Food Microbiology 94 (2021) 103646

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Bacterial diversity in six species of fresh edible seaweeds submitted to high pressure processing and long-term refrigerated storage

Antonia Picon *, Ana del Olmo, Manuel Nuñez

INIA, Departamento de Tecnología de Alimentos, Carretera de La Coruña Km 7, 28040, Madrid, Spain

ARTICLE INFO

Keywords:
Microbiota
Seaweed
High pressure processing
16S rDNA sequencing
Biodiversity
Bacillaceae

ABSTRACT

Seaweeds are highly perishable foods due to their richness in nutrients. High pressure processing (HPP) has been applied for extending the shelf life of fresh seaweeds but there is no information on the effect of HPP on the bacterial diversity of seaweeds. The culturable bacteria of six species of fresh edible seaweeds (green seaweeds *Codium fragile* and *Ulva lactuca*, brown seaweeds *Himanthalia elongata*, *Laminaria ochroleuca* and *Undaria pinnatifida*, and red seaweed *Chondrus crispus*) were investigated and compared to those of HPP-treated (400 and 600 MPa for 5 min) seaweeds, at the start and end of their refrigerated storage period. A total of 523 and 506 bacterial isolates were respectively retrieved from untreated and HPP-treated seaweeds. Isolates from untreated seaweeds belonged to 18 orders, 35 families, 71 genera and 135 species whereas isolates from HPP-treated seaweeds belonged to 13 orders, 23 families, 43 genera and 103 species. HPP treatment significantly reduced the number of isolates belonging to 6 families and greatly increased the number of *Bacillaceae* isolates. At the end of storage, decreases in bacterial diversity at the genus and species level were observed for untreated as well as for HPP-treated seaweeds.

1. Introduction

Seaweeds have traditionally been a major element of the human diet in Asian countries, and are extensively used in Asian cuisine. Although seaweeds occur with high diversity and abundance along European and North American coasts, they have not been a significant food resource in Western societies throughout the past centuries, excepting a few coastal Atlantic communities (Chapman et al., 2015; Wells et al., 2017). Considering that seaweeds are a bountiful, but poorly exploited, marine food resource (Buschmann et al., 2017), it is likely that, in the future, the use of seaweeds for human consumption will be fostered to reach the challenge of finding new food and sustainable resources with a smaller carbon footprint (Mouritsen et al., 2018). On top of that, seaweeds are an estimable source of functional ingredients and bioactive compounds, fibre, vitamins, amino acids and minerals (Ibañez and Cifuentes, 2013; Kumar et al., 2008).

Seaweeds provide a protected niche favourable for bacterial colonization and reproduction, resulting in complex and highly dynamic microbial communities (Goecke et al., 2010). These bacterial epiphytic communities supply nutrients and growth factors and shape the morphology and life cycle of their seaweed hosts. Furthermore, quorum sensing inhibitors and antimicrobial compounds produced by numerous epiphytic bacteria work in concert with seaweed-derived metabolites to protect the seaweed surface from pathogens, herbivores, and fouling organisms (Hollants et al., 2013). After reviewing numerous studies on seaweed-associated bacterial communities, these authors concluded that γ-proteobacteria were the most common bacterial clade associated with seaweeds (37% relative abundance), followed by the Cytophaga-Flavobacterium-Bacteroides (CFB) group (20%), α-proteobacteria (13%), Firmicutes (10%), Actinobacteria (9%) and β-proteobacteria (1%).

Although fresh seaweeds can be stored at 4 °C for several days without modification of their textural or sensory characteristics, microbial growth limits the length of their commercial life (Liot et al., 1993). In order to restrain microbial growth, high pressure processing (HPP) at 400 or 600 MPa was applied for the preservation of different seaweed species (del Olmo et al., 2019, 2020). The shelf life of HPP-treated seaweeds (established as the period to reach 7 log cfu/g) was thus extended up to at least 180 days whereas the shelf life of untreated seaweeds ranged from 3 to 60 days depending on the seaweed species (del Olmo et al., 2019, 2020). However, there is no information on the effect of HPP on the microorganisms present in seaweeds. The

* Corresponding author.
E-mail address: apicon@inia.es (A. Picon).

https://doi.org/10.1016/j.fm.2020.103646
Received 21 May 2020; Received in revised form 11 September 2020; Accepted 15 September 2020
Available online 24 September 2020
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Table 1
Taxonomical distribution of the 523 bacterial isolates from six species of untreated (control) seaweeds[a] into phylum, class, order and family.

Phylum	Class	Order	Family	Start of storage						End of storage						No. of isolates
				CF	UL	HE	LO	UP	CC	CF	UL	HE	LO	UP	CC	
Proteobacteria	α-Proteobacteria	Rhizobiales	*Hyphomicrobiaceae*	1												1
		Rhodobacterales	*Rhodobacteraceae*	17	3	9	5		4	8	3	4			5	58
		Sphingomonadales	*Erythrobacteraceae*	3				2								5
			Sphingomonadaceae	3		2						1				6
	β-Proteobacteria	Burkholderiales	*Oxalobacteraceae*		1											1
	γ-Proteobacteria	Alteromonadales	*Alteromonadaceae*						4							4
			Pseudoalteromonadaceae						3		1					4
			Psychromonadaceae				3									3
			Shewanellaceae				3					5	5			13
		Enterobacteriales	*Enterobacteriaceae*		1		2						3			6
			Erwiniaceae				3		2				3		1	9
			Yersiniaceae						1							1
		Oceanospirillales	*Halomonadaceae*	16	9	13	18	9	7	4	4	9	13	13	8	123
			Oceanospirillaceae					3								3
		Pseudomonadales	*Moraxellaceae*	9	10	8	14	8	13	3	13	6	3		7	94
			Pseudomonadaceae					4			2					6
		Thiotrichales	*Thiotrichaceae*			2										2
		Vibrionales	*Vibrionaceae*	1												1
		Xanthomonadales	*Xanthomonadaceae*			2										2
Bacteroidetes	Cytophagia	Cytophagales	*Cyclobacteriaceae*							3						3
	Flavobacteria	Flavobacteriales	*Flavobacteriaceae*	9	6	8	10	4	15	9	4	1	4	8	7	85
Actinobacteria		Corynebacteriales	*Dietziaceae*	2												2
			Nocardiaceae			2										2
		Micrococcales	*Brevibacteriaceae*		2											2
			Microbacteriaceae				2			7	1	3	3		4	20
			Micrococcaceae	1				4	2							7
		Propionibacteriales	*Nocardioidaceae*						1							1
Firmicutes	Bacilli	Bacillales	*Bacillaceae*		3	5	2	4	2		4	2	2	2	3	29
			Paenibacillaceae			1					1					2
			Planococcaceae		2						2				2	6
			Staphylococcaceae		2				1						1	4
			XII. Incertae sedis		3		5		1		1					10
		Lactobacillales	*Carnobacteriaceae*		1											1
			Enterococcaceae									3				3
			Lactobacillaceae											4		4

[a] Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed). Start of storage was 2 days after collection. End of storage was 15 days after collection for UL and CC, 30 days after collection for CF, LO and UP, and 60 days after collection for HE.

Table 2
Taxonomical distribution of the 342 Proteobacteria isolates from six species of untreated (control) seaweeds.[a].

