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Diversity of the metabolic profiles of a broad range of lactic acid bacteria in soy juice fermentation

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ABSTRACT

This study explores the ability of lactic acid bacteria (LAB) to ferment soy juice. The ability of 276 LAB strains from 25 species to ferment the principal soy carbohydrates, sucrose, raffinose or stachyose was tested in synthetic media and a soy juice. Fermented soy juices (FSJs) were characterized for their odor. Selected FSJs were characterized by targeted metabolomics. All *Streptococcus*, 83% of *Leuconostoc* and *Lactobacillus* and 41% of *Lactococcus* strains were sucrose-positive, while only 36% of all the LAB strains tested were raffinose-positive and 6% stachyose-positive. Nearly all (97%) the sucrose-positive strains fermented soy juice, indicating that an ability to use sucrose is a good criterion to select strains for soy juice fermentation. Among the most efficient acidifying strains, 46 FSJs had an odor deemed to be acceptable. FSJ composition was dependent on both species and strains: 17/46 strains deglycosylated soy juice isoflavones, the 27 *S. thermophilus* strains converted a mean 4.4 ± 0.1 g/L of sucrose into 3.0 ± 0.1 g/L of lactic acid *versus* 5.2 ± 0.1 g/L into 2.2 ± 0.1 g/L for the 18 *Lactobacillus* and one *Lactococcus* strains. This study highlights the diversity of the metabolic profiles of LAB strains in soy juice fermentation.

1. Introduction

Protein source in Western diets is mainly composed of animal-based protein (60% *versus* 40% plant-based protein) ("FAOSTAT", 2018). A more balanced diet containing 50% of plant-based protein is healthier, will help to reduce the environmental impacts of food systems and may contribute to meeting worldwide protein needs (Guéguen et al., 2016; Springmann et al., 2018). Among all the plants used as a protein source, soybean [*Glycine max*] is interesting because of its high protein content (40% in dry matter). In this context, soy juice, also called soymilk, represents an interesting alternative to animal milk as a sustainable food. It could also be a valuable protein source for lactose-intolerant and vegan populations. However, two main bottlenecks limit the consumption of soy juice. First, soy "off-flavors" ("beany" and "green" flavors) are not appreciated by consumers (Kaneko et al., 2011). Secondly, oligosaccharides such as raffinose and stachyose are poorly digestible by humans, causing digestive discomfort and flatulence (Guillon and Champ, 2002). The lactic fermentation of soy juice by lactic acid bacteria (LAB) to produce a yogurt-type fermented soy product is a sustainable and inexpensive process for the preservation of soy juice. Moreover, lactic acid fermentation can contribute to improving the organoleptic properties of soy juice by reducing "off-flavors" and/or increasing "hedonic-flavor" compounds (Mital and Steinkraus, 1979; Siroli et al., 2019) and to lowering the content of non-digestible oligosaccharides (Singh and Vij, 2018).

The soy juice fermentation profile is dependent on several parameters: the composition of the soy juice, including its carbohydrate content, the LAB strains used as a starter and the fermentation parameters applied. Soy juice contains different carbohydrates: sucrose (50%), a disaccharide of glucose and fructose linked in β-1,2, raffinose (10%), a trisaccharide of sucrose and galactose linked in α-1,6, and stachyose (40%), a tetrasaccharide of raffinose and galactose linked in α-1,6 (Mital and Steinkraus, 1979). The carbohydrate content of soy juice can range from 1 g/L (Hati et al., 2014) to 23 g/L (Champagne

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et al., 2009), depending on soybean quality and the different soybean treatments used to produce soy juice (such as soaking soybeans in water, grinding, mixing, heating or filtering). Several classes of enzymes have been shown to be implicated in the catabolism of these carbohydrates. LAB levansucrases are enzymes that hydrolyze β-1,2 linkages (Gänzle and Follador, 2012). LAB α-galactosidases are enzymes that hydrolyze α-1,6 linkages and convert raffinose and stachyose (LeBlanc et al., 2004; Yoon and Hwang, 2008).

Although LAB have been used to ferment soy juice for decades, the roughly 300 LAB strains (from the *Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus* genera) that have previously been studied across 50 scientific publications have not provided a full description of the metabolic profiles of LAB used in soy juice fermentation (due to the diversity of soy juice composition and fermentation parameters). Furthermore, generally, most publications do not report "negative" results; *i.e.* results that would indicate an inability of LAB strains to ferment soy juice. The ability of different LAB to use raffinose and stachyose in soy juice also remains poorly understood (Gänzle and Follador, 2012; Wang et al., 2003), as the diversity of the primary and secondary metabolites they produce during soy juice fermentation (Li et al., 2014).

The objective of the present study was therefore to explore the diversity of LAB metabolic profiles in soy juice fermentation. The screening was organized in three successive selecting steps (Fig. 1). During the first step, we screened strains abilities to acidify media with different carbon sources and soy juice. We investigated the ability of 276 LAB strains from 25 species to acidify a synthetic medium containing either sucrose, raffinose or stachyose as the only carbohydrate source, or a soy juice (SJ). In the second step, we screened strains through sensory evaluation of their FSJs by sniffing. We produced 112 fermented soy juices (FSJs) using the LAB strains that could acidify the SJ. Their odor was evaluated from a sensory point of view, which led to a third selection of 46 FSJs that had odors deemed to be acceptable. Finally, selected FSJs were characterized through metabolomics approaches targeting carbohydrates, organic acids, isoflavones and volatiles.

2. Materials and methods

2.1. Bacteria and reactivation conditions (from frozen culture)

Twenty five LAB species have been selected thanks to bibliography and Florilege database (Chaix et al., 2019, http://migale.jouy.inra.fr//Florilege) for their capacity to use soy main carbohydrates. Two hundred seventy-six strains from the 25 LAB species provided by the International Centre for Microbial Resources-Food Associated Bacteria (CIRM-BIA, https://www6.inra.fr/cirm_eng/Food-Associated-Bacteria) were assessed in this study. The strains were 57 *Streptococcus thermophilus*, 46 *Lactococcus lactis*, 5 *Leuconostoc mesenteroides* and 168 *Lactobacillus* sp. (44 *L. plantarum*, 21 *L. delbrueckii*, 9 *L. pentosus*, 16 *L. helveticus*, 14 *L. rhamnosus*, 9 *L. paraplantarum*, 9 *L. paracasei*, 7 *L. johnsonii*, 5 *L. casei/L. zeae*, 8 *L. acidophilus*, 5 *L. sanfranciscencis*, 7 *L. curvatus*, 3 *L. amylovorus*, 3 *L. mali*, 1 *L. coryneformis*, 1 *L. xiangfangensis*, 1 *L. diolivorans*, 1 *L.pontis*, 1 *L. rossiae*, 1 *L. hominis*, 1 *L. kunkeei* and 1 *L. sakei)*.

The strains were activated from frozen glycerol stocks (−80 °C) in broth medium for 24 h. They were inoculated at 1% v/v in M17 broth for *Streptococcus* and *Lactococcus* strains and in Man, Rogosa and Sharpe (MRS) broth for *Lactobacillus* and *Leuconostoc* strains. Cultures were incubated at 30 °C, 37 °C or 43 °C according to its specie affiliation (Supplementary Table 1).

2.2. Culture media

LAB were tested for their capacity to acidify a synthetic medium, either M17 or MRS, containing 5 g/L of sucrose (M-suc), 5 g/L of raffinose (M-raf) or 2 g/L of stachyose (M-sta) as the sole source of carbohydrate. Control media were prepared with no sugar (M-ns). Synthetic media had an initial pH of 6.9 ± 0.2.

The soy juice used for fermentation was a commercial stabilized organic soy juice *Sojade,* (Triballat Noyal SAS, France), referred to as SJ below. The SJ used had a pH of 7.2 and contained 5.5 g/L of sucrose, 0.9 g/L of raffinose, 3.1 g/L of stachyose, 0.7 g/L of succinic acid and 1.1 g/L of citric acid. It did not contain any monosaccharide, nor lactic, acetic, or pyruvic acids (Table 1).

