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Propionibacteria in fermented vegetables

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Abstract — This paper presents the results of the application of propionibacteria (PAB) to the production of fermented vegetables: sauerkraut, red beet juice and vegetable salads. The addition of PAB to the lactic acid bacteria (LAB) used in the fermentation led to increases in folacin, vitamin B_{12}, propionic and acetic acid contents, inhibition of harmful and pathogenic microorganisms and the extension of the shelf-life of the products. Products made with PAB usually had better organoleptic characteristics than products made only with LAB. In long fermentations, such as for sauerkraut, substantial amounts of metabolites from PAB were produced during processing and storage. On the other hand, in short fermentations, such as for red beet juice, the concentration of metabolites in the final product depended predominantly on their concentration in the inoculum of PAB used. © Inra/Elsevier, Paris.

***Propionibacterium* / folacin / vitamin B_{12} / plant product quality / pathogen**

Résumé — Bactéries propioniques dans les produits végétaux fermentés. Cet article présente l'application de bactéries propioniques pour la production de choucroute, de jus de betteraves rouges et de salade végétale. L'addition de bactéries propioniques aux bactéries lactiques utilisées pour la fermentation ont mené à l'augmentation de folacine, de vitamine B_{12}, des acides propioniques et acétiques ainsi qu'à l'inhibition de microbes pathogènes et à la prolongation de la durée de vie des produits obtenus. Des produits fabriqués avec des bactéries propioniques avaient généralement de meilleures caractéristiques organoleptiques que des produits fabriqués uniquement avec des bactéries lactiques. La quantité de métabolites formés dépendaient du levain et de la composition du milieu, ainsi que des interactions entre les souches. Pour les fermentations longues comme la choucroute, des quantités de métabolites augmentaient considérablement pendant la fabrication et le stockage. D'autre part, pendant les fermentations courtes comme le jus de betteraves rouges et la salade végétale, la concentration de métabolites dans le produit fini dépend en grande partie de leur concentration dans l'inoculum de bactérie propionique utilisé. © Inra/Elsevier, Paris.

***Propionibacterium* / folacine / vitamine B_{12} / microbe pathogène / produit végétal**

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* Correspondence and reprints.

1. INTRODUCTION

For many years, propionibacteria (PAB) have been used in the food industry, particularly the dairy industry, because of their ability to produce propionic and acetic acid and CO_2, which is responsible for eye formation in Swiss-type cheese. Folacin and vitamin B_{12} are also produced by PAB. Metabolites of PAB propionates, are used in food, particularly bread, to inhibit the development of moulds.

The use of PAB in the production of fermented plant foods could result in several desirable properties including enhancement of their nutritional value through an increase in vitamin contents particularly folacin and vitamin B_{12}, inhibition of harmful and pathogenic microorganisms, extension of shelf-life and improvement of organoleptic characteristics. Propionic and acetic acids are powerful mould inhibitors and their presence in foods prevents or, at least, delays the growth of fungi which can cause development of unacceptable tastes and flavours in food and which can be a possible source of hazardous substances, e.g. aflatoxins.

The present investigation was aimed at the development of a new use of PAB in the manufacture of fermented vegetable products without artificial preservatives, with enhanced nutritional value, acceptable sensory characteristics and prolonged shelf-life.

2. MATERIALS AND METHODS

2.1. Organisms and media

All strains of PAB and lactic acid bacteria (LAB) used in this study were from the culture collection of the Institute of Food Biotechnology at Olsztyn University of Agriculture and Technology.

Stock cultures of PAB and LAB were grown at 30 °C in sodium lactate broth (NLB) and MRS broth respectively and maintained at 4 °C. Before use, PAB and LAB were transferred three times at daily intervals in enriched whey medium (EWM) and MRS broth, respectively. The inocula were added to the vegetable material at levels of 1 to 2 %.

Sodium lactate broth (NLB) contained 1.0 % sodium lactate, 1.0 % yeast extract, 1.0 % tryptone and 1.5 % peptone (all from OXOID, Basingstoke, Hampshire, UK). EWM contained 9.0 % whey powder, 1.5 % yeast extract and 1.0 % glucose. The pH of both media was adjusted to 6.8 with 1.0 mol·L^{-1} NaOH before sterilisation at 121 °C for 20 min.