Family	Genus and species	Start of storage						End of storage						No. of isolates
		CF	UL	HE	LO	UP	CC	CF	UL	HE	LO	UP	CC	
Hyphomicrobiaceae	*Candidatus Devosia euplotis*	1												1
Rhodobacteraceae	*Albirhodobacter marinus*							1						1
	Aliiroseovarius halocynthiae							1						1
	Amylibacter sp.							1						1
	Cereibacter changlensis							1						1
	Celeribacter naphthalenivorans				2				2	4				8
	Falsirhodobacter sp.			1										1
	Loktanella agnita	1												1
	Loktanella hongkongensis			1										1
	Loktanella koreensis	1												1
	Loktanella ponticola												1	1
	Octadecabacter ascidiaceicola			1										1
	Paracoccus homiensis	5		4	3		3						3	18
	Paracoccus koreensis								1					1
	Pseudophaeobacter sp.	2						2						4
	Ruegeria atlántica			2										2
	Ruegeria faecimaris	1												1
	Ruegeria meonggei	1												1
	Sulfitobacter donghicola		1											1
	Sulfitobacter dubius		1										1	2
	Sulfitobacter indolifex						1							1
	Sulfitobacter litoralis	4												4
	Sulfitobacter mediterraneus	1												1
	Sulfitobacter pontiacus		1											1
	Sulfitobacter undariae							2						2
	Tateyamaria pelophila	1												1
Erythrobacteraceae	*Erythrobacter flavus*					2								2
	Erythrobacter longus	3												3
Sphingomonadaceae	*Novosphingobium gossypii*	1												1
	Sphingomonas aquatilis	1		1										2
	Sphingomonas mucosissima	1												1
	Sphingomonas panni			1						1				2
Oxalobacteraceae	*Janthinobacterium agaricidamnosum*		1											1
Alteromonadaceae	*Paraglaciecola mesophila*						4							4
Pseudoalteromonadaceae	*Pseudoalteromonas agarivorans*								1					1
	Pseudoalteromonas distincta						3							3
Psychromonadaceae	*Psychromonas aquatilis*				3									3
Shewanellaceae	*Shewanella baltica*									4				4
	Shewanella gaetbuli									1				1
	Shewanella algicola				2						3			5
	Shewanella inventionis				1						2			3
Enterobacteriaceae	*Citrobacter freundii*				2						3			5
	Raoultella planticola		1											1
Erwiniaceae	*Pantoea gaviniae*						2						1	3
	Erwinia endophytica				3						3			6
Yersiniaceae	*Serratia liquefaciens*						1							1
Halomonadaceae	*Cobetia crustatorum*		3						1					4
	Cobetia litoralis/amphilecti	12	4	8	13	9	5	4	2	5	7	9	5	83
	Cobetia marina/pacifica	4		5	5					4	6	4		28
	Halomonas alkaliantarctica						2						2	4
	Halomonas lionensis		2						1					3
	Halomonas titanicae												1	1
Oceanospirillaceae	*Marinomonas arenícola*					3								3
Moraxellaceae	*Acinetobacter beijerinckii*			1										1
	Acinetobacter bereziniae/guillouiae		1											1
	Acinetobacter junii			1										1
	Psychrobacter alimentarius									2				2
	Psychrobacter aquimaris			2			2							4
	Psychrobacter celer				2									2
	Psychrobacter cibarius	1												1
	Psychrobacter fozii		5			8			7					20
	Psychrobacter nivimaris	8	4	4	8		4	3	5	4	3		3	46
	Psychrobacter piscatorii						7						4	11
	Psychrobacter vallis								1					1
Pseudomonadaceae	*Pseudomonas brenneri*				4									4
	Pseudomonas plecoglossicida					1								1
	Pseudomonas sabulinigri								2					2
	Pseudomonas synxantha					3								3
Thiotrichaceae	*Leucothrix pacifica*			2										2
Vibrionaceae	*Vibrio diabolicus*	1												1
Xanthomonadaceae	*Stenotrophomonas rhizophila*			2										2

[a] Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed).

Table 3
Taxonomical distribution of the 88 Bacteroidetes and 34 Actinobacteria isolates from six species of untreated (control) seaweeds.[a].

Phylum	Family	Genus and species	Start of storage						End of storage						No. of isolates
			CF	UL	HE	LO	UP	CC	CF	UL	HE	LO	UP	CC	
Bacteroidetes	*Cyclobacteriaceae*	*Algoriphagus chordae*							2						2
		Cyclobacterium amurskyense							1						1
	Flavobacteriaceae	*Algibacter lectus*						2							2
		Algibacter undariae		1		3				1					5
		Cellulophaga algicola			1		4						8		13
		Cellulophaga fucicola						7						4	11
		Cellulophaga geojensis				1				1		3			5
		Cellulophaga lytica							4						4
		Dokdonia genika	1	1	1										3
		Flavobacterium glycines		1											1
		Formosa undariae										1			1
		Leeuwenhoekiella aequorea						1						1	2
		Maribacter forsetii	2												2
		Maribacter spongiicola							2						2
		Marixanthomonas ophiurae												1	1
		Mesonia mobilis				3									3
		Nonlabens dokdonensis												1	1
		Nonlabens ulvanivorans	5	1					1						7
		Nonlabens xylanidelens			2			2							4
		Olleya namhaensis	1	1	3			2							7
		Polaribacter atrinae								1					1
		Polaribacter litorisediminis		1											1
		Polaribacter vadi						1							1
		Psychroserpens mesophilus							1						1
		Tamlana nanhaiensis			1										1
		Winogradskyella arenosi									1				1
		Winogradskyella thalassocola				3			1						4
		Winogradskyella rapida								1					1
Actinobacteria	*Dietziaceae*	*Dietzia maris*	2												2
	Nocardiaceae	*Rhodococcus erythropolis*			2										2
	Brevibacteriaceae	*Brevibacterium casei*		2											2
	Microbacteriaceae	*Agrococcus jenensis*												2	2
		Salinibacterium amurskyense				2			7	1	3	3		2	18
	Micrococcaceae	*Kocuria palustris*					4	2							6
		Paenarthrobacter nicotinovorans	1												1
	Nocardioidaceae	*Nocardioides basaltis*						1							1

[a] Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed).

objective of the present study was to investigate the bacterial diversity of six species of commercially available fresh edible seaweeds before and after HPP treatments, and at the end of their shelf life.

2. Materials and methods

2.1. Seaweeds and HPP treatments

Six species of fresh edible seaweeds, two Chlorophyta (*Codium fragile*, CF, and *Ulva lactuca*, UL), three Ochrophyta (*Himanthalia elongata*, HE, *Laminaria ochroleuca*, LO, and *Undaria pinnatifida*, UP) and one Rhodophyta (*Chondrus crispus*, CC), were collected at Galicia (NW Spain) coastal areas and shipped under cooling to the laboratory in Madrid (Spain). Two batches of fresh seaweeds collected three weeks apart were used in experiments as previously described (del Olmo et al., 2019, 2020). Seaweeds were dispensed (150 g per tray) into expanded polystyrene trays, which were introduced into CN300 plastic bags (Cryovac Grace S.A., Barcelona, Spain). Seaweeds for HPP treatments were vacuum-packed and subjected to 400 or 600 MPa for 5 min, as previously described (del Olmo et al., 2019). Untreated seaweeds were stored in unsealed bags. After treatments, carried out on day 2 after collection, seaweeds (untreated and HPP-treated) were kept at 4 °C.

2.2. Bacterial isolation and preliminary characterization

Representative 10-g samples were homogenized with 90 ml of sterile 2% NaCl aqueous solution and analyzed as previously described (del Olmo et al., 2019). Total viable counts were determined on plate count agar (Biolife, Milano, Italy) and heterotrophic marine bacteria on Marine agar (BD Difco; Fisher Scientific, Madrid, Spain).

Representative colonies (generally 15 to 30 colonies per sample) were isolated from Marine agar plates at the start of storage (on day 2 after collection) and at the end of storage. After purification by at least three transfers, pure cultures were cryopreserved in tryptic soy yeast extract broth (Biolife) supplemented with 15% glycerol (v/v) at - 40 °C. Preliminary characterization of isolates based on cell morphology, and catalase and oxidase tests was carried out as previously described (del Olmo et al., 2018).

2.3. DNA extraction, 16S rDNA sequencing and biochemical tests

Genomic DNA from all isolates was extracted with the GenElute bacterial genomic DNA kit (Merck Life Science, Tres Cantos, Spain). An approximately 800 base-pair region of the 16S rDNA gene was amplified using the universal primers W01 and 800R, as previously described (Campos et al., 2011). The amplified PCR products were purified using the GenElute PCR clean-up kit (Merck Life Science) and sequenced at the Genomic Unit of Complutense University (CAI Genómica y Proteómica, Madrid, Spain). The obtained sequences (forward and reverse) were edited and aligned with the BioEdit program (© Hall TA, USA) and compared to those deposited in databases BLAST (http://blast.ncbi.nlm.nih.gov/), Eztaxon (www.ezbiocloud.net) and RDP (http://rdp.cme.mus.edu).

Isolates for which sequence homology identity in databases was lower than 99% and/or for which sequencing yielded several species indistinguishable by 16S rDNA were submitted to different biochemical

Table 4
Taxonomical distribution of the 59 Firmicutes isolates from six species of untreated (control) seaweeds.[a].