2.3. Evaluation of strains abilities to acidify media with different carbon sources and soy juice

Acidification tests were run after activation from frozen glycerol stocks and two sub-cultures inoculated at 1% v/v in 10 mL of M17/MRS. The first sub-culture lasted 24 h, the second 14 ± 1 h. Acidification tests were run using either 2 mL of SJ, M-ns, M-suc, M-raf or M-sta, which were inoculated at 1% v/v. All the experimentations were performed with the same batch of soy juice and were performed in duplicate from independent sub-cultures. The pH was measured for each sample after 10 h and 48 h of incubation using a pH meter (Cyberscan pH110, EUTECH Instruments). The criterion used to consider a strain sucrose-, raffinose- or stachyose-positive in 10 h and 48 h of fermentation was a difference superior to 0.3 pH unit between controls (M-ns) and M-suc, M-raf or M-sta. A pH below 6, an arbitrary criterion, was used to consider a strain positive to acidify soy juice in

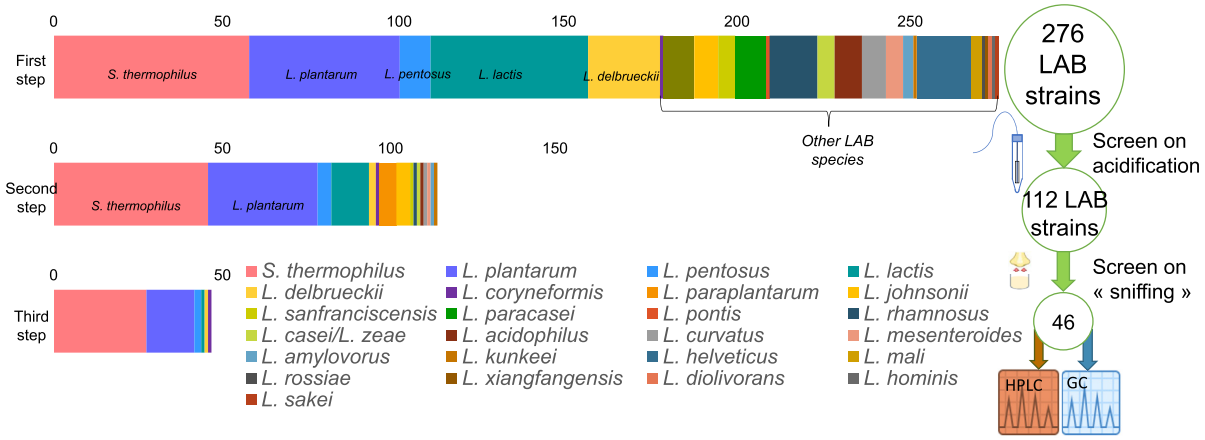


Fig. 1. Number of strains tested by species during the different steps of the present study. Color scales are used for each step to clarify visualization of the number of strains. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
pH values, oligosaccharides, organic acids and isoflavone concentrations in soy juices after 10 h of fermentation by 46 strains, means of duplicates inoculated at 1% v/v. The color gradient is used by metabolites to highlight differences between products. The products are classified according to their pH. Letters after the concentrations indicate statistical differences from a Tukey test with an alpha error of 0.1. If there is more than 5 letters, letters are written as "1st letter to last letter" (ex. "abcdef" is written as "a to f").

	Measures realised on soy juices fermented for 10h			Carbohydrates concentrations (g/L)														Acids concentrations (g/L)										Isoflavones concentrations (mg/L equivalent aglycones)									
Ref. CIRM-BIA	Species	Names	pH	Sucrose		Raffinose		Stachyose		Glucose		Fructose		Melibiose		Galactose		Total sugars		Citrate		Lactate		Acetate		Succinate		Pyruvate		glycosiled isoflavones		deglycosylated isoflavones		acetyled isoflavones		malonyled isoflavones		Total isoflavones	
258	*S. thermophilus*	S.th258	4.5	0.7	cdef	0.7	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.4	c	1.3	abc	3.6	ab	0.1	g to n	0.2	c	0.02	a to g	123	a	8	g	0	g	16	a	147	ab
251	*S. thermophilus*	S.th251	4.6	1.1	bcdef	0.7	a	2.7	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.7	bc	1.3	abc	3.5	abc	0.0	h to o	0.2	c	0.02	a to h	127	a	10	g	1	defg	12	a	150	ab
1051	*S. thermophilus*	S.th1051	4.6	0.5	def	0.6	a	3.1	a	0.0	b	0.5	bcd	0.0	b	0.0	a	4.8	bc	1.3	abc	3.4	a to f	0.1	g to n	0.2	c	0.03	abcd	124	a	9	g	1	defg	18	a	151	ab
1363	*S. thermophilus*	S.th1363	4.7	0.2	def	0.7	a	2.9	a	0.0	b	0.5	bcd	0.0	b	0.0	a	4.5	c	1.3	abc	3.3	b to g	0.1	g to n	0.2	c	0.02	c to j	128	a	8	g	1	defg	8	a	145	ab
257	*S. thermophilus*	S.th257	4.7	1.1	bcdef	0.7	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.8	bc	1.4	ab	3.5	abcd	0.0	mnop	0.6	ab	0.03	abc	124	a	11	g	0	fg	12	a	147	ab
1358	*S. thermophilus*	S.th1358	4.7	1.1	bcdef	0.8	a	3.0	a	0.0	b	0.3	cdef	0.0	b	0.0	a	5.4	bc	1.3	abc	3.4	abcde	0.0	j to p	0.2	c	0.01	c to j	123	a	10	g	1	efg	16	a	149	ab
2103	*S. thermophilus*	S.th2103	4.7	0.2	def	0.6	a	2.8	a	0.0	b	0.5	bc	0.0	b	0.0	a	4.3	c	1.2	abc	3.0	f to k	0.0	h to o	0.2	c	0.01	d to k	123	a	10	g	1	efg	16	a	149	ab
26	*S. thermophilus*	S.th26	4.8	1.0	bcdef	0.7	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.8	bc	1.3	abc	3.6	ab	0.0	j to p	0.2	c	0.02	a to f	128	a	9	g	1	defg	11	a	149	ab
1035	*S. thermophilus*	S.th1035	4.8	0.7	cdef	0.5	a	2.8	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.1	c	1.5	a	3.9	a	0.1	h to o	0.2	c	0.03	a to f	121	a	9	g	0	g	14	a	144	ab
18	*S. thermophilus*	S.th18	4.9	1.4	bcdef	0.6	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	5.1	bc	1.2	abc	2.4	h to m	0.0	klmn	0.2	c	0.02	b to h	124	a	10	g	0	g	14	a	147	ab
67	*S. thermophilus*	S.th67	4.9	1.1	bcdef	0.7	a	2.9	a	0.0	b	0.1	def	0.0	b	0.0	a	4.9	bc	1.4	ab	3.2	b to h	0.0	nop	0.6	ab	0.01	c to j	121	a	10	g	0	g	12	a	143	ab
1860	*S. thermophilus*	S.th1860	4.9	0.6	def	0.7	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.3	c	1.4	abc	3.4	abcd	0.0	mnop	0.3	c	0.02	c to h	121	a	9	g	0	g	19	a	150	ab
34	*S. thermophilus*	S.th34	4.9	1.3	bcdef	0.8	a	3.0	a	0.0	b	0.2	cdef	0.0	b	0.0	a	5.4	bc	1.3	abc	2.7	ijklm	0.0	j to p	0.2	c	0.04	a	120	a	12	g	0	g	15	a	147	ab
261	*S. thermophilus*	S.th261	4.9	1.0	bcdef	0.7	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.8	bc	1.3	abc	3.5	abcd	0.0	i to o	0.6	ab	0.03	ab	121	a	9	g	2	cdefg	11	a	143	ab
772	*S. thermophilus*	S.th772	4.9	0.9	bcdef	0.7	a	2.9	a	0.0	b	0.3	bcdef	0.0	b	0.0	a	5.0	bc	1.4	a	3.7	ab	0.0	op	0.3	c	0.02	b to h	120	a	8	g	0	fg	16	a	145	ab
1056	*S. thermophilus*	S.th1056	5.0	1.1	bcdef	0.7	a	3.0	a	0.0	b	0.4	bcde	0.0	b	0.0	a	5.3	bc	1.4	a	3.1	d to i	0.0	op	0.2	c	0.01	hijk	118	a	9	g	1	efg	17	a	144	ab
1053	*S. thermophilus*	S.th1053	5.1	1.5	bcde	0.5	a	2.8	a	0.0	b	0.1	cdef	0.0	b	0.0	a	5.0	bc	1.3	abc	1.8	st	0.0	mnop	0.2	c	0.02	c to i	122	a	10	g	0	g	13	a	145	ab
1046	*S. thermophilus*	S.th1046	5.1	1.2	bcdef	0.8	a	3.0	a	0.0	b	0.3	bcdef	0.0	b	0.0	a	5.4	bc	1.2	abc	2.5	lmnop	0.0	lmnop	0.2	c	0.02	c to i	118	a	17	g	5	abc	9	a	148	ab
1364	*S. thermophilus*	S.th1364	5.1	0.9	bcdef	0.6	a	2.3	a	0.0	b	0.2	cdef	0.0	b	0.0	a	4.1	c	1.3	abc	3.0	e to j	0.0	mnop	0.3	c	0.02	b to h	130	a	10	g	0	fg	8	a	149	ab
1128	*S. thermophilus*	S.th1128	5.1	2.2	b	0.8	a	3.1	a	0.0	b	0.7	b	0.0	b	0.0	a	7.1	ab	1.1	bc	3.0	e to k	0.0	mnop	0.2	c	0.01	e to k	129	a	9	g	0	g	11	a	150	ab
23	*S. thermophilus*	S.th23	5.1	1.6	bcd	0.6	a	2.9	a	0.0	b	0.5	bcde	0.0	b	0.0	a	5.7	bc	1.1	bc	2.7	jklmn	0.0	h to o	0.2	c	0.01	g to k	125	a	10	g	1	defg	12	a	147	ab
1135	*S. thermophilus*	S.th1135	5.2	2.0	bc	0.7	a	3.0	a	0.0	b	0.2	cdef	0.0	b	0.0	a	6.1	bc	1.4	abc	2.6	jklmn	0.1	d to k	0.2	c	0.01	c to j	121	a	11	g	0	g	15	a	147	ab
1864	*S. thermophilus*	S.th1864	5.2	0.5	def	0.7	a	2.8	a	0.0	b	0.3	bcdef	0.0	b	0.0	a	4.4	c	1.3	abc	3.2	c to i	0.0	j to p	0.3	c	0.01	c to j	122	a	11	g	2	cdefg	19	a	154	ab
2102	*S. thermophilus*	S.th2102	5.2	1.3	bcdef	0.8	a	2.8	a	0.0	b	0.1	cdef	0.0	b	0.0	a	5.2	bc	1.0	bc	3.0	f to k	0.0	h to o	0.2	c	0.01	d to k	125	a	9	g	0	g	10	a	145	ab
20	*S. thermophilus*	S.th20	5.3	1.1	bcdef	0.8	a	2.8	a	0.0	b	0.1	cdef	0.0	b	0.0	a	4.9	bc	1.1	bc	2.9	g to l	0.0	k to p	0.2	c	0.01	d to k	124	a	9	g	1	defg	19	a	153	ab
1490	*L. pentosus*	L.pe1490	5.3	0.0	f	0.8	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	3.8	c	1.0	bc	1.9	qrst	0.0	i to p	0.5	b	0.02	a to h	48	c	85	d	2	b to g	14	a	148	ab
36	*S. thermophilus*	S.th36	5.4	1.3	bcdef	0.7	a	2.5	a	0.0	b	0.2	cdef	0.0	b	0.0	a	4.7	bc	1.3	abc	2.8	g to l	0.0	k to p	0.2	c	0.01	d to k	126	a	9	g	0	fg	12	a	146	ab
653	*L. plantarum*	L.pl653	5.4	0.4	def	0.8	a	2.8	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.1	c	1.1	bc	2.6	jklmn	0.1	ab	0.2	c	0.02	b to h	49	c	90	d	4	a to e	10	a	152	ab
1420	*L. plantarum*	L.pl1420	5.4	0.9	bcdef	0.8	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.8	bc	1.0	bc	2.1	pqrst	0.1	ab	0.2	c	0.02	c to j	38	cd	91	d	1	defg	13	a	143	ab
1108	*L. plantarum*	L.pl1108	5.4	0.5	def	0.8	a	2.8	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.3	c	1.1	bc	2.3	mnopq	0.1	ab	0.2	c	0.02	a to h	22	de	123	ab	2	b to g	10	a	158	ab
2184	*L. plantarum*	L.pl2184	5.4	0.4	def	0.9	a	3.0	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.4	c	1.1	bc	2.2	nopqr	0.1	bcde	0.2	c	0.02	c to h	23	de	106	c	3	a to g	8	a	140	b
777	*L. plantarum*	L.pl777	5.4	0.0	f	0.8	a	2.8	a	0.0	b	0.0	ef	0.0	b	0.0	a	3.7	c	1.0	d	2.9	u	0.2	op	0.2	d	0.03	jk	17	e	122	ab	2	b to g	9	a	151	ab
1111	*L. plantarum*	L.pl1111	5.4	0.0	f	0.8	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	3.8	c	1.1	bc	2.1	opqrs	0.1	b to g	0.2	c	0.02	c to i	10	e	124	ab	1	defg	15	a	150	ab
2107	*L. plantarum*	L.pl2107	5.4	0.4	def	0.7	a	2.8	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.0	c	1.1	bc	2.2	n to s	0.1	abc	0.2	c	0.02	c to h	11	e	121	ab	2	cdefg	19	a	152	ab
313	*L. delbrueckii*	L.de313	5.5	0.0	ef	0.7	a	2.8	a	0.8	a	1.3	a	0.2	a	0.0	a	5.9	bc	1.3	abc	1.6	st	0.1	c to j	0.3	c	0.00	jk	118	a	14	g	2	b to g	14	a	148	ab
1568	*L. lactis*	L.la1568	5.5	0.9	bcdef	0.7	a	3.1	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.9	bc	1.1	abc	2.9	ghijk	0.1	b to i	0.2	c	0.00	ijk	123	a	12	g	6	ab	9	a	149	ab
2115	*L. plantarum*	L.pl2115	5.5	0.2	def	0.8	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.0	c	1.0	c	2.9	g to l	0.2	a	0.2	c	0.02	abcde	19	e	126	ab	7	a	9	a	160	ab
845	*L. plantarum*	L.pl845	5.5	0.2	def	0.7	a	2.7	a	0.0	b	0.0	ef	0.0	b	0.0	a	3.7	c	1.2	abc	1.9	rst	0.1	b to h	0.2	c	0.02	a to h	26	de	114	bc	3	a to g	9	a	152	ab
2185	*L. plantarum*	L.pl2185	5.5	0.7	cdef	0.8	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.5	c	1.5	ab	2.6	klmno	0.1	ab	0.3	c	0.02	a to h	21	de	119	abc	3	a to g	14	a	157	ab
2104	*S. thermophilus*	S.th2104	5.5	1.5	bcde	0.7	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	5.3	bc	1.2	abc	1.5	t	0.1	g to n	0.2	c	0.01	f to k	124	a	9	g	1	defg	11	a	145	ab
855	*L. plantarum*	L.pl855	5.6	0.0	f	0.8	a	2.8	a	0.0	b	0.0	ef	0.0	b	0.0	a	3.8	c	1.1	abc	2.2	nopqr	0.1	b to i	0.2	c	0.02	a to h	23	de	122	ab	5	abcd	9	a	159	ab
2169	*L. pentosus*	L.pe2169	5.6	0.0	f	0.7	a	3.0	a	0.0	b	0.0	ef	0.0	b	0.0	a	3.9	c	1.1	bc	2.0	qrst	0.1	e to l	0.2	c	0.02	c to h	113	a	32	f	4	a to f	12	a	162	a
2239	*L. coryniformis*	L.co2239	5.6	0.0	f	0.8	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	3.8	c	1.1	bc	2.0	pqrst	0.1	bcd	0.2	c	0.02	c to h	82	b	54	e	3	a to g	15	a	154	ab
2183	*L. plantarum*	L.pl2183	5.7	0.6	cdef	0.8	a	3.0	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.5	c	1.2	abc	2.0	pqrs	0.1	bcdef	0.2	c	0.02	c to j	14	e	129	a	3	a to g	11	a	157	ab
2210	*L. plantarum*	L.pl2210	5.7	0.3	def	0.7	a	2.6	a	0.0	b	0.0	ef	0.0	b	0.0	a	3.7	c	1.2	abc	1.9	pqrst	0.1	f to m	0.2	c	0.01	c to j	15	e	127	ab	3	a to g	11	a	157	ab
2186	*L. plantarum*	L.pl2186	5.7	0.6	cdef	0.8	a	2.9	a	0.0	b	0.0	ef	0.0	b	0.0	a	4.4	c	1.4	abc	1.9	st	0.1	d to k	0.3	c	0.02	c to j	15	e	129	a	3	a to g	11	a	158	ab
t43	Controls	t43	7.2	5.6	a	0.8	a	3.1	a	0.0	b	0.0	f	0.0	b	0.0	a	9.6	a	1.2	bc	0.0	u	0.0	p	0.7	a	0.00	k	127	a	11	g	3	a to g	8	a	149	ab
t30	Controls	t30	7.2	5.4	a	0.9	a	3.1	a	0.0	b	0.0	f	0.0	b	0.0	a	9.7	a	1.1	bc	0.0	u	0.0	p	0.6	a	0.00	k	127	a	11	g	3	a to g	8	a	149	ab