2.2. Raw materials

The raw materials used were good quality vegetables purchased from a local market. Red beet juice was squeezed from grated beets and pasteurised at 80 °C for 10 min before use. Sauerkraut was made in the traditional manner from white cabbage. The clean cabbage was shredded into small pieces, about 0.5 × 4.0 cm, mixed with 1.5 % NaCl and pressed in 5-L glass jars until juice appeared on the surface. PAB and LAB were mixed with the shredded cabbage before addition of NaCl.

Vegetable salad was made of the following mixture of shredded vegetables: 58 % white cabbage, 12 % carrot, 12 % red sweet pepper, 10 % celery, 6 % leek, 2 % onion and 2 % salt (all w/w). The shredded vegetables were mixed with the salt, placed in glass jars and pressed until juice appeared on the surface. PAB and LAB were mixed with the shredded vegetables before addition of NaCl.

2.3. Growth and storage conditions

Red beet juice was held at 22 °C for the whole period of the experiment. Sauerkraut and vegetables were held at 22 °C for the first 14 d of the experiment after which they were stored at 8 °C.

2.4. Analytical methods

Bacteria were enumerated on the following media (MERCK, Darmstadt, Germany). Total bacterial count, nutrient agar, 30 °C, 72 h; lactobacilli, MRS agar, 30 °C, 24 h and Rogosa agar, pH 5.5, 30 °C, 24 h; lactococci, M17 agar, 30 °C, 24 h; proteolytic bacteria, calcium

caseinate agar, 25 °C, 48 h; fungi, yeast extract glucose chloramphenicol agar, 25 °C, 96 h; clostridia, reinforced clostridial medium, 37 °C, 48 h; enterococci, bromocresol-purple azide broth, 37 °C, 48 h; coliform, lauryl sulphate broth, 30 °C, 24–48 h later transferred for confirmation to brilliant green bile agar broth, 30 °C, 24–48 h.

For PAB counts, NLB containing agar (15 $g \cdot L^{-1}$) and bromocresol purple (0.032 $g \cdot L^{-1}$) as an indicator with anaerobic incubation at 30 °C was used. Both PAB and LAB grew on NLB agar. LAB formed big, white colonies after 48 h and PAB formed small, yellow colonies with yellow zones after 96 h incubation.

Vitamins were determined using biological methods with *L. casei* ATCC 7469 for determination of folacin and *L. leichmanii* ATCC7830 for determination of vitamin B_{12} [8].

Propionic and acetic acids were analysed according to the method described by Babuchowski et al. [2, 3].

Sensory characteristics of the products were noted according to the 'relative-to-ideal' method described by Amerine et al. [1] and Solheim and McEwan [18].

3. RESULTS AND DISCUSSION

3.1. Red beet

Red beet (*Beta vulgaris esculenta suprodes*) has been cultivated for many years for the production of juices, pickles, soups and salads. These products have great nutritive value and favourable organoleptic and health promoting properties [6, 10, 11]. Their nutritive value is due to the presence of substantial amounts of mineral salts, e.g. Fe, K, Mg, exogenous amino acids, vitamins and carbohydrates. As a result red beet juices are recommended for convalescents and people with different disorders, e.g. anemia, tumours, gastrointestinal problems, etc. Particularly beneficial are fermented juices called 'bio-juices', which contain probiotics. In this respect, the proper combination of microorganisms in the starter cultures used for production of juice can have an additional beneficial influence on the quality of the final product [12, 13, 19].

In this study, seven strains of PAB were evaluated for the production of fermented red beet juices. Except for *P. thoenii* 119, the growth rates of the PAB and final cell concentrations after 14 d of fermentation, were similar (*figure 1a, b*). PAB entered the stationary phase of growth after 4 d of incubation. After 14 d of incubation, large differences were observed in the final pH values of the fermented juices (*figures 1c, d*) with pH values of 4.13 for *P. jensenii* 118 and 4.96 for *P. freudenreichii* 111.

Regarding organoleptic properties, the best characteristics and the tastiest juices were made with *P. freudenreichii* 111 and *P. thoenii* 119 (*table I*). Both obtained 4.9 points out of 5 for taste, 4.6 out of 5 for colour as well as 4.9 and 4.6 out of 5 for flavour, respectively. On the 14th d of fermentation, *P. freudenreichii* 111 and *P. thoenii* produced 1.74 mg and 1.58 mg of vitamin $B_{12} \cdot L^{-1}$, 15.6 g and 21.8 g of propionic $acid \cdot L^{-1}$, and 6.5 g and 9.1 g of acetic $acid \cdot L^{-1}$, respectively (data not shown). Based on this, these strains in combination with LAB selected in previous studies [7, 14, 16, 17] were used for further experiments.