Family	Genus and species	Start of storage						End of storage						No. of isolates
		CF	UL	HE	LO	UP	CC	CF	UL	HE	LO	UP	CC	
Bacillaceae	*Bacillus algicola*					1						2		3
	Bacillus altitudinis												1	1
	Bacillus amyloliquefaciens										2			2
	Bacillus butanolivorans			1						2				3
	Bacillus firmus/muralis				2									2
	Bacillus humi								1					1
	Bacillus hwajinpoensis		1											1
	Bacillus idriensis			1										1
	Bacillus licheniformis								1					1
	Bacillus luteus						2							2
	Bacillus megaterium		2						2				2	6
	Bacillus simplex			1										1
	Bacillus vietnamensis			1										1
	Fictibacillus nanhaiensis					3								3
	Halobacillus locisalis			1										1
Paenibacillaceae	*Paenibacillus sinopodophylli*			1										1
	Paenibacillus woosongensis								1					1
Planococcaceae	*Planococcus donghaensis*		1						1					2
	Planococcus maritimus												2	2
	Planococcus plakortidis		1						1					2
Staphylococcaceae	*Staphylococcus epidermidis*		1											1
	Staphylococcus equorum												1	1
	Staphylococcus hominis						1							1
	Staphylococcus pasteuri/warneri		1											1
XII. Incertae sedis	*Exiguobacterium oxidotolerans*		3		5		1		1					10
Carnobacteriaceae	*Marinilactibacillus psychrotolerans*		1											1
Enterococcaceae	*Enterococcus faecium*									3				3
Lactobacillaceae	*Pediococcus pentosaceus*											4		4

[a] Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed).

tests by using API 20E, API 20NE, API ZYM, API 20strep and/or API 50CH strips (bioMérieux España, Madrid, Spain) following the manufacturer's instructions. API databases (https://apiweb.biomerieux.com) and literature on the subject were consulted for the identification of those isolates.

2.4. Biodiversity and statistical analysis

Changes in seaweed bacterial biodiversity at the start and the end of storage, as well as with and without HPP treatments, were evaluated by means of several indexes as previously described (Lucena-Padrós et al., 2014). Menhinick index (I_{MN}) was used as a measurement of species richness, Shannon-Weaver index (H') as a measurement of diversity, Pielou index (J') as a measurement of evenness, and Simpson index (D) as a measurement of dominance. Bacterial species represented by just one isolate (singletons) were not considered for biodiversity analysis, in order to conservatively estimate diversity (Zhou et al., 2013).

Analysis of variance (ANOVA) was carried out by means of SPSS program version 22.0 (IBM Corporation, Armonk, NY, USA), with HPP treatment and seaweed as main effects, on the number of isolates belonging to each genus or species at the start and the end of storage. Means were compared by Tukey's test, with the significance assigned at $P < 0.05$. Principal component analysis (PCA), with Varimax rotation, was carried out on the presence/absence of the different phyla and orders using the same statistical package.

3. Results and discussion

3.1. Diversity of culturable bacteria in untreated seaweeds

A total of 523 bacterial isolates were retrieved from the six untreated (control) seaweeds, 318 isolates at the start of storage and 205 isolates at the end of storage (Table 1). Seaweeds included in the present study showed at the start of storage, on day 2 after collection, microbial loads ranging from 4.60 log cfu/g for UP to 6.74 log cfu/g for UL (del Olmo et al., 2019, 2020). Storage ended when the threshold of 7 log cfu/g, the limit set for seaweed shelf life, was exceeded. That limit was reached in samples of untreated CF, UL, HE, LO, UP and CC seaweed analyzed after 30, 15, 60, 30, 30 and 15 days, respectively (del Olmo et al., 2019, 2020).

Bacterial isolates obtained from the six species of untreated seaweeds belonged to 18 orders and 35 families whereas only 8 families were represented by the 177 isolates obtained from commercial dehydrated CC, HE, LO, PU, UL and UP seaweeds (del Olmo et al., 2018). In the present study, families harbouring the higher number of isolates were *Halomonadaceae* (123 isolates), *Moraxellaceae* (94 isolates) and *Rhodobacteraceae* (58 isolates) within Proteobacteria, *Flavobacteriaceae* (85 isolates) within Bacteroidetes, *Microbacteriaceae* (20 isolates) within Actinobacteria, and *Bacillaceae* (29 isolates) within Firmicutes.

The 175 isolates from Chlorophyta seaweeds (CF and UL) belonged to 23 families, the 254 isolates from Ochrophyta (HE, LO and UP) to 22 families and the 94 isolates from Rhodophyta (CC) to 15 families (Table 1). Regarding individual seaweeds, 12, 16, 13, 11, 9 and 15 families were represented in CF, UL, HE, LO, UP and CC, respectively. A clear relationship between the microbial load of untreated seaweeds at the start of storage and their bacterial diversity at the family level was observed. Seaweeds UL and UP, which respectively had the highest (6.74 log cfu/g) and lowest (4.60 log cfu/g) bacterial counts on Marine agar at day 2 after collection (del Olmo et al., 2019, 2020), ranked first and last according to the number of represented bacterial families.

At a lower taxonomical level, bacterial isolates from untreated seaweeds belonged to a total of 71 genera and 135 species (Tables 2–4). This diversity was higher than that found for the 177 isolates obtained from commercial dehydrated CC, HE, LO, PU, UL and UP seaweeds, in which only 22 genera and 47 species were represented (del Olmo et al., 2018). In the present study, the highest diversity at the genus level on day 2 after collection was found for HE and CC, each with 20 genera represented by isolates, and the highest diversity at the species level for

Table 5
Taxonomical distribution of the 506 bacterial isolates from six species of HPP-treated edible seaweeds[a] into phylum, class, order and family.

Phylum	Class	Order	Family	Start of storage						End of storage						No. of isolates
				CF	UL	HE	LO	UP	CC	CF	UL	HE	LO	UP	CC	
Proteobacteria	α-Proteobacteria	Caulobacterales	*Caulobacteraceae*									2				2
		Rhizobiales	*Methylobacteriaceae*					1								1
		Sphingomonadales	*Sphingomonadaceae*					2				1				3
	γ-Proteobacteria	Alteromonadales	*Pseudoalteromonadaceae*	4	2		5		7						1	19
			Psychromonadaceae	1	2	1	5		2		1		2			14
		Enterobacteriales	*Erwiniaceae*				2						1			3
		Oceanospirillales	*Halomonadaceae*	2	1	11	13	4	3	1		1	12	3		51
			Oceanospirillaceae					5		2	2	4		7	1	21
		Pseudomonadales	*Moraxellaceae*	3	1		2		3	3	8	3			5	28
			Pseudomonadaceae					1								1
		Vibrionales	*Vibrionaceae*				1					1				2
Bacteroidetes	Flavobacteria	Flavobacteriales	*Flavobacteriaceae*	2	3		2		4		3					14
Actinobacteria		Corynebacteriales	*Dietziaceae*		1											1
		Micrococcales	*Microbacteriaceae*				3					1				4
			Micrococcaceae					2	3			1			1	7
Firmicutes	Bacilli	Bacillales	*Bacillaceae*	23	26	18	10	25	17	9	30	10	25	22	8	223
			Brevibacteriaceae						2				2			4
			Paenibacillaceae	3	2						1				1	7
			Planococcaceae		2	1			2	2	7	2				16
			XII. Incertae sedis		1		2						1			4
		Lactobacillales	*Carnobacteriaceae*								1			4		5
			Enterococcaceae			3				17	6	15			11	52
			Lactobacillaceae					5			1			12	6	24

[a] Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed). Start of storage was 2 days after collection. End of storage 180 days after collection.

HE and UL, each with 26 species represented by isolates. Bacterial diversity previously reported for fresh seaweeds considerably varied depending on the seaweed species, the location and season of collection, and the methods used for the study of bacterial communities associated with seaweeds. Fifteen genera and 17 bacterial species were represented in the microbiota of the red seaweed *Palmaria palmata* (Moore et al., 2002) and 14 genera in the microbiota of two brown and two red seaweed species (Beleneva and Zhukova, 2006). The brown seaweed *Ascophyllum nodosum*, with 36 genera, showed a higher bacterial diversity (Martin et al., 2015) while only 10 genera were represented in the microbiota of two brown seaweed *Lobophora* species (Vieira et al., 2016) and 12 genera in the microbiota of two green seaweed *Ulva* species and two red seaweed *Gracilaria* species (Singh et al., 2015). The use of 16S RNA gene library analysis permitted the detection of 79 bacterial species in red seaweed *Delisea pulchra* and 36 bacterial species in green seaweed *Ulva australis* (Longford et al., 2007), and 17 genera in the microbiota of *U. australis* (Burke et al., 2011).