10 h or 48 h.

2.4. Fermented soy juices production used for sensory and metabolomics analyses

Soy juice fermentations were run after strain reactivation from frozen culture and two consecutive sub-cultures. SJ was inoculated at 1% v/v in 20 mL and incubated 10 h at the specified temperature. Fermented soy juices (FSJs) for 10 h were cooled in ice and stored for 14 ± 4 days at 4 °C, then frozen at −20 °C until biochemical analyses. We kept FSJs 14 days at 4 °C before frozen to represent in the closest way the FSJs when they are consumed, i.e. 14 days after production. Two control samples were prepared with non-inoculated SJ incubated for 10 h at 30 and 43 °C.

2.5. Sensory evaluation of FSJs by "sniffing"

FSJs stored for 14 ± 4 days at 4 °C were smelled by a panel composed of 10–14 untrained judges. The FSJs were prepared and evaluated in biological duplicates. FSJs were stored in 40 mL straight containers with screw cap (Corning® Gosselin™). Sensory session took place at ambient temperature and products were at 8 ± 4 °C. 8 to 12 FSJs were evaluated by judges by session. Caps were removed at the beginning of the sessions.

Judges described FSJ odors using a free vocabulary. In addition, judges deemed whether the FSJ odors were acceptable or not for a yogurt-type soy product. The criteria of "acceptability" was subjective and evaluated through the answer (Yes/No) to the question "Do you judge the odor unacceptable for eating the fermented soy juice as a "yogurt-like" product?". The FSJs that had odors deemed to be unacceptable by more than two out of ten judges were not further characterized.

2.6. Organic acid quantification in FSJs

Lactic, acetic, citric, propionic, butyric, succinic and pyruvic acids were analyzed by High Performance Liquid Chromatography (HPLC, Dionex, P680, Sunnyvale California). Trials were prepared by 2-fold dilution of FSJs in H_2SO_4 0.01 M, freezing at −20 °C, centrifugation at 7500 × *g* for 15 min at 4 °C, and filtration (chromafil Xtra PVDF 45/13, Macherey Nagel) and were kept frozen at −20 °C until analysis. Analysis was run using a Rezek ROA organic acid H + column (300*7.8 mm, Phenomenex, California), with H_2SO_4 0.005 M as the mobile phase at a flow rate of 0.4 mL/min at 60 °C. Two detectors were used: UV detector (DIONEX-UVD 1704) operated at 210 nm and refractometer (RI 2103 Plus Jasco). Quantification was performed with an external calibration. Standards of lactic, citric, propionic, butyric, succinic, and pyruvic acids were from Merck, St. Quentin Fallavier, France, and acetic acid from PanReac, Lyon, France.