The initial pH of all juices, after addition of each starter culture, was 6.26. After 24 h of fermentation, the pH decreased to about 3.5 and the first differences in taste and appearance were observed. Juices prepared with yeast and PAB were strongly effervescent and had poor organoleptic properties during the later stages of storage. After 14 d at 22 °C, they were considered organoleptically unacceptable (data not shown). The best results were obtained with juice inoculated with a starter culture of the following composition: *L. plantarum* 2L, *L. lactis* subsp. *lactis* 5k, *L. acidophilus* 294 and *P. freudenreichii* 111 or *P. thoenii* 119 (*table II*).

During the 14 d fermentation, changes in organoleptic properties of the juices were observed, but juices made with *L. plantarum* 2L, *L. lactis* subsp. *lactis* 5k, *L. acidophilus*

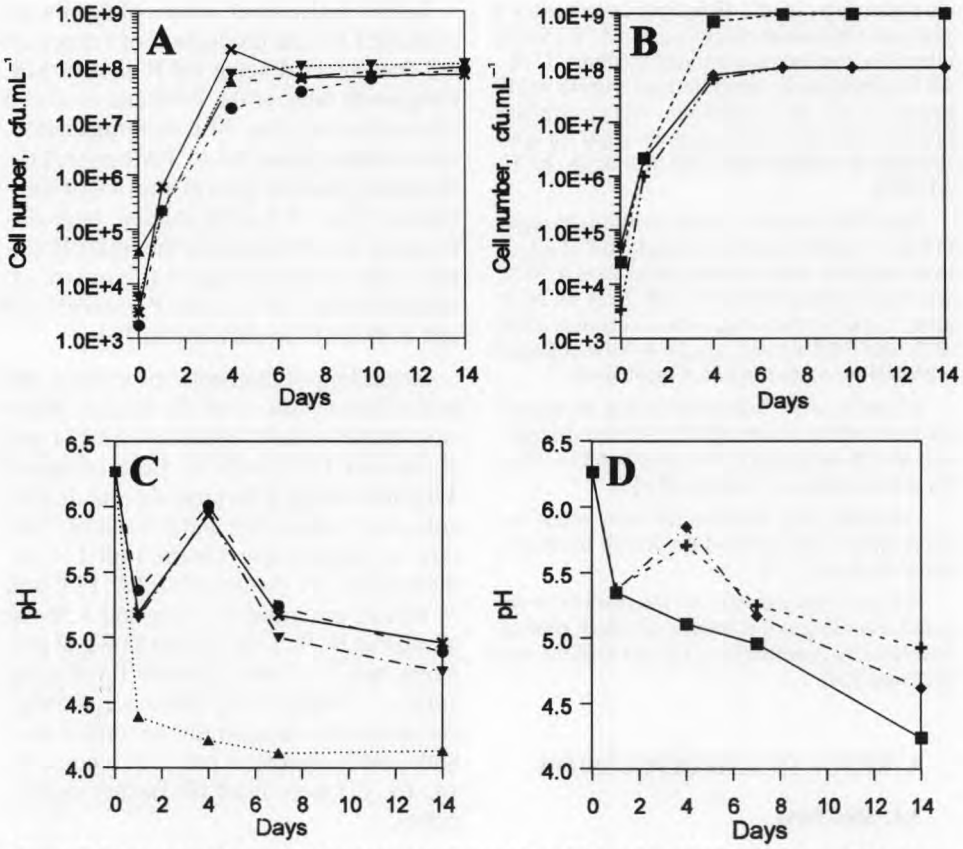

Figure 1. Changes in the propionibacteria population (**A** and **B**) and in pH (**C** and **D**) in red beet juice during fermentation.

Figure 1. Changements dans la population (**A** et **B**) et évolution du pH (**C** et **D**) dans le jus de betteraves rouges fermenté.

A: (×) *P. freudenreichii* 111, (●) *P. acidipropionici* 114, (▼) *P. acidipropionici* 117, (▲) P. *jensenii* 118. **B**: (■) *P. thoenni* 119, (✚) *P. acidipropionici* 122, (◆) P. *thoenii* 124. **C**: (✖) *P. freudenreichii*, 111, (●) *P. acidipropionici* 114, (▼) *P. acidipropionici* 117, (▲) *P. jensenii* 118. **D**, (■) *P. thoenii* 119, (✚) *P. acidipropionici* 122, (◆) *P. thoenii* 124.