Refrigerated storage decreased the bacterial diversity of untreated seaweeds. At the start of storage, isolates from untreated seaweeds belonged to 17 out of the 18 orders, 32 out of the 35 families, 58 out of the 71 genera and 100 out of the 135 species whereas at the end of storage only 11 orders, 19 families, 41 genera and 67 species were represented. Storage time had a significant ($P < 0.05$) effect on the number of isolates of *Pseudoalteromonadaceae*, *Psychromonadaceae*, *Micrococcaceae*, *Enterococcaceae* and *Lactobacillaceae*, according to the ANOVA. Few or no representative isolates from the first three families were found at the end of storage, and none from the last two families were isolated from samples analyzed at the beginning of storage. Lactic acid bacteria such as *Enterococcaceae* and *Lactobacillaceae* are tolerant to the pH decrease observed during refrigerated storage of some of the untreated seaweeds (del Olmo et al., 2019, 2020), which probably favoured their growth comparatively to less acid tolerant bacteria.

3.2. Diversity of culturable bacteria in HPP-treated seaweeds

A total of 506 bacterial isolates were obtained from the six HPP-treated seaweeds, 246 at the start of storage and 260 at the end of storage (Table 5). Isolates from HPP-treated seaweeds belonged to 13 orders and 23 families, including *Halomonadaceae* (51 isolates) and *Moraxellaceae* (28 isolates) in the phylum Proteobacteria, *Flavobacteriaceae* (14 isolates) in the phylum Bacteroidetes, Micrococcaceae (7 isolates) in the phylum Actinobacteria, and *Bacillaceae* (223 isolates) and *Enterococcaceae* (52 isolates) in the phylum Firmicutes.

Bacterial diversity at the family level decreased with HPP treatments. The number of families represented in HPP-treated CF, UL, HE, LO, UP and CC seaweeds (Table 5) were 10, 14, 12, 10, 8 and 13, respectively, while 12, 16, 13, 10, 9 and 15 families, respectively, were isolated from untreated seaweeds (Table 1). It must be noted that HPP treatment 2 days after harvest lowered microbial loads of the six seaweeds by 1.70–3.06 log CFU/g at 400 MPa and by 2.22–3.66 log CFU/g at 600 MPa (del Olmo et al., 2019, 2020). HPP treatment had a significant ($P < 0.05$) effect on the number of isolates belonging to the *Rhodobacteraceae*, *Shewanellaceae*, *Halomonadaceae*, *Moraxellaceae*, *Flavobacteriaceae*, *Microbacteriaceae* and *Bacillaceae* families, according to the ANOVA. No isolate belonging to the first two families was retrieved from HPP-treated seaweeds, the number of isolates belonging to the next four families greatly declined, and the number of isolates belonging to the *Bacillaceae* family greatly increased. *Bacillaceae* accounted for 44.1% of all isolates from HPP-treated seaweeds, which could be due to the high pressure resistance of sporeformers.

Bacterial diversity at the genus and species level also decreased with HPP treatment, with 43 genera and 103 species isolated from HPP-treated seaweeds (Tables 6–8) in contrast with the 71 genera and 135 species isolated from untreated seaweeds (Tables 2–4). Similarly to this reduction in bacterial diversity caused by HPP, heat treatment (85 °C/15 min) of three brown seaweeds prior to analysis resulted in low

Table 6
Taxonomical distribution of the 145 Proteobacteria isolates from six species of HPP-treated seaweeds.[a].

Family	Genus and species	Start of storage						End of storage						No. of isolates
		CF	UL	HE	LO	UP	CC	CF	UL	HE	LO	UP	CC	
Caulobacteraceae	*Brevundimonas intermedia*									2				2
Methylobacteriaceae	*Methylobacterium oryzae*					1								1
Sphingomonadaceae	*Sphingomonas faeni*									1				1
	Sphingomonas mali					1								1
	Sphingomonas yunnanensis					1								1
Pseudoalteromonadaceae	*Pseudoalteromonas agarivorans*	1	1				2							4
	Pseudoalteromonas carrageenovora		1											1
	Pseudoalteromonas distincta	3			5		5						1	14
Psychromonadaceae	*Psychromonas aquatilis*		2		5				1		2			10
	Psychromonas arctica	1		1			2							4
Erwiniaceae	*Erwinia endophytica*				2						1			3
Halomonadaceae	*Cobetia litoralis/amphilecti*	2	1	8	9	4	2				7	3		36
	Cobetia marina/pacifica			3	4			1		1	5			14
	Halomonas alkaliantarctica						1							1
Oceanospirillaceae	*Marinomonas arenícola*					5		2	2	4		7		20
	Neptunomonas naphthovorans												1	1
Moraxellaceae	*Psychrobacter adeliensis*								1					1
	Psychrobacter aquimaris									3				3
	Psychrobacter celer							3	1					4
	Psychrobacter cibarius								6					6
	Psychrobacter fozii	3	1		2									6
	Psychrobacter nivimaris						3						5	8
Pseudomonadaceae	*Pseudomonas azotoformans*					1								1
Vibrionaceae	*Vibrio metschnikovii*									1				1
	Vibrio splendidus-like group				1									1

[a] Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed).

Table 7
Taxonomical distribution of the 14 Bacteroidetes and 12 Actinobacteria isolates from six species of HPP-treated seaweeds.[a].

Phylum	Family	Genus and species	Start of storage						End of storage						No. of isolates
			CF	UL	HE	LO	UP	CC	CF	UL	HE	LO	UP	CC	
Bacteroidetes	*Flavobacteriaceae*	*Algibacter undariae*				2									2
		Cellulophaga fucicola						2							2
		Dokdonia genika		2											2
		Formosa algae								3					3
		Lacinutrix undariae	1												1
		Maribacter forsetii		1											1
		Mesonia algae	1					1							2
		Olleya namhaensis						1							1
Actinobacteria	*Dietziaceae*	*Dietzia schimae*		1											1
	Microbacteriaceae	*Okibacterium fritillariae*									1				1
		Salinibacterium amurskyense				3									3
	Micrococcaceae	*Kocuria carniphila*												1	1
		Kocuria koreensis						3							3
		Kocuria palustris					2								2
		Micrococcus yunnanensis									1				1

[a] Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed).

bacterial diversity, with only 4 genera represented in their microbiota (Gupta et al., 2010), while 19 genera were represented in the microbiota of one green, one brown and one red seaweeds submitted to a less severe (55 °C/6 min) heat treatment (Alvarado et al., 2018).

Refrigerated storage of HPP-treated seaweeds affected bacterial diversity. At the start of storage, isolates from HPP-treated seaweeds belonged to 12 out of the 13 orders, 21 out of the 23 families, 35 out of the 43 genera and 75 out of the 103 species, whereas at the end of storage only 11 orders, 20 families, 29 genera and 60 species were represented. Storage had a significant ($P < 0.05$) effect on the number of isolates of the *Pseudoalteromonadaceae*, *Psychromonadaceae*, *Halomonadaceae*, *Flavobacteriaceae* and *Enterococcaceae* families in HPP-treated seaweeds, according to the ANOVA. A reduced number of isolates from the first four families were retrieved from HPP-treated seaweeds at the end of storage whereas the number of *Enterococcaceae* isolates at the end of storage greatly increased with respect to the start of storage.

The relative abundances of the different bacterial clades in untreated and HPP-treated seaweeds are shown in Table 9. At the start and end of storage, higher ($P < 0.05$) relative abundances of α-Proteobacteria, γ-Proteobacteria and the CFB group were found in untreated than in HPP-treated seaweeds, whereas Firmicutes was the most abundant group in HPP-treated seaweeds accounting for 66.2% of all bacterial isolates. In untreated seaweeds, γ-Proteobacteria were the most common bacterial clade followed in decreasing order by the CFB group, α-Proteobacteria, Firmicutes and Actinobacteria. These results are in general agreement with those of Hollants et al. (2013), although the relative abundance of γ-Proteobacteria was higher in the present study. Storage only had a significant ($P < 0.05$) effect on the different groups in seaweeds treated at 400 MPa, with higher relative abundances of Firmicutes and lower relative abundances of γ-Proteobacteria, the CFB group and Actinobacteria at the end of storage.