2.7. Oligosaccharide quantification in FSJs

Oligosaccharides were quantified in FSJs by High-Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC, Spectra System, Thermo Scientific – Antex Leyde, Decade II). Aliquots (1 g) were diluted in 100 mL of Carrez solution ($C_6FeK_4N_6$ 0.5 mM, $ZnSO_4$ 2.0 mM, NaOH 0.01 M), sonicated and filtered (UPTIDISC RC 25 mm, 0.20 µm, T38111 Interchim). The column used was a CarboPac

PA1, 250 mm × 4 mm i. d., particles 10 μm, 035391, Thermo Scientific, volume injected 20 μl. The eluents used were ultrapure water (A) from PURELAB Option Q. of ELGA (High Wycombe, United Kingdom) and NaOH 0.2 M (B). Both eluents were degassed with helium. HPLC-EC was run at 20 °C with a flow rate of 1 mL/min and the gradient was as follows: initial conditions 8% B, maintained for 31 min, then a linear rise to 65 min up to 100% B, maintained from 65 min to 80 min, followed by reversion to the initial conditions with a linear decrease from 80 min to 95 min down to 8% B, which was held for 15 min. The total run time was 110 min. Quantification was performed with an external calibration using lactose, raffinose, melibiose, stachyose, galactose, glucose, sucrose, maltose and fructose (Merck, St. Quentin Fallavier, France).

2.8. Yield of conversion calculation

The yield of conversion of oligosaccharides into acids was calculated from the sum of concentrations of every oligosaccharide consumed (sucrose, raffinose and stachyose) minus the concentrations of every oligosaccharide released (glucose, fructose, melibiose and galactose), expressed in molar equivalent carbon, divided by the sum of concentrations of lactic and acetic acid produced, expressed in molar equivalent carbon.

2.9. Isoflavone quantification

Aliquots of 1 g of sample were mixed in 100 mL of methanol 80%, sonicated and filtered (UPTIDISC RC 25 mm, 0.45 μm, T38101 Interchim). Isoflavones were separated by HPLC Finnigan Surveyor, Thermo Scientific. Analysis was run on a reverse phase C18 column (ODS-AM, 250 mm × 4.6 mm i. d., particles 5 μm, pores 120 Å, YMC-Pack ODS AM12S05-2546WT) injection of 12.5 μL and detection using an UV detector at 260 nm (PDA Plus Detector). Elution rated at 1 mL/min, at 20 °C with a gradient of acetic acid (A) and acetonitrile (B) as follows: initial conditions 15% B, maintained for 5.5 min, then a linear rise to 40 min up to 40% B then a decrease from 40 min to 50 min down to 15% B, held for 10 min. The total run time was 60 min. Quantification was performed for malonyl, acetyl, glucoside and aglycone forms of glycitin, genistin and daidzin using external calibrations with daidzin, acetyldaidzein, genistin, glycitin, glycitein (LC LAB, Woburn, USA), daidzein, and genistein (Merck, St. Quentin Fallavier, France). Results were expressed in equivalent aglycones with corresponding molar conversion factors (Peñalvo et al., 2004; Song et al., 1998).

2.10. Volatile compound profile of FSJs

Volatile compounds were analyzed by headspace (HS) gas chromatography-mass spectrometry (GC-MS) using Turbomatrix HS-40 trap, Clarus 680 gas chromatograph, and Clarus 600 T quadrupole mass spectrometer (PerkinElmer, Courtaboeuf). The principle was previously described in detail (Pogačić et al., 2015) Samples of 2.5 ± 0.1 g were placed in 20 mL PerkinElmer vial) and stored at −20 °C until analysis. Compounds were eluted on an Elite WAX ETR column (30 m × 0.25 mm x 0.25 μm; PerkinElmer, Waltham, MA, USA), with helium as the mobile phase, in the following conditions: initial temperature 35 °C maintained for 10 min, then increase at 5 °C/min up to 230 °C. MS was operated within a mass range of m/z 29–206 and detection by ionisation impact at 70 eV. Volatile compounds were identified by comparison to retention indexes, mass spectral data of standards and from the NIST 2008 Mass Spectral Library data (Scientific Instrument Services, Ringoes, NJ, USA). GC-MS data were processed as described by Pogačić et al. (2015). Volatile compounds were quantified using the abundance of one selected mass fragment (m/z), in arbitrary units.

2.11. Statistical analysis

Concentrations of compounds and pH values were analyzed as repeated measures with the function *aov* and compared using the Tukey *HSD. test* from the package *agricolae* on R studio (Version 1.0.153 – © 2009–2017 RStudio, Inc.). These analyses were used for each compound to determine if the means of concentrations of distinct FSJs or groups of FSJs significantly differ (p-value < 0.1). Multiple Factorial analysis (MFA) from the FactoMineR package (Lê et al., 2008) was used to present the main differences between FSJs according to their pH value and their profile in oligosaccharides and organic acids. Data from GC-MS were centered and scaled by compound and hierarchically clustered by Ward's minimum variance method and Euclidean distance metric with the *hclust* function before being plotted by the function *pheatmap*. Data collected from sensory evaluations were analyzed with the functions *textual* and *descfreq* of the package SensoMineR (Lê and Husson, 2008) with a p-value < 0.1 to determine FSJ characteristic odors. Some of the odor descriptors were merged according to the list given in Supplementary Table 2.

3. Results

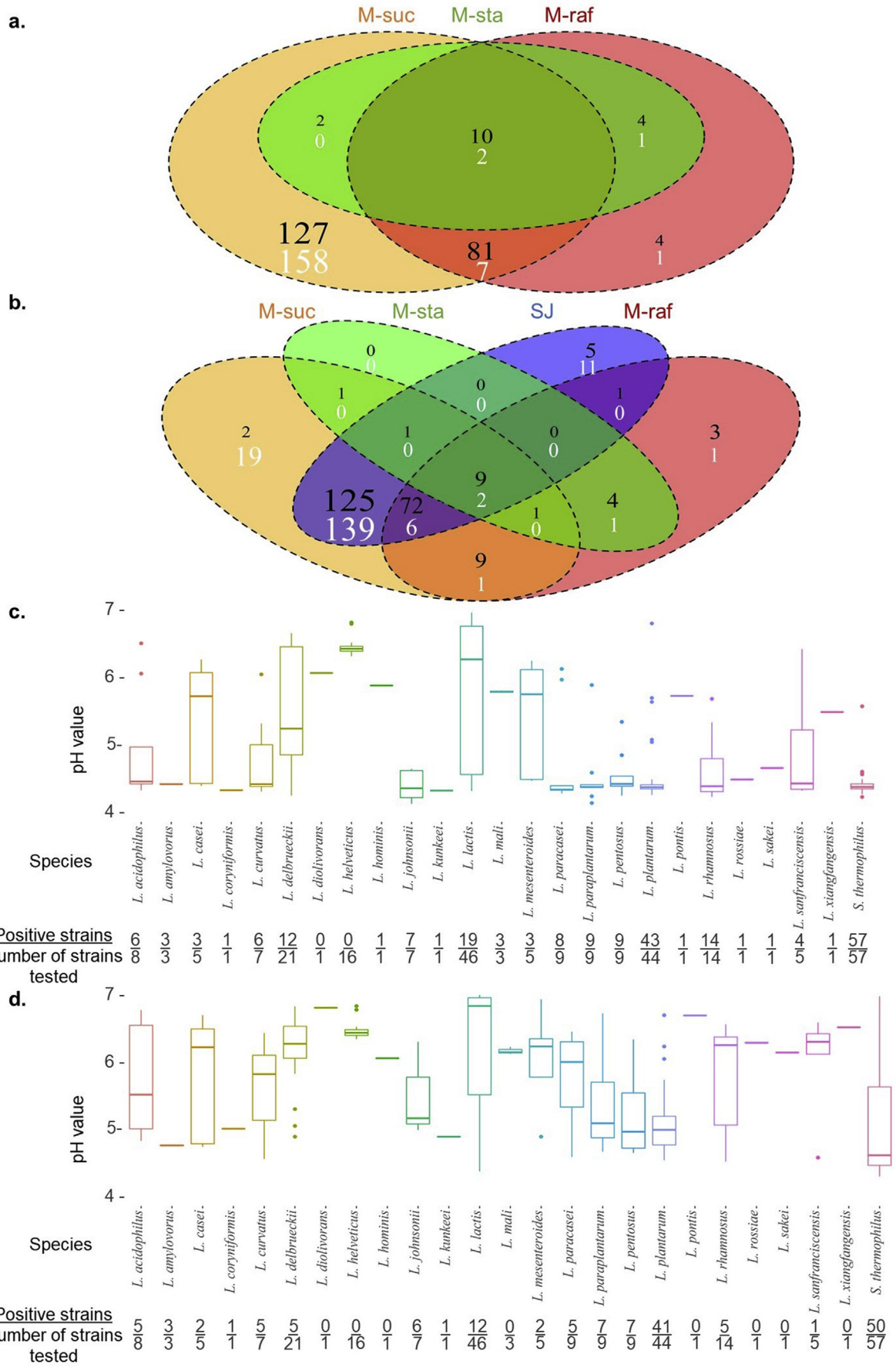
3.1. Ability of 276 LAB strains to acidify synthetic media containing different oligosaccharides in 48 and 10 h

Among the 276 LAB strains tested, 83% (228/276) were sucrose-, raffinose- or stachyose-positive within 48 h of fermentation (difference superior to pH 0.3 between M-ns and M-suc, M-raf or M-sta) (Fig. 2a). Two hundred and twenty strains (96% of acidifying strains within 48 h) were sucrose-positive, including all *Streptococcus,* 4/5 *Leuconostoc*, 19/46 *Lactococcus* (41%) and 140/168 *Lactobacillus* strains tested (83%) (Supplementary Table 1). Ninety-nine strains were raffinose-positive (Fig. 2a): 8/57 *Streptococcus,* 2/5 *Leuconostoc* and 89/168 *Lactobacillus* strains. None of the *Lactococcus* strains was raffinose-positive (Supplementary Table 1). Sixteen strains were stachyose-positive (Fig. 2a): 4/57 *Streptococcus*, 12/168 *Lactobacillus* strains but no *Lactococcus* nor *Leuconostoc* strains (Supplementary Table 1). Ten of the 276 strains tested were simultaneously sucrose-, raffinose- and stachyose-positive (Fig. 2a).