294 and *P. freudenreichii* 111 or *P. thoenii* 119 were still the best ones. These juices had vitamin B_{12} contents of 18.1 and 16.6 $\mu g \cdot L^{-1}$ for juices fermented with *P. freudenreichii* 111 and *P. thoenii* 119, respectively, after 14 d of fermentation, compared to 0.93 $\mu g \cdot L^{-1}$ in the control.

The shelf-life of the products was also improved, probably due to the presence of propionic and acetic acids (*figure 2*). Acetic acid concentrations reached their peak on day 7 of the fermentation and propionic acid on day 1 after which the concentrations of both acids decreased steadily. However, there was still sufficient propionic acid present to inhibit growth of added fungi, e.g. numbers of *Geotrichum candidum* in red beet juice fermented with *P. freudenreichii* 111 were 3.0×10^1 cfu·mL^{-1} at the beginning of incubation, 6.2×10^1 cfu·mL^{-1} after

Table I. Influence of different *Propionibacterium* strains on organoleptic properties of red beet juice.

Tableau I. Influence de différentes souches de *Propionibacterium* sur les propriétés organoleptiques de jus de betteraves rouges.

Strain	Sensory assessment (points)					
	After 1 d External appearance			After 14 d External appearance		
	Colour	Flavour	Taste	Colour	Flavour	Taste
Control	4.1	4.1	3.9	1.9	1.8	1.7
P. freudenreichii 111	4.6	4.9	4.9	4.6	4.9	4.9
P. acidipropionici 114	4.2	4.1	4.9	3.9	4.1	4.1
P. acidipropionici 117	4.6	4.1	4.1	4.6	4.1	4.1
P. jensenii 118	4.6	4.1	4.1	3.3	3.1	3.1
P. thoenii 119	4.6	4.5	4.9	4.6	4.6	4.9
P. acidipropionici 122	3.9	3.5	4.1	3.3	3.8	3.1
P. thoenii 124	4.6	4.1	4.1	3.1	2.6	2.2

1 d of fermentation and 3.0×10^2 cfu·mL^{-1} after 7 and 10 d (data not shown). In the juice fermented with LAB only, there were 3.0×10^1 cfu·mL^{-1} at the beginning of incubation and 3.4×10^4 cfu·mL^{-1} of *Geotrichum candi*dum after 10 d (data not shown).

3.2. Sauerkraut

Sauerkraut is another popular fermented product in Poland. Cabbage used for its production is a poor source of B vitamins, including B_{12} and folacin [6]. Earlier research indicated that fodder silage inoculated with LAB and PAB had a higher content of vitamin B_{12} compared to silage inoculated with LAB only [9]. The influence of LAB on the vitamin B_{12} content in fermented products is not well established. Some workers [5] have claimed that lactic acid bacteria utilise vitamin B_{12}, while other [4] suggested that they produce vitamin B_{12} during fermentation. It is probable that this property is species- or strain-dependent.

In our research, sauerkraut was made in the traditional manner and the only bacteria intentionally added to the cabbage was *P. jensenii* 112 to give an initial level of 3.2×10^5 cfu·g^{-1} of cabbage [15]. During the fermentation, changes in microbial population were observed (*table III*). In standard sauerkraut, PAB were present at low and constant levels during the fermentation. Total bacteria, LAB and PAB counts increased until the 6th d of the fermentation, after which they slowly decreased. The number of proteolytic bacteria decreased from an initial level of 2.2×10^4 cfu·g^{-1} on day 1 of the fermentation, to $< 10 \cdot g^{-1}$ on day 9. A much greater decrease in the number of proteolytic bacteria was observed in sauerkraut fermented with PAB, from 2.2×10^4 cfu·g^{-1} on day 1 to 1.2×10^2 cfu·g^{-1} after 64 d of fermentation than in the sauerkraut made without addition of PAB, from 2.2×10^4 cfu·g^{-1} on day 1 to 3.7×10^3 cfu·g^{-1} after 64 d of fermentation. This was correlated with an increase of acidity of the product (data not shown).

In sauerkraut made with and without added PAB, the growth of moulds was suppressed until day 9 of the fermentation when their numbers began to increase.