Table 8
Taxonomical distribution of the 335 Firmicutes isolates from six species of HPP-treated seaweeds[a].

Family	Genus and species	Start of storage						End of storage						No. of isolates
		CF	UL	HE	LO	UP	CC	CF	UL	HE	LO	UP	CC	
Bacillaceae	*Bacillus algicola*		4			10			7			9		30
	Bacillus altitudinis	4	4						1					9
	Bacillus amyloliquefaciens				4				1		7			12
	Bacillus aryabhattai					6						3		9
	Bacillus berkeleyi					1			1	1		5		8
	Bacillus butanolivorans		1	2			3			3				9
	Bacillus cereus	3						3						6
	Bacillus clausii	1	1	2										4
	Bacillus composti		1											1
	Bacillus drentensis						3							3
	Bacillus firmus/muralis				2					1	5			8
	Bacillus halosaccharovorans								1					1
	Bacillus hemicentroti	1		1										2
	Bacillus hwajinpoensis		1			5	1		4			5	1	17
	Bacillus idriensis	1		7						3				11
	Bacillus lentus								2					2
	Bacillus licheniformis	5	4					4	4					17
	Bacillus litoralis								1					1
	Bacillus megaterium		1				5						5	11
	Bacillus nanhaiensis/arsenicus										2			2
	Bacillus oceanisediminis	1												1
	Bacillus patagoniensis												1	1
	Bacillus selenatarsenatis	1												1
	Bacillus simplex			2	2				1		4			9
	Bacillus soli	1												1
	Bacillus subterraneus		1											1
	Bacillus thermotolerans								1					1
	Bacillus thioparans			2										2
	Bacillus vietnamensis		1		2		1				5			9
	Bacillus xiamenensis		6						3					9
	Fictibacillus nanhaiensis	1									2			3
	Fictibacillus phosphorivorans									1				1
	Halobacillus litoralis			2										2
	Halobacillus locisalis	2												2
	Halobacillus profundi							1						1
	Halobacillus salinus	2												2
	Halobacillus trueperi						2						1	3
	Halobacillus yeomjeoni					3		1						4
	Lysinibacillus macroides								2					2
	Oceanobacillus chironomi						1							1
	Oceanobacillus profundus		1											1
	Psychrobacillus psychrotolerans						1		1	1				3
Brevibacteriaceae	*Brevibacterium frigoritolerans*						2				2			4
Paenibacillaceae	*Chengkuizengella sediminis*		1											1
	Paenibacillus alba	1												1
	Paenibacillus barengoltzii	1												1
	Paenibacillus fonticola	1												1
	Paenibacillus macquariensis								1					1
	Paenibacillus rhizoplanae		1											1
	Paenibacillus segetis												1	1
Planococcaceae	*Bhargavaea cecembensis*								1					1
	Paenisporosarcina macmurdoensis			1										1
	Paenisporosarcina quisquiliarum						2			1				3
	Solibacillus isronensis									1				1
	Solibacillus silvestris		1											1
	Sporosacina aquimarina							2	3					5
	Sporosarcina koreensis		1											1
	Sporosarcina luteola								2					2
	Sporosarcina saromensis								1					1
XII. Incertae sedis	*Exiguobacterium oxidotolerans*		1		2						1			4
Carnobacteriaceae	*Carnobacterium mobile*								1				4	5
Enterococcaceae	*Enterococcus faecium*			3				17	6	15			11	52
Lactobacillaceae	*Pediococcus pentosaceus*					5			1			12	6	24

[a] Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed).

A PCA carried out on the relative abundances of the different bacterial clades of untreated and HPP-treated seaweeds explained 81.5% of the total variance. Component 1 including α-Proteobacteria, Actinobacteria and the CFB group explained 49.1% of the variance, component 2 including γ-Proteobacteria and Firmicutes (with a negative sign) explained 17.2%, and component 3 including β-Proteobacteria explained 15.3%. When untreated and HPP-treated samples were plotted in the plane defined by the first two components of the PCA, most of the untreated samples were located in the first quadrant or in its near vicinity and most of the HPP-treated samples in the third and fourth quadrants (Fig. 1). The negative contribution of Firmicutes to component 2 explained the location of most HPP-treated samples, with higher

Table 9
Mean relative abundances of the different bacterial groups proposed as bacterial core community in seaweed-bacteria interactions[a] in untreated and HPP-treated (400 or 600 MPa) fresh seaweed samples.

Group	Start of storage			End of storage			Reference percentages[a]
	Untreated	400 MPa	600 MPa	Untreated	400 MPa	600 MPa	
α-Proteobacteria	14.5^{aA}	0.0^{bA}	2.3^{abA}	13.4^{aA}	0.0^{bA}	2.5^{bA}	13.0
β-Proteobacteria	0.4^{aA}	0.0^{aA}	0.0^{aA}	0.0^{aA}	0.0^{aA}	0.0^{aA}	1.0
γ-Proteobacteria	52.9^{aA}	42.1^{abA}	20.1^{bA}	46.5^{aA}	25.1^{abB}	21.3^{bA}	37.0
CFB group[b]	16.0^{aA}	6.0^{bA}	1.9^{bA}	13.2^{aA}	0.0^{bB}	1.6^{bA}	20.0
Actinobacteria	5.4^{aA}	5.0^{aA}	1.0^{aA}	8.8^{aA}	0.7^{bB}	1.7^{abA}	9.0
Firmicutes	10.9^{cA}	46.8^{bB}	74.8^{aA}	18.0^{bA}	74.3^{aA}	72.9^{aA}	10.0

[a,b,c] Values at the same time point followed by a different lowercase letter are significantly ($P < 0.05$) different.
[A,B] Values at two time points followed by a different uppercase letter are significantly ($P < 0.05$) different.
[a] Reference percentages from Hollants et al. (2013).
[b] Cytophaga-Flavobacterium-Bacteroides group.

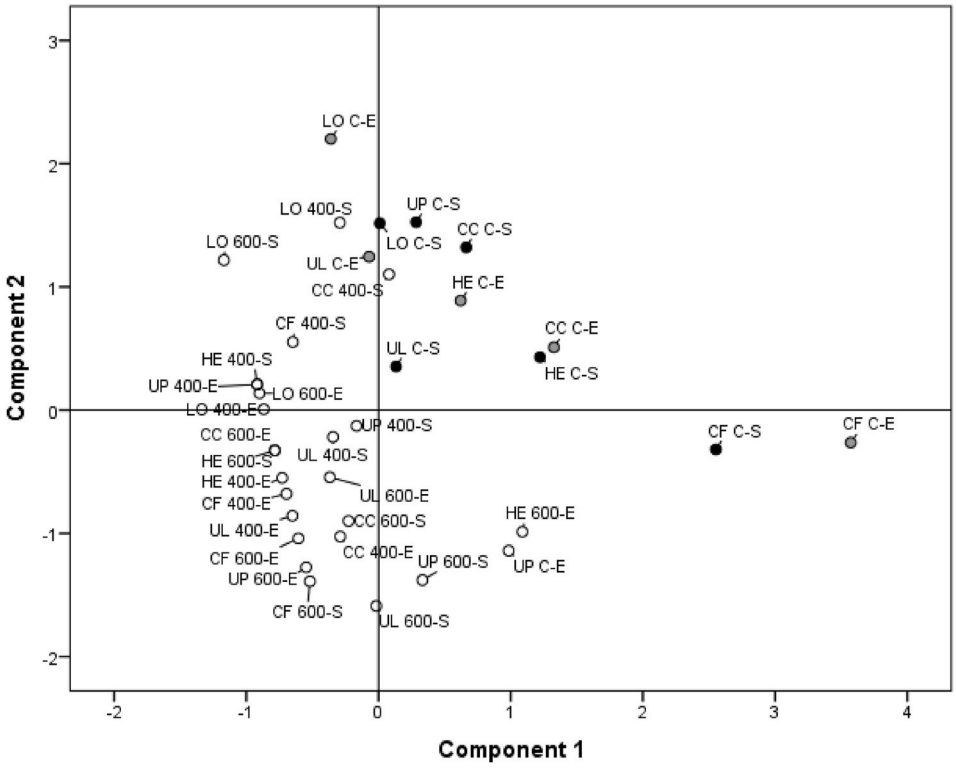

Fig. 1. Distribution of untreated (control) and HPP-treated fresh seaweed samples in the planes defined by components 1 and 2 of the PCA carried out on the relative abundances of the different bacterial groups. Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed). C, Control; 400 or 600, HPP-treated at 400 or 600 MPa; S or E, start or end of storage.

relative abundances of this group, in the third quadrant.