The number of strains capable of acidifying synthetic media containing oligosaccharides within 10 h of fermentation fell to 169 (61% of the 276 strains tested; 74% of the 228 acidifying strains within 48 h of fermentation). Among these strains, 167 were sucrose-positive (51/57 *Streptococcus,* 2/5 *Leuconostoc*, 13/46 *Lactococcus* and 101/168 *Lactobacillus*), Eleven were raffinose-positive (1/57 *Streptococcus*, 10/168 *Lactobacillus* and no *Leuconostoc* or *Lactococcus* strains) and three belonging to the *Lactobacillus* genus were stachyose-positive (Fig. 2a and Supplementary Table 1).

3.2. Ability of 276 LAB strains to acidify soy juice in 48 and 10 h

Among the 276 strains tested, 77% (213/276) representing 23/25 species were positive in terms of acidifying SJ within 48 h (pH below 6) (Figs. 2b and 1d and Supplementary Table 1). This ability appeared to be species-dependent. Indeed, all strains belonging to the *L. pentosus*, *L. plantarum*, *S. thermophilus*, *L. rhamnosus*, *L. amylovorus*, *L. coryniformis, L. kunkeei* and *L. curvatus* species acidified SJ (Fig. 2c and Supplementary Table 1). Further, *L. helveticus* and *L. diolivorans* strains tested managed to acidify SJ. Moreover, strains of *S. thermophilus*, *L. amylovorus, L. coryniformis, L. kunkeei, L. pentosus,* and *L. plantarum* only acidified to average pH values of 4.4 ± 0.1, which was significantly (p < 0.1) lower than *L. lactis,* and *L. mali* which acidified to average pH values of 5.8 ± 0.1 (Fig. 2c and Supplementary Table 1). The number of strains able to acidify soy juice within 10 h of fermentation fell to 158 (57% of the 276 strains tested; i.e. 74% of the strains acidifying SJ within 48 h), representing 17 species (Figs. 2b and 1c and



(caption on next page)

Fig. 2. Acidification capacities of 276 strains of lactic acid bacteria, from 25 different species in **(a.)** a synthetic medium containing sucrose (M-suc), raffinose (M-raf) or stachyose (M-sta) as the carbon source, **(b.)** a synthetic medium containing sucrose (M-suc), raffinose (M-raf), stachyose (M-sta) as the carbon source or in soy juice (SJ). Venn diagram showing positive acidifying strains in M-suc (orange), M-raf (red), M-sta (green) or SJ (blue) during 48 h of fermentation (numbers in black) or 10 h of fermentation (numbers in white). **c.** pH values and proportions of positive acidifying strains in SJ (n = 213), i.e. able to acidify SJ to a pH < 6 within 48 h of fermentation. **d.** pH values and proportions of strains (n = 158) that could acidify SJ within 10 h, i.e. able to acidify SJ to a pH < 6 within 10 h of fermentation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Supplementary Table 1). In this case, the ability to acidify SJ was strain-dependent. Indeed, 89% of *L. pentosus, L. plantarum* and *S. thermophilus* strains and 27% of *L. rhamnosus*, *L. delbrueckii* and *L. lactis* strains acidified SJ within 10 h of fermentation (Fig. 2d and Supplementary Table 1). The acidification rates were also strain-dependent. For example, within 10 h, the first quartile of *S. thermophilus* strains acidified SJ to below pH 4.5 and the fourth quartile acidified it to higher than pH 5.6 (Fig. 2d).

3.3. Odor profiles of FSJs

One hundred and twelve strains were selected from the 158 identified as being able to acidify SJ to a pH lower than 6 in 10 h. The FSJs were prepared using these 112 strains. About 4000 words were cited, leading to 244 descriptors, which were further merged in 164 descriptors by grouping those which were similar (for details, see Supplementary Table 2).

The control was characterized by the descriptors “green” and “hay”, which were used significantly more frequently when compared to comments on the FSJs ($p < 0.05$, Supplementary Table 2).

Some species-specific odors were produced by the FSJs during fermentation. The odor of *S. thermophilus* FSJs was more frequently associated with “nuts”, “soy”, “fresh”, “caramel” and “hay” descriptors ($p < 0.001$). These FSJs were also more appreciated, with a higher frequency of “good” descriptors ($p < 0.001$). The odor of *L. plantarum* FSJs was significantly more frequently described as “acid”, “sour”, “floral”, “pineapple”, “spicy”, “cheesy”, “kefir” and “sorrel” ($p < 0.001$), while that of *L. pentosus* FSJs was described as “plastic” ($p < 0.001$), and *L. lactis* FSJ odors were associated with “soy sauce”, “black bread”, “cabbage”, “salty” and “broth” descriptors ($p < 0.001$).

A few FSJs also displayed some peculiar, strain-specific odors; for example, FSJs fermented by *L. acidophilus* CIRM-BIA2087 had a “goat” odor ($p < 0.001$), and seven FSJs fermented by *L. lactis* had a “cabbage” and/or a “broth” odor (Supplementary Table 2; $p < 0.001$). Four other strains belonging to diverse species: *L. lactis* CIRM-BIA1551, *L. plantarum* CIRM-BIA1522, CIRM-BIA2180 and CIRM-BIA2115, produced “floral” odors ($p < 0.001$).

Forty-seven out of 112 FSJs had an odor deemed to be acceptable. These 46 FSJs, fermented by 27 *S. thermophilus* strains, 14 *L. plantarum* strains, 2 *L. pentosus* strains, one *L. lactis* strain, one *L. coryniformis* strain and one *L. delbrueckii* strain were then further characterized.

3.4. Carbohydrates consumption and organic acids production in FSJs

Whichever strain was used to produce FSJs, sucrose was the predominant carbohydrate consumed. Sucrose consumption was accompanied by the production of lactic acid (Table 1). The pH of the 46 FSJs reached values ranging from 4.5 to 5.7. Most strains (35/46) produced significant concentrations of pyruvic acid when compared to the control SJs ($p < 0.1$; Table 1). Most strains (43/46) significantly lower the concentrations of succinic acid in FSJs ($p < 0.1$; Table 1).

The multifactorial analysis (MFA) performed using these results produced three groups of FSJs (Fig. 3). The first and second dimensions accounted for 31% and 19% of total variance, respectively. Dim 1 separated FSJs on the basis of acidification intensity, and the lactic acid content. Dim 2 was positively associated with total sugars, fructose, sucrose, citric acid and succinic acid concentrations and negatively associated with those of acetic acid. Dim 1 clearly separated the controls (unfermented SJs) from all FSJs. The three groups of FSJs, separated on Dim 1 and Dim 2, were i) a *S. thermophilus*group, composed of all 27 *S. thermophilus* FSJs, ii) a group containing the only FSJ fermented by a *L. delbrueckii* strain, and iii) an *Lb/Lc* group containing the 18 other FSJs (fermented by 14 *L. plantarum,* two *L. pentosus,* one *L. coryniformis* and one *L. lactis*) (Fig. 3). The 27 *S. thermophilus* FSJs were characterized by the presence of residual sucrose at 1.1 ± 0.1 g/L, 3.0 ± 0.1 g/L lactic acid, 0.03 ± 0.01 g/L acetic acid, 1.3 ± 0.1 g/L citric acid and an average pH of 4.9 ± 0.1 (Table 1). *L. delbrueckii* CIRM-BIA313, which completely hydrolyzed sucrose, released significant concentrations of glucose and fructose and was the only melibiose-releasing strain ($p < 0.1$ Table 1). The *L. delbrueckii* FSJ was also characterized by a pH of 5.5, 1.6 g/L lactic acid and 0.1 g/L acetic acid. The 18 FSJs in the *Lb/Lc* group contained 0.3 ± 0.1 g/L sucrose, 2.2 ± 0.1 g/L lactic acid and 0.11 ± 0.01 g/L acetic acid; they did not modify the citric acid content in SJ (1.1 ± 0.1 g/L) and had a pH of 5.5 ± 0.1. In the *Lb/Lc* group, one *L. coryniformis*, two *L. pentosus* and three *L. plantarum* strains consumed the totality of sucrose available within 10 h of fermentation. In the present work, no peculiar strain was able to use stachyose in 10 h of soy juice fermentation.