Table II. Changes in pH and microbial population in fermented red beet juice inoculated with different propionibacteria and lactic acid bacteria.
Tableau II. Changements dans le pH et la population du jus de betteraves rouges fermentées inoculé par bactéries propionique et lactique.

Starter culture	0 d				After 1 d								After 14 d							
	Cell	Vitamin	Cell		Volatile fatty acids		Vitamin	Sensory evaluation (points)				Cell		Volatile fatty acids		Vitamin	Sensory evaluation (points)			
Composition	number	B_{12}	number	pH	(mg·L^{-1})		B_{12}					number	pH	(mg·L^{-1})		B_{12}				
	(cfu·L^{-1})	(mg·L^{-1})	(cfu·L^{-1})		C3	C2	(µg·L^{-1})	Colour	Flavour	Taste	General	(cfu·L^{-1})		C3	C2	(µg·L^{-1})	Colour	Flavour	Taste	General
Lb. plantarum 2L	3.2×10^7	ND	6.0×10^9									2.2×10^7		ND	ND					
L. lactis subsp. *lactis* 5k	5.8×10^7		1.4×10^9	3.60	ND	ND	ND	4.8	4.6	4.9	4.7	4.4×10^7	3.79			0.93	4.1	4.5	4.8	4.3
Lb. acidophilus 294	3.8×10^7		4.0×10^9									4.3×10^7								
Lb. plantarum 2L	3.6×10^7		2.0×10^9									3.4×10^7								
L. lactis subsp. *lactis* 5k	2.1×10^7	trace	8.0×10^9	3.56	1180	320	trace	4.7	4.8	4.9	4.8	6.9×10^7	3.80	422	256	18.12	4.2	4.5	4.8	4.5
Lb. acidophilus 294	3.8×10^7		4.0×10^9									5.1×10^7								
P. freudenreichii 111	2.9×10^6		1.0×10^7									1.5×10^8								
Lb. plantarum 2L	3.4×10^7		3.0×10^9									3.3×10^7								
L. lactis subsp. *lactis* 5k	2.8×10^7	trace	6.3×10^9	3.50	1180	320	trace	4.7	4.5	4.9	4.7	3.4×10^7	3.79	475	198	16.60	4.3	4.5	4.7	4.5
Lb. acidophilus 294	3.6×10^7		5.1×10^9									2.7×10^7								
P. thoenii 119	2.8×10^6		1.4×10^7									3.1×10^8								
Lb. plantarum 2L	3.5×10^7	ND	1.1×10^9	3.56	ND	ND	trace	4.5	4.1	4.9	4.5	3.2×10^7	3.74	ND	ND	1.14	3.9	4.1	4.8	4.2
L. lactis subsp. *lactis* 5k	2.8×10^7		8.0×10^8									4.2×10^7								
Lb. plantarum 2L	3.3×10^7	trace	5.2×10^9									3.9×10^7								
L. *lactis* subsp. *lactis* 5k	2.5×10^7		9.3×10^9	3.52	965	290	trace	4.4	4.2	4.1	4.2	3.3×10^7	3.73	318	121	22.58	4.1	4.2	4.6	4.3
P. freudenreichii 111	3.2×10^6		4.0×10^7									5.1×10^8								
Lb. plantarum 2L	3.4×10^7		7.2×10^9									4.3×10^7								
L. *lactis* subsp. *lactis* 5k	2.9×10^7	trace	7.1×10^9	3.54	925	285	trace	4.4	4.2	4.1	4.2	4.4×10^7	3.75	309	119	19.20	4.1	4.1	4.7	4.3
P. thoenii 119	3.9×10^6		3.0×10^7									3.3×10^8								

C2 – acetic acid / acide acétique. C3 – propionic acid / acide propionique. ND – not detected / non détecté.