3.3. Main bacterial species isolated from seaweeds

Paracoccus homiensis was the most common α-Proteobacteria species recovered from the seaweed samples. It was found in untreated green, brown and red seaweeds (Table 2), with most isolates retrieved at the start of storage, but not in HPP-treated seaweeds (Table 6). *P. homiensis* was first isolated from a sea-sand sample in South Korea (Kim et al., 2006). Bacteria belonging to the genus *Paracoccus* show high metabolic versatility. One *Paracoccus* sp. isolate with antibacterial activity was retrieved from the brown seaweed *Padina pavonica* (Ismail et al., 2016). The β-Proteobacteria class was poorly represented, with just one isolate of *Janthinobacterium agaricidamnosum*, obtained from untreated UL at the start of storage.

In contrast, several γ-Proteobacteria species were abundant in both untreated and HPP-treated seaweeds (Tables 2 and 6). *Pseudoalteromonas distincta* was isolated at the start of storage from untreated CC and from HPP-treated CF, LO and CC. This species was first isolated from a marine sponge collected near the Komandorskie Islands, Russia, and named *Alteromonas distincta* by Romanenko et al. (1995), but after phylogenetic analysis it was assigned to the genus *Pseudoalteromonas* (Ivanova et al., 2000). Members of this genus possessing algal polysaccharide-degrading activity have been isolated from *Ascophyllum nodosum* and could play a key role in algal biomass recycling (Martin et al., 2015). *Psychromonas aquatilis* was isolated from untreated LO at the start of storage and from HPP-treated UL and LO at the start and end of storage. *P. aquatilis* was first isolated from an Antarctica water sample (Kämpfer et al., 2017). *P. arctica*, its closest relative, has been isolated from brown seaweeds *U. pinnatifida* (Lee et al., 2006) and *Splachnidium rugosum* (Albakosh et al., 2016).

Cobetia litoralis/amphilecti was isolated in high numbers from untreated and HPP-treated samples of all seaweeds (Tables 2 and 6) and *Cobetia marina/pacifica* from untreated samples of four seaweeds and HPP-treated samples of three seaweeds. Most *Cobetia* strains are motile and require salt for growth, with optimal growth in the presence of 5% salt (Arahal et al., 2002). *C. marina* was isolated from marine environments (Arahal et al., 2002), C. *litoralis*, *C. amphilecti* and *C. pacifica* from

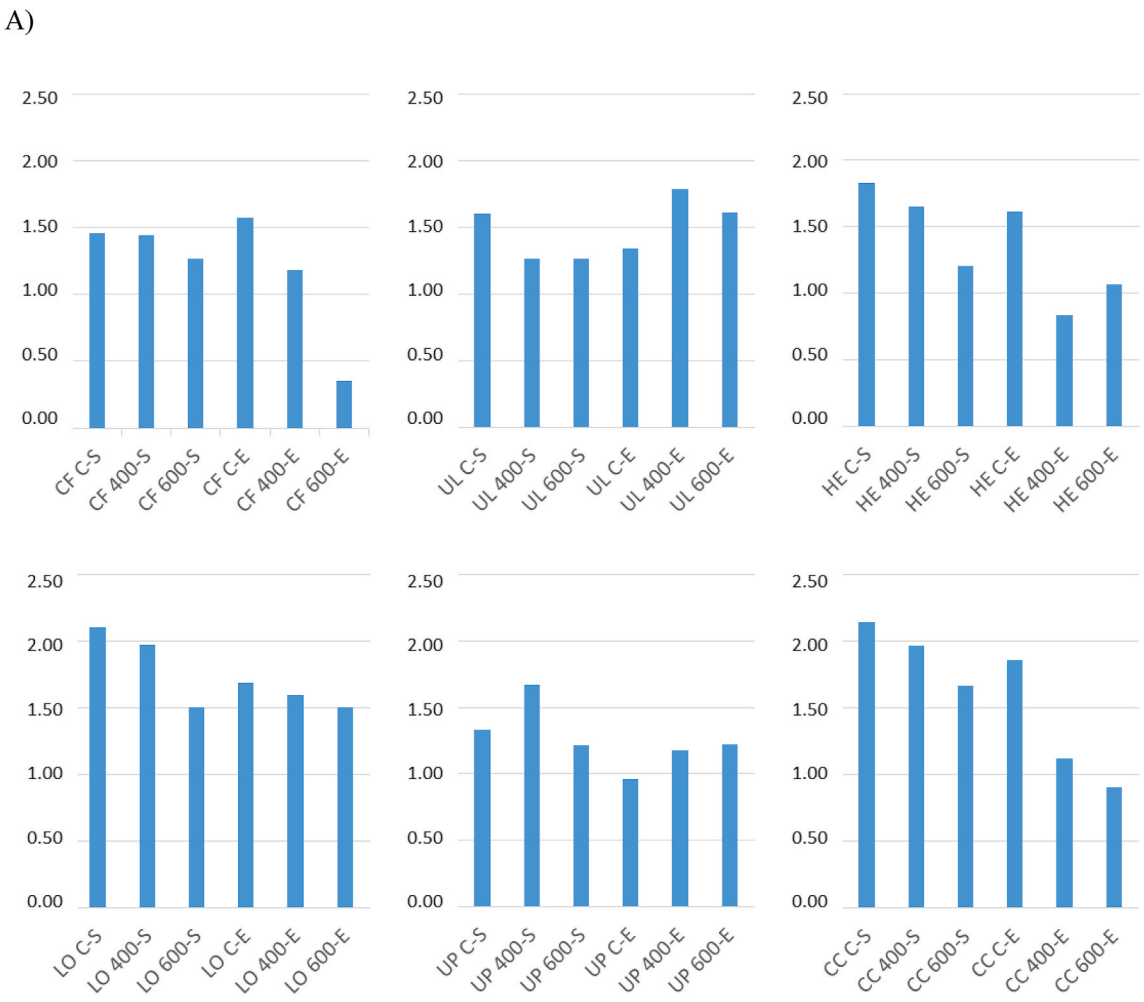

Fig. 2. Diversity indexes for untreated (control) and HPP-treated fresh seaweed samples at the start and end of a cold storage period. A) Menhinick index (I_{MN}) used as a measurement of species richness; B) Shannon-Weaver index (H'), as a measurement of diversity; C) Pielou index (J'), as a measurement of evenness, and D) Simpson index (D), as a measurement of dominance. Seaweeds were CF (*Codium fragile*, green seaweed), UL (*Ulva lactuca*, green seaweed), HE (*Himanthalia elongata*, brown seaweed), LO (*Laminaria ochroleuca*, brown seaweed), UP (*Undaria pinnatifida*, brown seaweed) and CC (*Chondrus crispus*, red seaweed). C, Control; 400 or 600, HPP-treated at 400 or 600 MPa; S or E, start or end of storage.

sandy sediment at the Sea of Japan and ascidian internal tissue, from sponge internal tissues at the Gulf of Alaska, and from sandy sediment at the Sea of Japan, respectively (Romanenko et al., 2013), and *C. amphilecti* from brown seaweed *S. rugosum* (Albakosh et al., 2016). *Cobetia* spp. strains with algal polysaccharide-degrading activity were isolated from *A. nodosum* (Martin et al., 2015).

Marinomonas arenicola was mainly isolated from HPP-treated seaweeds at the end of storage (Table 6). The genus *Marinomonas* consists of Gram-negative motile rods and is widely distributed in marine environments (Romanenko et al., 2009; Sanchez-Amat and Solano, 2005). Strains with algal polysaccharide-degrading activity were isolated from brown seaweed *A. nodosum* (Martin et al., 2015). One *Marinomonas* sp. isolate was highly effective in inducing morphogenesis and growth in green seaweed *U. fasciata* (Singh et al., 2011).