3.5. Isoflavones in FSJs

The control SJ contained 127 mg/L glycosylated isoflavones, 11 mg/L aglycone isoflavones, 3 mg/L acetylated isoflavones and 8 mg/L malonylated isoflavones, *i.e.* a total of 149 mg/L of equivalent aglycones (Table 1). The 46 FSJs were analyzed for their isoflavone contents and whatever the strain implemented to produce the FSJ, no significant modifications were found to the concentrations of acetylated, malonylated or total isoflavones. The ratio between glycosylated and aglycone isoflavones was however modified in 16 FSJs (*i.e.* 14/14 *L. plantarum*, 2/2 *L. pentosus*, 1/1 *L. coryniformis*, 0/27 *S. thermophilus*, 0/1 *L. lactis* and 0/1 *L. delbrueckii* strains), which indicates that these strains displayed isoflavone deglycosylation activity ($p < 0.1$; Table 1). Among these, 12 strains deglycosylated more than 80%, three strains deglycosylated about 60% and one strain about 30% of SJ glycolysated isoflavones (Table 1).

3.6. Volatile compounds in FSJs

Thirty-five volatile compounds were identified in FSJs (Table 2). Four groups of strains were distinguished by hierarchical clustering as a function of their volatile compound profiles (Fig. 4), referred to as A, B, C and D. Group A only contained the controls (unfermented SJs) and was characterized by significantly more 2-pentylfuran than the other groups ($p < 0.1$). Most other volatile compounds (32/35) were present at significantly lower concentrations ($p < 0.1$) in group A than in at least one other FSJ. Group B contained four *S. thermophilus* FSJs and *L. delbrueckii* CIRM-BIA313 and Group C contained the other 23 *S. thermophilus* FSJs. Group D corresponded to the *Lb/Lc* group identified by MFA analysis (section 3.4) and was made up of the 18 FSJs fermented by fourteen *L. plantarum,* two *L. pentosus,* one *L. coryniformis* and one *L. lactis* strains. Groups B and C were distinguished from groups A and D by significantly higher quantities ($p < 0.1$) of a range of aldehydes (benzaldehyde and butanal), carbonyl compounds (2-hydroxypentan-3-one, 2,4-dimethylpentan-3-one, butane-2,3-dione) and alcohols (propan-1-ol, furan-2-ylmethanol). Groups B and C differed from each other by significantly higher concentrations of pentane-2,3-dione,

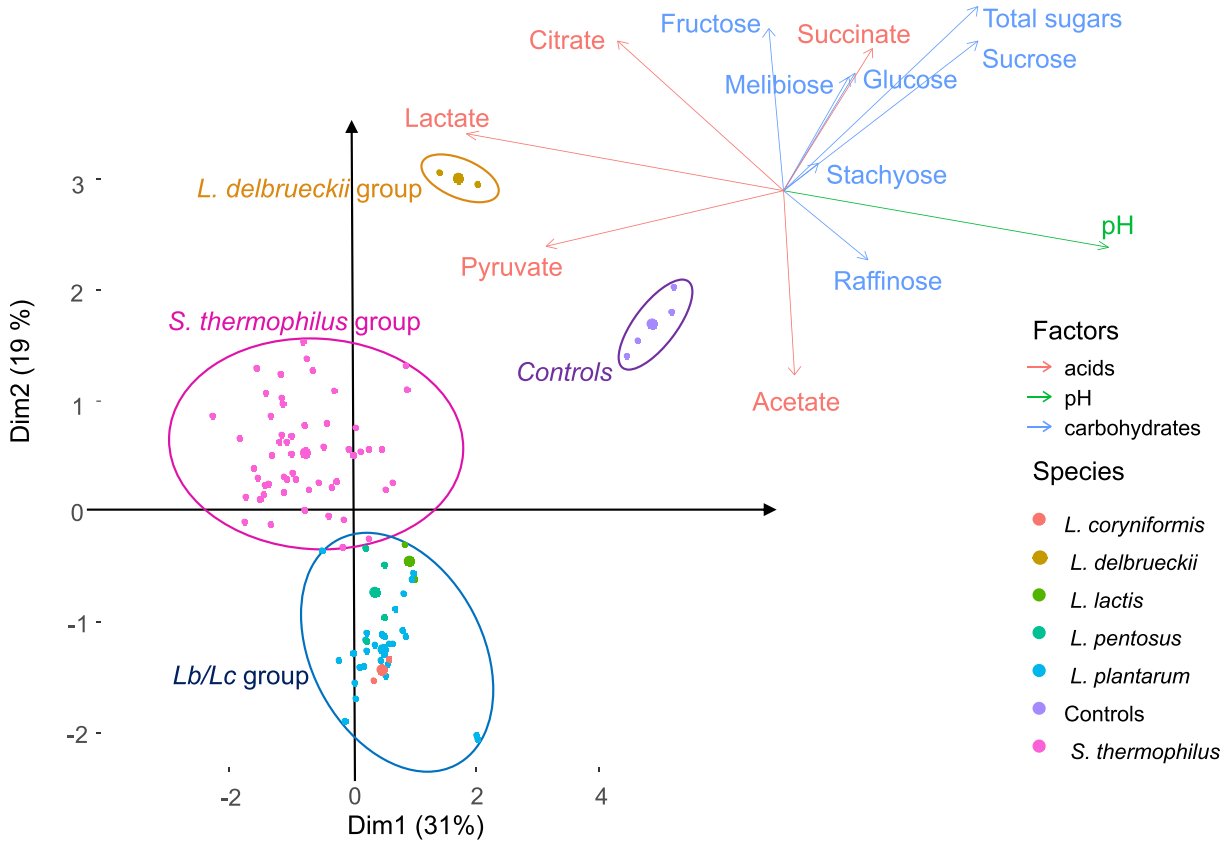


Fig. 3. Multiple Factor Analysis (MFA) of oligosaccharides, acids concentrations and pH values in soy juices fermented for 10 h by 46 strains of lactic acid bacteria. Results of replicate experiments by strain are represented with dots, and the barycenter of results by species shown using larger dots.

heptane-2,3-dione, methyl acetate and ethyl acetate ($p < 0.1$) in group C, and significantly higher concentrations of 2,4-dimethylbenzaldehyde ($p < 0.1$) in group B. Group D was differentiated from the other groups by its significantly higher concentrations of four acids (acetic, butanoic, pentanoic and hexanoic acids), two carbonyl compounds (1-hydroxypropan-2-one and 3-hydroxybutan-2-one) and two alcohols (2-methylpropan-1-ol and ethanol) ($p < 0.1$).

In addition, few compounds were only produced by specific strains. For example, two compounds: 1-phenylethan-1-ol, 3-methylbutanoic acid were each present at significantly higher concentrations in only one FSJ. Similarly, 1-pentanol was present at significant higher concentrations in two FSJs, heptane-2-one in three FSJs, butanoic-acid, butanal and hexanal in four FSJs, and ethanol, 4-methylpentan-1-ol, 3methylbutanoic acid and 1-hydroxypropane-2-one in five FSJs (Table 2 and Fig. 4).

4. Discussion

The fermentation of soy juice depends on the composition of the juice, the LAB strains used as a starter and the fermentation parameters applied. Our study was the first to have compared the ability of 276 LAB strains to ferment soy carbohydrates, *i.e.* sucrose, raffinose and stachyose, in both a synthetic medium and a commercial soy juice (SJ), during two fermentation periods. These strains were relatively representative of LAB diversity as they included strains isolated over a long period (1950–2016) from a variety of products (including different dairy, meat and plant-based products) and different geographic locations (23 countries), and they belonged to 25 LAB species.

4.1. The ability of LAB to ferment soy juice is related to their use of sucrose but not of raffinose and stachyose

The utilization of soy carbohydrates by LAB is a prerequisite to the acidification of soy juice. In synthetic media, the catabolism of sucrose, raffinose and stachyose was shown to be both species- and strain-specific. Concerning sucrose, *S. thermophilus*, *L. plantarum*, *L. pentosus*, *L. johnsonii*, *L. rhamnosus*, and *L. acidophilus* appeared to be the species most capable of degrading sucrose, with 100% of sucrose-positive strains within 48 h of fermentation ($n \geq 8$). This result was in line with the data in Bergey's Manual (Vos et al., 2009; Gänzle and Follador, 2012), who reported that most *S. thermophilus*, *L. plantarum*, *L. pentosus*, *L. johnsonii*, *L. rhamnosus* and *L. acidophilus* strains are sucrose-positive. Concerning raffinose, *L. plantarum*, *L. johnsonii*, *L. acidophilus* and *L. helveticus* appeared to be appropriate species to degrade it (50%–75% of raffinose-positive strains, $n \geq 6$). Some results agreed with the data in Bergey's Manual data (Vos et al., 2009), which reported that 11%–89% of *S. thermophilus*, *L. johnsonii*, *L. acidophilus* strains can use raffinose, but others did not agree with these data (Vos et al., 2009) which reported that more than 90% of *L. plantarum* and *L. pentosus* strains and fewer than 10% of *L. helveticus* strains can use raffinose. *L. acidophilus* also appeared to be efficient in using stachyose (50% of stachyose-positive strains, $n \geq 6$), in accordance with the findings of Wang et al., (2003), who reported stachyose consumption in SJ by another *L. acidophilus* strain. As for the results obtained during 10 h of fermentation, few scientific publications have reported LAB acidification rates, so our study is the first to have determined a broad range of LAB rates regarding the use of sucrose, raffinose and stachyose and the fermentation of soy juice.