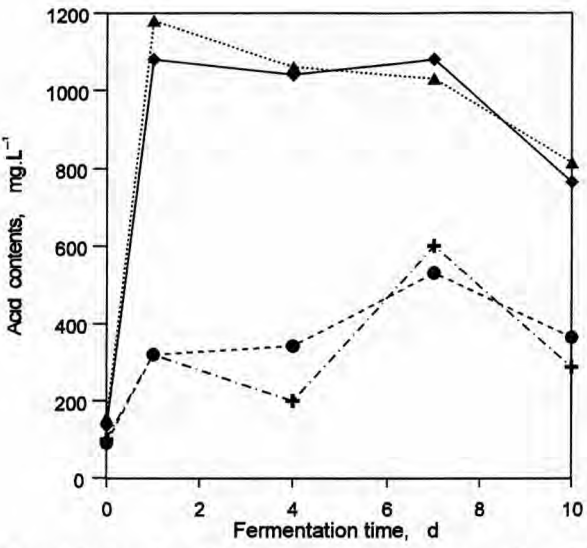

Figure 2. Changes in propionic and acetic acid contents during fermentation at 22 °C of red beet juice inoculated with selected starter cultures. Starter culture **A:** *L. plantarum* 2L, *L. lactis* subsp. *lactis* 5k, *L. acidophilus* 294, *P. freudenreichii* 111, (◆) propionic acid, (●) acetic acid. Starter culture **B:** *L. plantarum* 2L, *L. lactis* subsp. *lactis* 5k, *L. acidophilus* 294, *P. thoenii* 119; (▲) propionic acid, (✚) acetic acid.

Figure 2. Changements dans la teneur en acides acétique et propionique pendant la fermentation de jus de betteraves rouges avec des cultures lactiques sélectionnées. Levain **A :** *L. plantarum* 2L, *L. lactis* subsp. *lactis* 5k, *L. acidophilus* 294, *P. freudenreichii* 111, (◆) acide propionique, (●) acide acétique. Levain **B :** *L. plantarum* 2L, *L. lactis* subsp. *lactis* 5k, *L. acidophilus* 294, *P. thoenii* 119; (▲) acide propionique, (✚) acide acétique.

From the 6th d of incubation the numbers of coliforms, enterococci and sporeformers were < $10 \cdot g^{-1}$ throughout the fermentation in both types of sauerkraut.

The results also indicated that the addition of PAB to the cabbage during production of sauerkraut resulted in higher concentrations of folacin and vitamin B_{12} (*figure 3*) in the final product. After 64 d of fermentation, the folacin content in the sauerkraut inoculated with PAB was 832 $\mu g \cdot kg^{-1}$ and the vitamin B_{12} content 72.1 $\mu g \cdot kg^{-1}$ compared with 327 $\mu g \cdot kg^{-1}$ and 20.1 $\mu g \cdot kg^{-1}$, respectively, in product made without PAB. Both vitamins are produced during normal production of sauerkraut but the addition of PAB significantly increased their levels. Commercially-made sauerkraut contained much lower levels of folacin (89.0 $\mu g \cdot kg^{-1}$) and vitamin B_{12} (1.3 $\mu g \cdot kg^{-1}$). This may be due to several factors including differences in microbial composition of the products, storage conditions, etc. The age of the commercially-made sauerkraut as well as the production method used were unknown.

Differences in organoleptic properties were also observed. Sauerkraut made with PAB had a pleasant, characteristic flavour and a slightly acidic and salty taste. The standard sauerkraut had a slightly yeasty taste and an off-flavour caused by the growth of fungi.

3.3. Vegetable salads

Fermented vegetable salads are gaining popularity in many countries and are made

Table III. Changes in microbial composition during production and storage of sauerkraut[1].
Tableau III. Changements dans la composition microbiologique pendant la production et le stockage de la choucroute.