Several *Psychrobacter* species were abundant in untreated and HPP-treated seaweeds (Tables 2 and 6), in particular *P. fozii* and *P. nivimaris,* while *P. piscatori* was only found in untreated CC samples, although at high frequency. This genus consists of Gram-negative, strictly aerobic, chemoheterotrophic, non-motile, cold-adapted, osmotolerant bacteria (Bowman, 2006). Some *Psychrobacter* strains have shown a wide range of enzymatic activities with potential applications in bioremediation or in the food industry (Lasa and Romalde, 2017). *P. fozii* was isolated from sea ice samples collected from Peter the Great Bay of the Sea of Japan (Romanenko et al., 2008). *P. nivimaris*, an aggregate-attached bacterium, was isolated at the southern Atlantic Ocean (Heuchert et al., 2004). *P. piscatorii* was first isolated from a drain in a fish-processing plant (Yumoto et al., 2010). *P. nivimaris* and *P. piscatorii* were associated with the soft coral *Alcyonium digitatum* from the Baltic Sea, with a *P. nivimaris* strain exhibiting antibacterial activity (Pham et al., 2016). This species was also isolated from dehydrated seaweeds UL, CC and *Palmaria palmata* (del Olmo et al., 2018).

Cellulophaga algicola and *C. fucicola* were the most common Bacteroidetes, although *C. algicola* was only retrieved from some untreated seaweeds (Tables 3 and 7). *C. fucicola* was first isolated from *Fucus serratus* L. at Hirsholm island, Denmark (Johansen et al., 1999). It is a Gram-negative, motile by gliding, psychrophilic, halophilic bacterium able to degrade agar, carrageenan, starch and cellulose (Johansen et al., 1999). Members of the *Cellulophaga* genus were associated with brown seaweed *Ascophyllum nodosum* and could play a key role in algal biomass recycling (Martin et al., 2015).

Salinibacterium amurskyense was the most common species of Actinobacteria (Tables 3 and 7), most commonly found in untreated seaweeds at the end of storage. This species was first isolated from seawater samples at Amursky Bay, Russia (Han et al., 2003), and was the

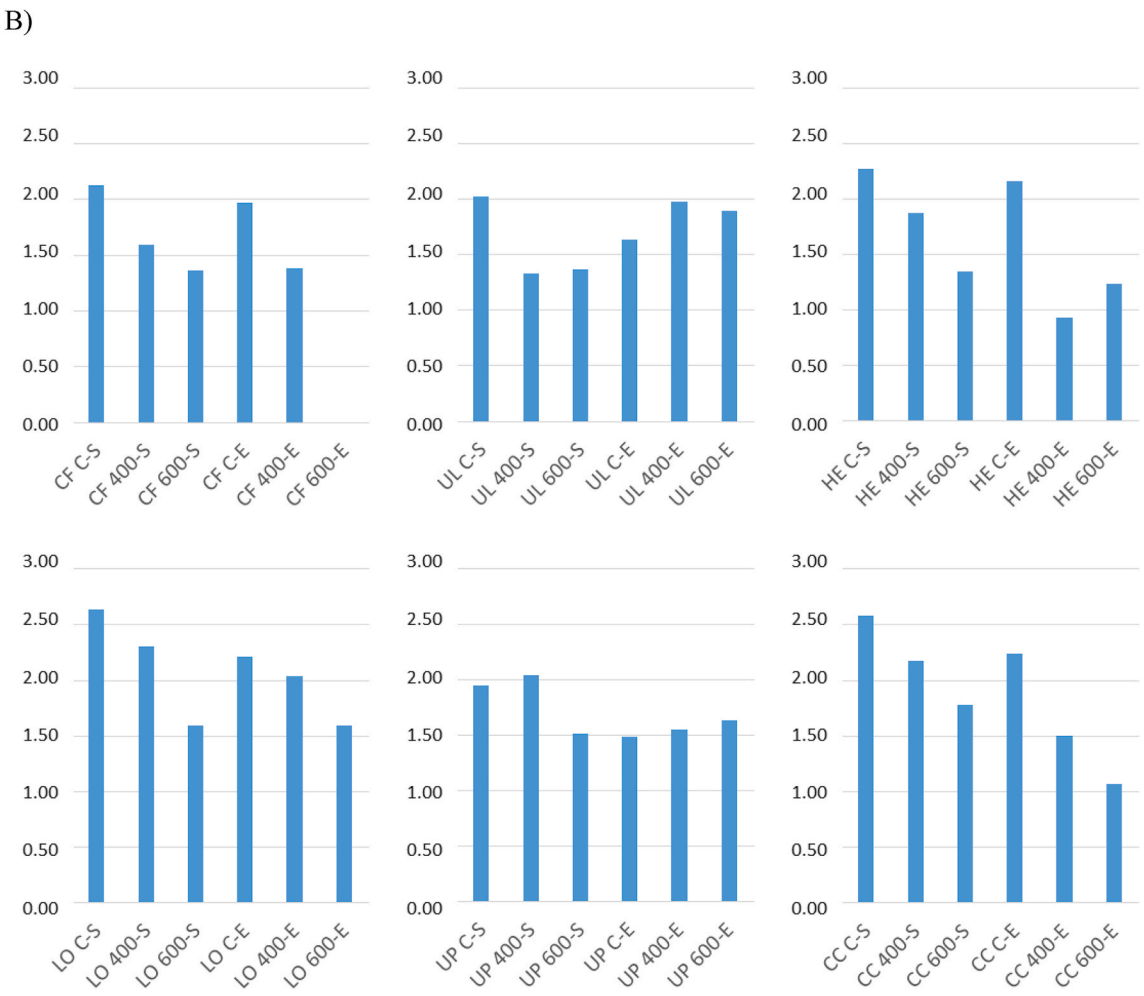

Fig. 2. (*continued*).

first member of the *Microbacteriaceae* family isolated from a marine environment. It is a Gram-positive, non-motile, irregular rod which grows in the presence of NaCl, at mesophilic temperatures and around neutral pH (Han et al., 2003).

Bacillus was the most diverse genus from the phylum Firmicutes with 33 different species, 11 of which were found in both untreated and HPP-treated seaweeds (Tables 4 and 8). A markedly higher number of isolates was retrieved from HPP-treated seaweeds (198) than from untreated seaweeds (25). *Bacillus* species are Gram-positive, aerobic or facultative anaerobic, endospore-forming, rod-shaped bacteria (Claus and Berkeley, 1986), with a wide range of physiological abilities that allow them to live in most natural environments. HPP treatments at 400–600 MPa, which cause high (>4 log) reductions of vegetative bacteria have little effect on *Bacillus* spores (Rendueles et al., 2011), which likely contributed to explain the higher number of *Bacillus* isolates in HPP-treated seaweeds. *B. algicola*, found in untreated and HPP-treated seaweeds (Tables 4 and 8), was first isolated from brown seaweed *Fucus evanescens* and is able to hydrolyze urea, alginate, starch, and gelatin (Ivanova et al., 2004). *B. altitudinis*, also found in untreated and HPP-treated seaweeds (Tables 4 and 8), was isolated from sediment samples collected at the abyssal plains of the Eastern South Atlantic Ocean (da Silva et al., 2013). *B. amyloliquefaciens*, mainly found in untreated and HPP-treated LO samples (Tables 4 and 8), forms extremely pressure-resistant spores (Margosch et al., 2004; Rajan et al., 2006). *B. aryabhattai* was only found in HPP-treated UP samples (Table 8). This species tolerates up to 11.6% NaCl and is resistant to UV radiation (Shivaji et al., 2009). *B. aryabhattai* has also been isolated from deep sea water at the South China Sea (Wen et al., 2015). *B. cereus* was only found in HPP-treated CF samples at start and end of storage (Table 8). This species is widespread in nature and has been frequently isolated from soil and growing plants. It may cause an emetic or a diarrhoeal type of food-associated illness and is considered a foodborne pathogen (Arnesen et al., 2008). This species was also found in dehydrated LO, CC and *Porphyra umbilicalis* (del Olmo et al., 2018). *B. hwajinpoensis* was isolated from both untreated and HPP-treated seaweeds (Tables 4 and 8). This species was first isolated from sea water at the East Sea, Korea (Yoon et al., 2004). Cells are aerobic, non-motile rods which may grow in the presence of up to 19% NaCl, but not without NaCl (Yoon et al., 2004). *B. idriensis*, a species not previously isolated from marine habitats, was mainly recovered from HPP-treated HE samples (Tables 4 and 8). *B. licheniformis*, a species which produces an assortment of extracellular enzymes that may contribute to nutrient cycling in nature (Rey et al., 2004), was mostly isolated from HPP-treated CF and UL samples (Tables 4 and 8). In previous studies, *B. licheniformis* was isolated from *Ulva* spp. and *Gracilaria* spp. (Singh et al., 2011), surimi products (Coton et al., 2011) and dehydrated UL, LO, CC, *Saccharina latissima* and *P. palmata* (del Olmo et al., 2018). Some *B. licheniformis* strains can produce a heat-stable toxin and it has been suggested the species may be a foodborne pathogen (Salkinoja-Salonen et al., 1999). *B. megaterium* was isolated from UL and CC untreated and HPP-treated samples (Tables 4 and 8). This species is found in diverse habitats including soil, seawater, sediment, rice paddies, honey, fish, and dried food, and can grow in simple media on more than 62 out of 95 carbon sources (Vary et al., 2007). Some strains produce heat-stable toxins similar to *B. cereus*