Most of the strains identified as sucrose-positive in a synthetic medium were also able to ferment SJ (Fig. 2b). The ability to use sucrose could therefore be a good criterion for the selection of strains for

Table 2
Volatile compounds identified in soy juices after 10 h of fermentation with 46 lactic acid bacteria strains. ("FooDB,"; "The Good Scents Company" 2019).

CAS Number	Volatile compounds (IUPAC Name)	Odor descriptor[a]	m/z	Identification[b]	Linear Retention Index, calculated	P value[c]
123-72-8	butanal	apple; brady; chocolate; earthy; fatty	72	DB,LRI,S	802	1.2E-19
79-20-9	methyl acetate	bitter; ether; fruity; sweet	43	DB,LRI	814	1.5E-07
141-78-6	ethyl acetate	anise; balsam; ethereal; fruity; green	43	DB,LRI,S	866	1.2E-05
590-86-3	3-methylbutanal	chocolate; ethereal; malt; fatty; peach;	58	DB,LRI,S	896	1.5E-09
431-03-8	butane-2,3-dione	butter; vinegar; coffee; caramel; creamy	86	DB,LRI,S	969	1.4E-21
71-23-8	propan-1-ol	alcohol; fermented; fusel; musty; peanut	31	DB,LRI	1041	7.2E-20
600-14-6	pentane-2,3-dione	butter; caramel; cheese; cream; nutty	100	DB,LRI	1058	3.3E-07
64-17-5	ethanol	alcoholic; ethereal	31	DB,LRI	1058	3.6E-10
66-25-1	hexanal	fatty; fruity; green; fresh; sweaty	44	DB,LRI	1075	1.1E-14
78-83-1	2-methylpropan-1-ol	bitter; ether; solvent; wine	33	DB,LRI	1119	3.1E-11
565-80-0	2,4-dimethylpentan-3-one	acetone	71	DB,LRI	1140	7.7E-18
96-04-08	heptane-2,3-dione	butter; cheese; oily	85	DB,LRI	1151	1.8E-04
71-36-3	1-butanol	balsam; fruit; oil; vanilla; fusel	56	DB,LRI	1161	5.1E-10
110-43-0	heptan-2-one	cinnamon; woody; coconut; fruity; herbal	71	DB,LRI,S	1177	5.8E-04
3777-69-3	2-pentylfuran	beany; butter; earthy; fruity; green	81	DB,LRI	1220	1.5E-01
71-41-0	1-pentanol	balsam; fusel; oil; sweet; vanilla	70	DB,LRI	1264	1.7E-01
513-86-0 ou 53584-56-8 (stereoisomère)	3-hydroxybutan-2-one	butter; cream; fatty; dairy; sweet	43	DB,LRI	1276	7.2E-11
116-09-6	1-hydroxypropan-2-one	caramelic; ethereal; pungent; sweet	43	DB,LRI	1292	2.9E-08
18829-55-5	(2E)-hept-2-enal	fatty; green	41	DB,LRI	1332	5.2E-08
626-89-1	4-methylpentan-1-ol	nutty	56	DB,LRI	1332	1.1E-08
5704-20-1	2-hydroxypentan-3-one	truffle, nutty,earthy	57	DB,LRI	1351	2.3E-11
64-19-7	acetic acid	sour; acetic; vinegar	60	DB,LRI,S	1447	1.1E-08
13679-85-1	2-methylthiolan-3-one	sulfur; fruit; berry	60	DB,LRI	1494	7.0E-23
100-52-7	benzaldehyde	almond; walnut; cinnamon, bitter; cherry	106	DB,LRI,S	1520	1.5E-10
15764-16-6	2,4-dimethylbenzaldehyde	almond; cherry; naphtyl; spice; vanilla	134	DB	1801	5.2E-10
107-92-6	butanoic acid	acetic; butter; cheese; fruit; rancid	60	DB,LRI,S	1625	1.5E-05
503-74-2	3-methylbutanoic acid	fusel; fruit; ether; ferment; creme	60	DB,LRI,S	1666	8.3E-01
98-00-0	furan-2-ylmethanol	alcoholic; bitter; bread; burnt; caramel	53	DB,LRI	1677	2.8E-04
109-52-4	pentanoic acid	acidic; putrid; rancid; sweat; animal	60	DB,LRI,S	1734	3.4E-01
142-62-1	hexanoic acid	cheese; fatty; sour; sweat	60	DB,LRI,S	1839	5.5E-01
111-14-8	heptanoic acid	cheese; rancid; sour; sweat	60	DB,LRI,S	1938	9.7E-01
118-71-8	3-hydroxy-2-methyl-4H-pyran-4-one	baked; bread; candy; caramel; cotton	126	DB,LRI	1946	9.8E-01
60-12-8	1-phenylethan-1-ol	hyacinth; gardenia; fresh; sweet	122	DB,LRI	1952	1.3E-27
124-07-2	octanoic acid	cheese; fatty; rancid; sweat; vegetable	60	DB,LRI,S	2034	1.0E+00
112-05-0	nonanoic acid	cheese; dairy; fat; green; dirty	60	DB,LRI	2124	1.0E+00

[a] Odor descripted from thegoodscentscompany.com and foodb. ca (2019).
[b] Identification based on: LRI, linear retention index and DB, mass spectral data Library NIST 2008 et S standards.
[c] p-value associated with ANOVA between the different products concentrations by volatile compounds.

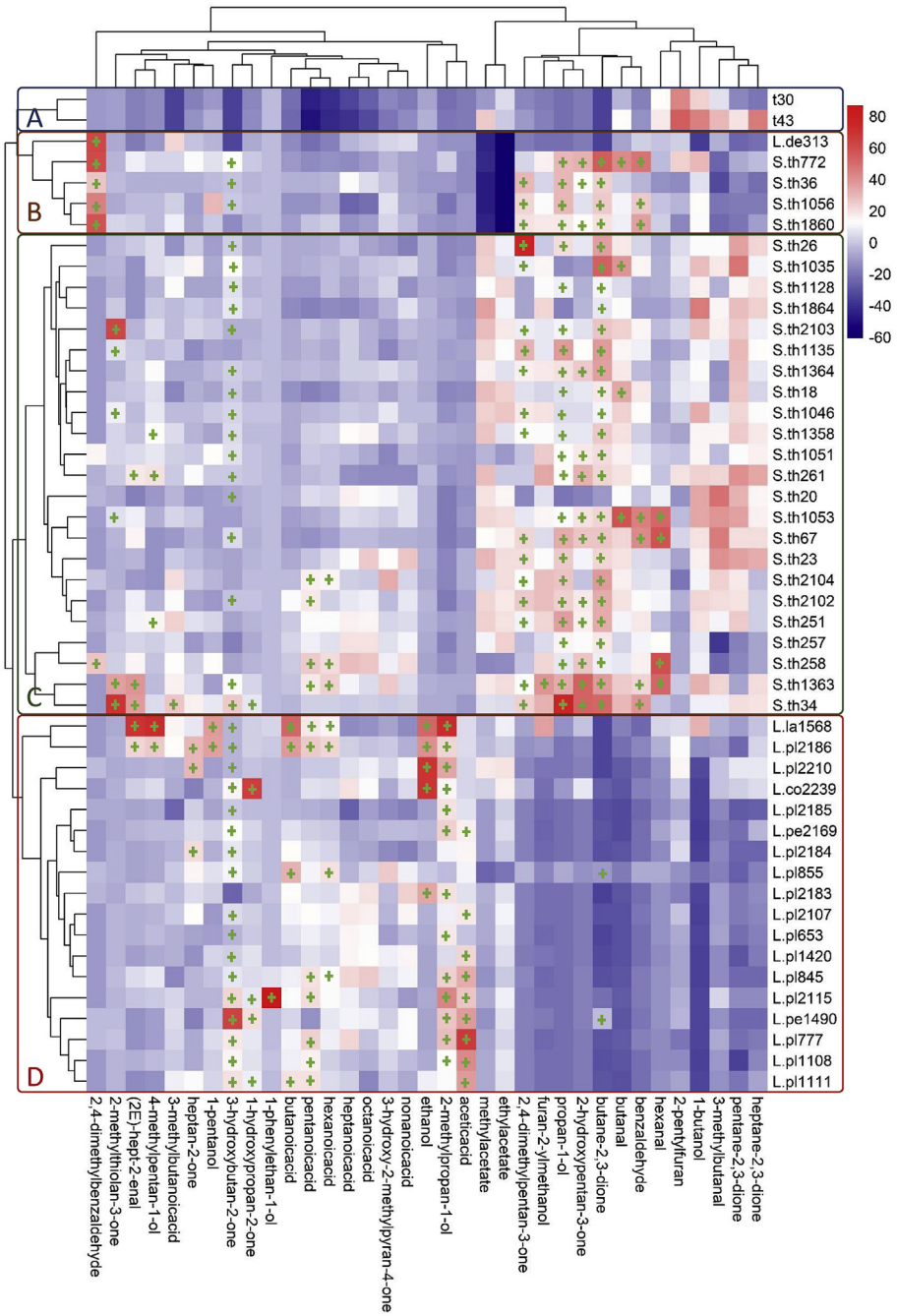


Fig. 4. Hierarchical clustering with a heatmap representation based on Ward's minimum variance method and a Euclidean distance metric of the relative concentrations of volatile compounds from 46 fermented soy juices obtained with strains of lactic acid bacteria, clustered in four groups, A, B, C and D. Green crosses notify a significantly higher concentration of volatile compounds in fermented soy juice ($p < 0.1$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

soy juice fermentation. Sucrose-positive strains mainly belong to ten species achieving fermentation within 48 h (Fig. 2c) and to *S. thermophilus, L. plantarum* and *L. pentosus* species with fermentation within 10 h (Fig. 2d). Only one strain out of the strains identified as sucrose-negative and raffinose and/or stachyose-positive was able to ferment SJ in 48 h. And for the first time, three species (*L. pontis*, *L. kunkeei* and *L. xiangfangensis*) were identified as being able to ferment SJ.