Bacteria	Inoculum	Storage time (d)							
Present	type	0 d	3 d	6 d	9 d	14 d	28 d	42 d	64 d
Total bacterial count	Control	8.4×10^4	4.2×10^8	6.1×10^8	5.9×10^7	5.3×10^6	9.2×10^6	5.7×10^5	1.7×10^5
(cfu·gL^{-1})	+ *P. jensenii* 112	8.4×10^4	5.8×10^8	7.2×10^8	4.2×10^7	9.4×10^6	6.2×10^6	4.2×10^5	4.0×10^5
Propionic acid bacteria	Control	12	64	82	71	61	60	51	62
(cfu·gL^{-1})	+ *P. jensenii* 112	12	4.2×10^5	3.6×10^8	3.2×10^8	2.3×10^7	1.2×10^7	6.7×10^6	5.2×10^6
Lactic acid bacteria	Control	4.4×10^3	1.1×10^8	1.7×10^8	2.5×10^7	2.6×10^6	1.2×10^6	4.4×10^5	5.0×10^4
(cfu·gL^{-1})	+ *P. jensenii* 112	4.4×10^3	3.2×10^8	4.5×10^8	3.2×10^7	5.4×10^6	3.2×10^6	1.2×10^5	4.0×10^4
Proteolytic bacteria	Control	2.2×10^4	2.1×10^3	1.9×10^2	< 10	< 10	2.1×10^2	4.6×10^3	3.7×10^3
(cfu·gL^{-1})	+ *P. jensenii* 112	2.2×10^4	1.3×10^3	1.2×10^2	< 10	< 10	1.6×10^2	3.2×10^2	1.2×10^2
Fungi	Control	62	< 10	< 10	< 10	2.3×10^3	4.4×10^4	1.2×10^5	2.1×10^4
(cfu·gL^{-1})	+ *P. jensenii* 112	62	< 10	< 10	< 10	2.9×10^4	4.5×10^4	3.2×10^4	3.1×10^3
Coliforms	Control	9.3	2.7×10^2	46	9.3	7.5	2.1	0.23	0.64
(mpn·g^{-1})	+ *P. jensenii* 112	7.5	38	9.3	7.5	4.3	0.92	0.23	0.36
Enterococci	Control	9.3	1.5×10^2	46	38	43	3.6	23	28
(mpn·g^{-1})	+ *P. jensenii* 112	9.3	46	29	7.5	4.3	4.3	3.5	2.9
Sporeformers (Clostridium)	Control	0.36	0.36	0.74	0.74	0.62	0.62	0.36	0.36
(mpn·g^{-1})	+ *P. jensenii* 112	0.36	0.32	0.62	0.72	0.62	0.62	0.36	0.36

[1] Sauerkraut was fermented for 14 d at 22 °C after which it was stored at 8 °C / la choucroute était fermentée 14 j à 22 °C puis stockée à 8 °C.

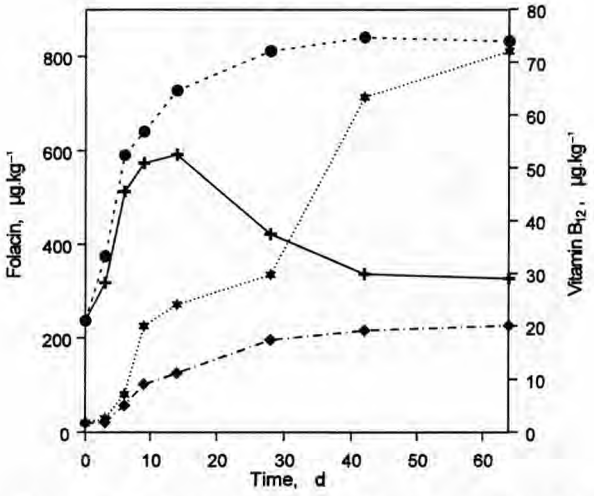

Figure 3. Changes in folacin and vitamin B_{12} content during production and storage of sauerkraut: (✚) folacin in control sauerkraut, (●) folacin in sauerkraut inoculated with propionibacteria, (◆) vitamin B_{12} in control sauerkraut, (✱) vitamin B_{12} in sauerkraut inoculated with propionibacteria.

Figure 3. Changements dans la teneur en folacine et en vitamine B_{12} pendant la production et le stockage de choucroute : (✚) folacine dans la choucroute témoin (●) folacine dans la choucroute contenant des bactéries propioniques, (◆) vitamine B_{12} dans la choucroute témoin, (✱) vitamine B_{12} dans la choucroute contenant des bactéries propioniques.

by natural fermentation of mixtures of freshly grated vegetables. Their shelf-life is limited due to the development of spoilage microorganisms, mainly fungi and proteolytic microflora. The addition of PAB to vegetable salads should not only lead to the extension of their shelf-life, inhibit the growth of yeasts, fungi and some pathogens but also improve their nutritive properties, by increasing the folacin content, and lead to improved sensory characteristics [20].

Data obtained with vegetable salads showed that the composition of the starter culture (*table IV*) had a significant influence on the growth or inhibition of some microbial groups. In general terms, a decrease in coliform counts was observed in all salads; however, in those inoculated with starter cultures, the decrease was faster than in naturally fermented ones (data not shown). In all cases, growth of yeast and LAB occurred. The numbers of LAB were greater in a mixture of *L. acidophilus* and *L. plantarum* than in *L. acidophilus* by itself. Perhaps, this was due to better exploitation of nutritional niches in the product. The opposite effect was observed in relation to the number of yeast which decreased to a small extent in the salads containing LAB but to a significant extent in the salads containing both LAB and PAB. This probably resulted from greater variability and concentration of volatile fatty acids in the product (*table IV*).

