, compared to the negative control). For pediococci and enterococci, only five strains were able to constrain *P. verrucosum* UBOCC-A-109221.

Production of Biogenic Amines

The search for biogenic amines, carried out by multiplex PCR, revealed no detection of the four target genes for 17 of the 48 strains; *P. pentosaceus* (P98, P111, P92, P100, P103), *L. coryniformis* (L145, L141), *L. buchneri* (L1), *L. pentosus* (L148), *L. namurensis* (L162), *L. curvatus* (L84), *W. paramesenteroides* (W99), *E. faecium* (E80, E192, E159, E157, E82). The genes, *odc* and *tdc* encoding ornithine decarboxylase and tyrosine decarboxylase, respectively, were not observed in any of the tested strains. On the other hand, for 9 strains of pediococci, the presence of *hdc* and *agdi* genes which, respectively, code for histidine carboxylase and agmatine deiminase, has been recorded but was never concomitant. The same case was encountered in 16 strains of enterococci, with 14 strains harboring the gene *hdc* and two strains the gene *agdi* (Table 1).

Discussion

48 LAB strains were investigated for the presence of technologically four relevant characteristics for cereal fermentation: acidifying ability, enzymatic activities, antifungal activity, and safety. Our results showed that many strains of pediococci and enterococci seem to dominate the kinetic acidification. This corroborates the previous study on the characterization of the *lemzeiet* microbiota, where *P. pentosaceus* and *E. faecium* were described as dominant from the first stage of the fermentation [17]. *L. curvatus* (L84), *L. brevis* (L120), and *L. mesenteroides* (L70) also showed good acidifying abilities. Among all the criteria considered for the selection of starters, the performance in terms of strains' acidification rate is a key factor; since it is a guarantee for the success of the first steps of the fermentation process. Indeed, acids inhibit undesirable flora and enhance the activity of some endogenous enzymes in cereals [25–28].

The hydrolytic profiles of the tested strains showed that the majority are able to hydrolyse casein but only 12% were amylolytics. The degradation of cereals proteins is of crucial importance for fermented cereals' flavor and rheology [29]. Many LAB from fermented cereals have been reported to have these activities [30–32]. The shift of the pH initiates the hydrolysis of the cereal's proteins by the endogenous proteases. LAB, by membrane and/or endogenous proteases, hydrolyze them into shorter peptides then into acids amines by the concerted action of various peptidases [33]. Nevertheless, starch hydrolysis is not common in LAB, but amylolytic LAB have often been isolated from fermented cereal foods and may represent about 10% of the LAB population [10]. Extracellular amylase activity was characterized in several lactobacilli and enterococci, notably in *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus manihotivorans*, *Lactobacillus amylovorus*, *Lactobacillus gasseri,* and *E. faecium* [34–36]. It plays an important role in the microbiota of fermented cereals by providing the substrates necessary for the growth of non-amylolytic LAB [10, 37]. Reduced sugars, peptides, and amino acids affect the taste of fermented foods and, particularly, are important precursors for volatile flavor compounds [17, 38]. In the present work, no strain with lipolytic activity was detected. In fact, LAB were reported as weakly lipolytic compared with other bacteria, but their presence in fermented foods during long periods can bring them to release appreciable amounts of free fatty acids which can contribute to flavor development [39, 40].

Fermentation also plays a key role in food biopreservation due to a variety of compounds and metabolites produced by the fermentative microbiota, especially by LAB [41, 42]. Thus, they can act synergistically in complex food ecosystems against alteration and/or pathogenic microorganisms [25]. In this study, from the 48 tested LAB, two strains, *L. fallax* (L91) and *L. buchneri* (L1) were able to inhibit three fungal targets frequently involved in food spoilage and mycotoxins production [43, 44]. In addition, strains belonging to *Lactobacillus* and *Leuconostoc* spp. represented the most active ones against the targets tested in WFH agar. In fact, strains of these species are known for their high antifungal activity [45]. *Lactobacillus* and *Leuconostoc* spp. have been described by many studies as the best antifungal agents compared to other LAB species [23, 46, 47]. Moreover, in our precedent study, on *lemzeie*t, we had highlighted that fermentation process allows the disappearance of fungi flora over time, which reinforces the fact that LAB strains exerted an antifungal activity and make the product safer for consumption [17]. Indeed, LAB are naturally present in many foods and have a long history of safe use in fermentation process [1]. They produce a wide range of antifungal compounds including organic acids such as acetic, lactic, phenyl lactic, propionic, and fatty acids as well as cyclic dipeptides or proteinaceous compounds [47–49]. Fungal inhibition generally results from the additive and/or synergistic activities of several of these compounds [50].

Biogenic amines are organic and nitrogenous compounds of low molecular weight, with biological activity, basically formed by the decarboxylation of amino acids [51]. They can be toxic if foods containing high

concentrations are ingested, and they may have a role as indicators of quality and/or acceptability in some foods [15]. They can be present in a variety of products like chocolate, meat, fish, and fermented foods [52]. Thus, when looking for strains with a potential starter application, their microbial safety and regulatory considerations such as Generally Recognized as Safe (GRAS) or Qualified Presumption of Safety (QPS) must be considered [1]. Among them LAB species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* are considered as potential producers of biogenic amines [53]. We have seen, in the present study, that 9 and 16 strains of pediococci and enterococci, respectively, present abilities to produce biogenic amines which reaffirms a previous finding that their formation is highly dependent on bacterial strains rather than the species, suggesting that horizontal gene transfer may account for their dissemination in LAB [18]. Thus, highlighting the importance of the careful selection of indigenous strains for inclusion in starter and adjunct cultures [54].

The synthesis of the obtained results, integrating acidification ability, proteolytic and amylolytic activities, antifungal potential, and molecular traits of biogenic amines production, is presented in table S1. Among the 48 studied strains, ten fulfill the required, complementary, functionalities to be potentially used as starter (4 strains of *P. pentosaceus*, 1 strain of *L. mesenteroides*, and 5 lactobacilli belonging to 5 different species) and appear in the list of species of the European Food Safety Authority (EFSA) as presumed to be safe (QPS). In fact, a consortium of species and strains is necessary to obtain the desirable properties of fermented cereal products [2, 11, 55].

Conclusion

This study, of selected technological features of 48 LAB strains previously isolated from fermented wheat *lemzeiet*, confirms the interest in exploiting the natural diversity of vegetal matrix to select safe, accurate, and efficient starters for fermented food production. As a result, 10 strains belonging to seven species (*P. pentosaceus*, *L. brevis*, *L. buchneri*, *L. namurensis*, *L. curvatus*, *L. pentosus*, *L. mesenteroides*) could be used as starter cultures for a future controlled fermentation process of *lemzeiet*. Moreover, in order to have multifunctional starter, this study reinforces the focus on using starter cultures composed of several strains, each one of them having a specific functionality. However, scale-up fermentation should be conducted to determine the expression and the interaction of the strains when used in association within wheat matrix and confirm the properties of the final fermented product.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Research Involving Human and Animal Participants This article does not contain any studies with human or animal subjects.

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