One drawback affecting the consumption of soy-based food is the presence of non-digestible oligosaccharides such as raffinose and stachyose, as highlighted by Guillon and Champ (2002). *L. delbrueckii* CIRM-BIA313 displayed a tendency towards raffinose hydrolysis in FSJs. *L. delbrueckii* CIRM-BIA313 released glucose, fructose and melibiose in FSJs ($p < 0.1$; Table 1), suggesting that it produces levansucrase. This strain could therefore be used to alleviate the gastrointestinal discomfort related to the presence of raffinose in fermented soy juice products. According to Baú et al. (2015); Yoon and Hwang (2008), who studied kefir cultures containing yeasts and LAB, one *L. mesenteroides* strain and one *L. curvatus* strain were able to reduce concentrations of raffinose and stachyose by up to 30% during 12 h of soy juice fermentation. In our study, it would have been interesting to know if a longer fermentation time could have enabled a more significant reduction of SJ raffinose and stachyose contents. Nevertheless, our results confirmed that the ability to use raffinose and stachyose by LAB in soy juice fermentation is strain-dependent.

4.2. Primary metabolites in FSJs

Two groups of strains could be distinguished from the MFA performed on LAB primary metabolites in FSJs (Fig. 3). The *S. thermophilus* group converted a mean 4.4 ± 0.1 g/L content in sucrose into 3.0 ± 0.1 g/L lactic acid, 0.03 ± 0.01 g/L acetic acid and 0.2 ± 0.1 g/L citric acid to reach an average pH of 4.9 ± 0.1 (Table 1). Surprisingly, group D of the FSJs fermented by 18 LAB strains (14 *L. plantarum*, two *L. pentosus*, one *L. lactis*, one *L. coryniformis*) converted more sucrose (5.2 ± 0.1 g/L) into less lactic acid (2.2 ± 0.1 g/L), 0.11 ± 0.01 g/L acetic acid and no citric acid to reach a higher pH of 5.5 ± 0.1 than *S. thermophilus* FSJs. We characterized this discrepancy by calculating their apparent conversion yields of carbohydrates into acids. The apparent yields of the *S. thermophilus* group and *Lb/Lc* group were 0.7 and 0.4 mol C/mol C, respectively. Our findings therefore agreed with those of Champagne et al., (2009), who reported that under similar conditions, a *S. thermophilus* strain and *L. helveticus* strain displayed similar apparent molar yields of conversion of 0.7 and 0.5 mol C/mol C, respectively. These results can be explained by the fact that group D strains displayed a heterolactic metabolism to ferment the SJ. Heterolactic fermentation is also known to produce CO_2 and ethanol (Holzapfel and Wood, 2014). CO_2 and ethanol were not quantified during our study, although ethanol was detected in some *Lb/Lc* group FSJs (Fig. 4). Heterolactic fermentation can be expected to occur with *Lactobacillus* strains when sucrose is limited, as suggested by Gänzle (2015), and in agreement with our results (Table 1). However, heterolactic fermentation is not expected with a *Lactococcus* strain (Table 1). By contrast, *S. thermophilus* strains mainly use homolactic metabolism, as shown by Hols et al., (2005) in synthetic media. In our study, *S. thermophilus* did not however display a strictly homolactic metabolism to ferment SJ because citric and/or acetic acid concentrations rose in *S. thermophilus* FSJs. Further, the succinic acid content fell in most FSJs, while to the best of our knowledge, the catabolism of succinic acid have been only observed during *togwa* (maize and malt) fermentation by a complex community containing yeasts and LAB (Mugula et al., 2003).

Isoflavones are soy constituents which might have effects on human health (Munro et al., 2003; Zaheer and Humayoun Akhtar, 2017). Soy contains mostly glycosylated isoflavones (90%) and aglycone isoflavones (10%). Both have the same effect on human health but aglycone isoflavones are more rapidly and better absorbed than their glycosylated forms (Kano et al., 2006). The results of the present study confirm that LAB can deglycosylate isoflavones, as had previously been reported for several *Streptococcus* and *Lactobacillus* strains (Champagne et al., 2010; Chien et al., 2006). Our results also indicate that isoflavone deglycosylation is prevalent in *Lactobacillus*, with 89% of the strains being able to deglycosylate SJ isoflavones while no *S. thermophilus* strain could deglycosylate isoflavones. To the best of our knowledge, no LAB strains have been shown to be able to decrease total isoflavone contents, as it has been described for *Bifidobacteria* (Champagne et al., 2010; Chien et al., 2006).

In brief, our findings show that LAB use a variety of metabolic pathways to ferment SJ. Among the FSJs that produced an odor deemed to be acceptable, *S. thermophilus* FSJs reached the lowest pH in 10 h (lower than 5.6).

4.3. Odor changes and volatile compounds produced during SJ fermentation

The "off-flavors" of soy limit the consumption of "yogurt-like" soy products and may be due to compounds such as hexanal and 2-pentylfuran (Kobayashi et al., 1995) which are known to have a "green" odor. As expected, our non-fermented soy juice contained 2-pentylfuran and hexanal, probably detected and described as "green" and "hay" by the judges ($p < 0.1$; Supplementary Table 2). Our results confirmed that fermentation can reduce the concentration of hexanal in a strain- and species-specific manner, as previously reported for diverse *Lactobacillus* and *Streptococcus* strains in SJ fermentation (Blagden and Gilliland, 2005). All but one strain from group D lowered levels of hexanal. Unexpectedly, only four of the *S. thermophilus* strains tested significantly reduced the hexanal content in SJ, and surprisingly, four S. *thermophilus* strains increased hexanal levels in SJ. This increase had never previously been reported. The odors of some *S. thermophilus* FSJs, characterized using "soy", "fresh" and "hay" descriptors may have been due to high hexanal contents. However, *S. thermophilus* FSJs were also characterized as having "nut", "caramel", "almond" and "yogurt" odors that must have been due to the aldehydes, alcohols and ketones, including 2,3-butanedione (diacetyl, butter related), present in its FSJs. Group D FSJs (10 *L. plantarum*, six *L. pentosus*, one *L. lactis* and one *L. coryniformis*) presented high levels of 3-hydroxybutan-2-one, with "buttery", "creamy" or "dairy" odors as in the *S. thermophilus* group. Group D FSJs could then present "yogurt-like" odors as in the *S. thermophilus* group and group D FSJs were more frequently described using a range of strong odors. The "sour", "acid", "cheese" and perhaps "sorrel" odors may have been due to the higher concentrations of acetic, butanoic and pentanoic acids in these FSJs, which would mask the "yogurt-like" odors. Sucrose limitation, which induces heterolactic fermentation in lactobacilli, may have been responsible for this marked acid production in Group D FSJs.

Each strain exhibited a specific profile of volatile compounds potentially associated with a peculiar "bouquet" of odors resulting from specific secondary metabolisms in the context of SJ fermentation. FSJ produced using *L. plantarum* CIRM-BIA2115 differed from all other FSJs because of its marked floral/hyacinth odor, which was probably related to the high 1-phenylethan-1-ol levels detected in its FSJ. 1-Phenylethan-1-ol had also been shown to be produced by another *L. plantarum* strain in a malt medium (Salmeron et al., 2009). The FSJ odors generated using *L. kunkeei* were associated with uncommon descriptors such as yeast, floral and honey ($p < 0.05$). Other strains in these species could therefore be tested for use in SJ fermentation in order to expand the range of flavors for yogurt-type fermented soy products. Strains that generated peculiar odors such as "cabbage" or "floral" could also be used to produce FSJ not used in yogurt-type products.

In brief, our results show that *S. thermophilus* strains globally generate a more "yogurt-like" odor in FSJ than *Lactobacillus* or *Lactococcus* strains. Even so, additional studies are required to evaluate acceptability according to the LAB metabolic profiles in soy juice fermentation.

5. Conclusion

In conclusion, our screening of a broad range of LAB constitutes an important step towards revealing species and strain specificities and selecting strains of interest to ferment soy juice. The focus on primary metabolites confirms that Streptococcus strains are the most effective in acidifying SJ. The screening of LAB strains will probably be of interest regarding the development of offbeat food products. Specific LAB could improve SJ fermentation rates and the health or organoleptic properties of FSJ. The use of strains able to utilize oligosaccharides with aromatic strains could be relevant to optimize plant-based product fermentation. We are currently investigating how pairs of LAB strains can cooperate thanks to their peculiar metabolic profiles to improve the plant-based products fermentation. This broad study confirms the primary metabolism of LAB used to ferment SJ and highlights the diversity of their secondary metabolism in the fermentation of SJ. Sucrose limitation needs to be investigated as it may induce heterolactic fermentation with *Lactobacillus* or *Lactococcus* strains and therefore be responsible for the production of strong odors. This work has revealed certain species- and strain-dependent characteristics and highlighted the diversity and richness of the metabolic profiles of LAB strains in soy juice fermentation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.103410.

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