A starter culture composed of *L. acidophilus* 127, *L. plantarum* 120 and *P. jensenii* 118 produced the highest concentration of volatile fatty acids (VFA) (560 $mg \cdot L^{-1}$) and folacin (344 $mg \cdot L^{-1}$) after 28 d of fermentation. The lowest concentration of VFA (150 $mg \cdot L^{-1}$) and folacin (150 $mg \cdot L^{-1}$) was found in the naturally fermented control salad. It is interesting that, in the vegetable salad made with only *L. acidophilus* 127, no nitrates were found after 28 d of fermentation (data not shown). A significant

Table IV. Changes in the numbers of microorganisms and concentrations of vitamins and fatty acids in fermented vegetable salads[1].
Tableau IV. Changements dans la population de microbes et dans la concentration en vitamines et en acides gras dans les salades végétales fermentées.

Product	pH			Lactic acid bacteria ($cfu \cdot g^{-1}$)			Yeast ($cfu \cdot g^{-1}$)		Propionibacteria ($cfu \cdot g^{-1}$)			Folacin $Mg \cdot kg^{-1}$		Volatile fatty acids $mg \cdot g^{-2}$		Sensory evaluation
	0 d	14 d	28 d	0 d	14 d	28 d	0 d	28 d	0 d	14 d	28 d	0 d	28 d	C2 28 d	C3 28 d	28 d
Control: naturally fermented salad	6.0	3.96	3.91	7.2×10^4	8.0×10^6	1.2×10^3	9.8×10^2	7.8×10^6	NE	NE	NE	trace	150.0	130.0	20.0	2.4
+ *L. acidophilus* 127	6.0	3.85	3.84	8.0×10^6	8.4×10^7	3.4×10^5	5.6×10^2	5.1×10^6	NE	NE	NE	trace	169.0	440.0	trace	3.8
+ *L. acidophilus* 127 + *L. plantarum* 120 + *L. acidophilus* 127	6.0	3.68	3.71	9.0×10^6	2.1×10^8	1.1×10^6	4.0×10^2	4.2×10^6	NE	NE	NE	trace	181.0	280.0	trace	3.4
+ *L. plantarum* 120 + *P. jensenii* 118	6.0	3.63	3.70	5.6×10^6	2.8×10^8	4.3×10^6	6.6×10^2	1.7×10^6	2.0×10^5	7.2×10^6	1.7×10^6	87.6	334.0	360.0	200.0	4.8

[1] Fermented at 22 °C for 14 d after which they were stored at 8 °C / Fermentées à 22 °C pendant 14 j puis stockées à 8 °C.
[2] C2 – Acetic acid / acide acétique. C3 – propionic acid / acide propionique. NE – not examined / non examiné.

reduction in nitrate level was also observed in the vegetable salad inoculated with a starter culture composed of *L. acidophilus* 127, *L. plantarum* 120 and *P. jensenii* T118. The other starters did not lead to a reduction in nitrate level (data not shown). Regardless of the starter culture used, a significant reduction in nitrite concentration was observed in all samples tested.

The vegetable salad inoculated with *L. acidophilus* 127, *L. plantarum* 120 and *P. jensenii* T118 had the best sensory characteristics (4.8 points out of a possible 5) followed by the salad inoculated only with *L. acidophilus* 127 and with both *L. acidophilus* 127 and *L. plantarum* 120. The naturally fermented salad had the lowest score as a result of the development of an unclean taste and a soft texture (*table IV*).

4. CONCLUSION

The data in this paper demonstrate that there is a distinct possibility of using PAB in the manufacture of fermented vegetable products. The advantages are increased vitamin B_{12} and folacin contents, extended shelf-life of the product, inhibition of pathogenic and harmful microflora and, in certain cases, better organoleptic properties. However, these properties are species- and strain-specific.

For products with long fermentation processes like silages, it is not necessary to modify technical processes but for products with short fermentations, like fermented vegetable juices, a process modification is necessary due to the slow growth rates of PAB. In most cases, the modification can be limited to an increased inoculum size. As a result, in the products with long fermentations, the vitamin contents increased during processing and storage. In the products with short fermentations, the vitamin contents probably result directly from the vitamin content in the PAB inoculum used.

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