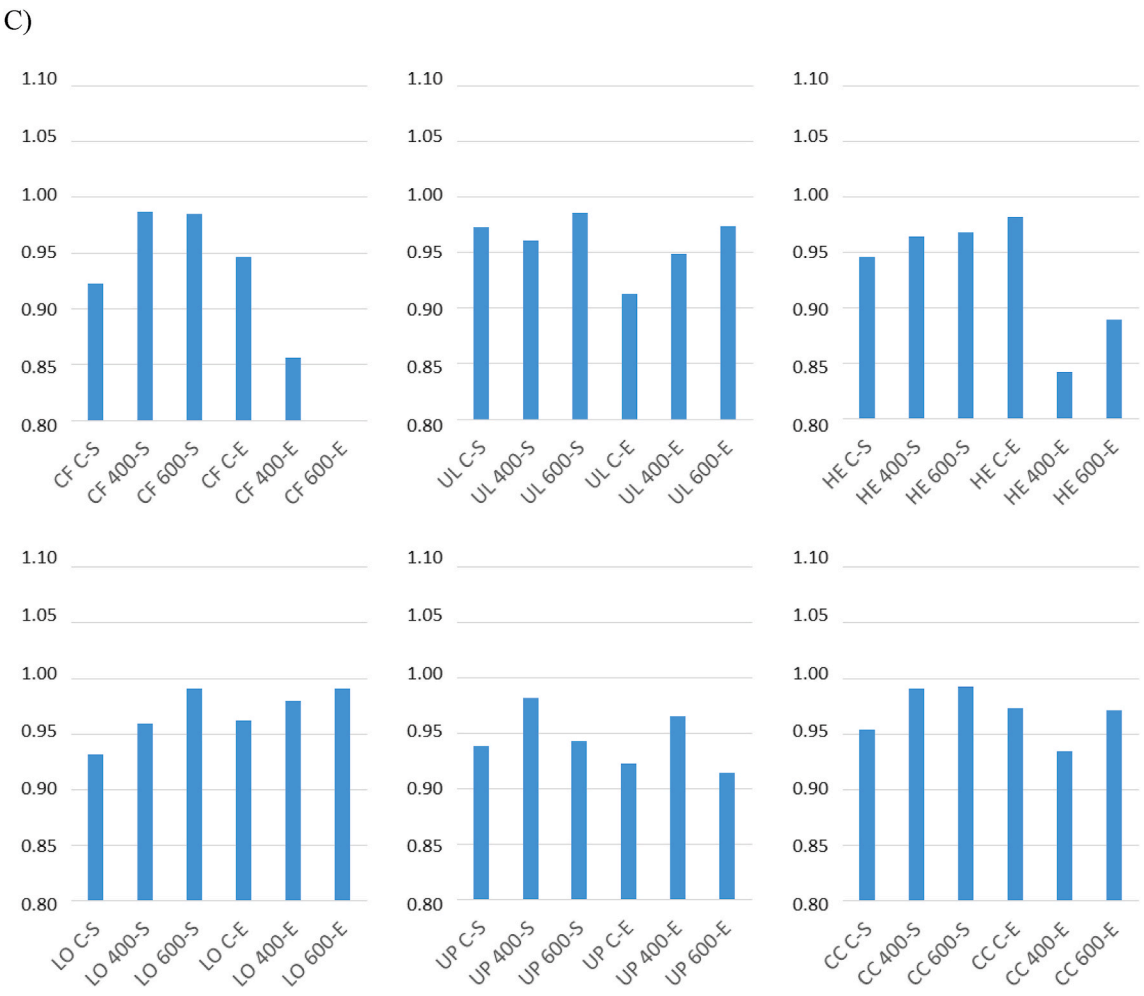

Fig. 2. (*continued*).

emetic toxin (Taylor et al., 2005). *B. megaterium* strains were also found in dehydrated UL, LO, CC, *P. palmata*, *P. umbilicalis* and *S. latissima* (del Olmo et al., 2018). *B. simplex* was isolated from untreated and HPP-treated seaweeds (Tables 4 and 8). One *B. simplex* strain produced a heat-stable toxin, similar in physical characteristics to cereulide (Taylor et al., 2005), which has also led to the suggestion the species may be a foodborne pathogen. *B. simplex* was another one of the three predominant *Bacillus* species isolated from surimi samples (Coton et al., 2011). This species was also isolated from dehydrated LO and *Saccharina latissima* (del Olmo et al., 2018). *B. xiamenensis* was only isolated from HPP-treated UL samples (Table 8). This species was first isolated from the intestinal tract of a flathead mullet at Xiamen Island, China (Lai et al., 2014). It is a motile, rod-shaped bacterium which grows up to 12% NaCl, up to 45 °C, and up to pH 11 (Lai et al., 2014).

Exiguobacterium oxidotolerans was isolated from both untreated and HPP-treated seaweeds (Tables 4 and 8). This genus, which was first described in 1983 (Collins et al., 1983), has been isolated from a wide range of habitats, including Siberian permafrost, Greenland glacial ice, Yellowstone National Park hot springs (Vishnivetskaya et al., 2009). *E. oxidotolerans*, which was first isolated from a drain of a fish processing plant, exhibited 567 times higher catalase activity than *Escherichia coli* (Yumoto et al., 2004). This species was also isolated from dehydrated UL and *P. palmata* (del Olmo et al., 2008).

Enterococcus faecium was isolated from untreated and, particularly, from HPP-treated seaweeds (Tables 4 and 8). The genus *Enterococcus* is present in many different habitats due to its ability to grow under low a_w conditions and within a relatively wide range of temperatures (Franz et al., 1999). High concentrations of enterococci, able to grow on algal leachates, were associated with green seaweed *Cladophora* at Lake Michigan (Byappanahalli et al., 2003). A virulent *E. faecalis* strain showed a great resistance to HPP treatment in dry-cured ham, with a reduction of only 4 log after treatment at 750 MPa for 9.5 min (Belletti et al., 2013). The pressure resistance of enterococci may explain their higher abundance in HPP-treated seaweeds (Tables 4 and 8).

Pediococcus pentosaceus was also isolated from untreated and, particularly, from HPP-treated seaweeds (Tables 4 and 8). A strain of *P. damnosus*, isolated from the spoilage microbiota of HPP-treated gilthead seabream, showed a considerable resistance to pressure, with a reduction of only 1.8 log after 10 min at 450 MPa (Panagou et al., 2007). As in the case of enterococci, the pressure resistance of pediococci may explain their higher abundance in HPP-treated seaweeds (Tables 4 and 8).

Some of the bacterial species isolated from seaweeds in the present study, such as *B. cereus*, *B. licheniformis* and *B. simplex*, may cause food-related illnesses. Other species, such as *Serratia liquefaciens*, *Vibrio diabolicus*, *V. metschnikovii* and *V. splendidus*, have been reported as opportunistic human pathogens or as animal pathogens. Therefore, precautions in the consumption of raw seaweeds should be taken in the case of at-risk populations.

3.4. Biodiversity analysis

Species richness in untreated seaweeds, evaluated through the Menhinick index (Fig. 2A) once singletons were removed, ranged from

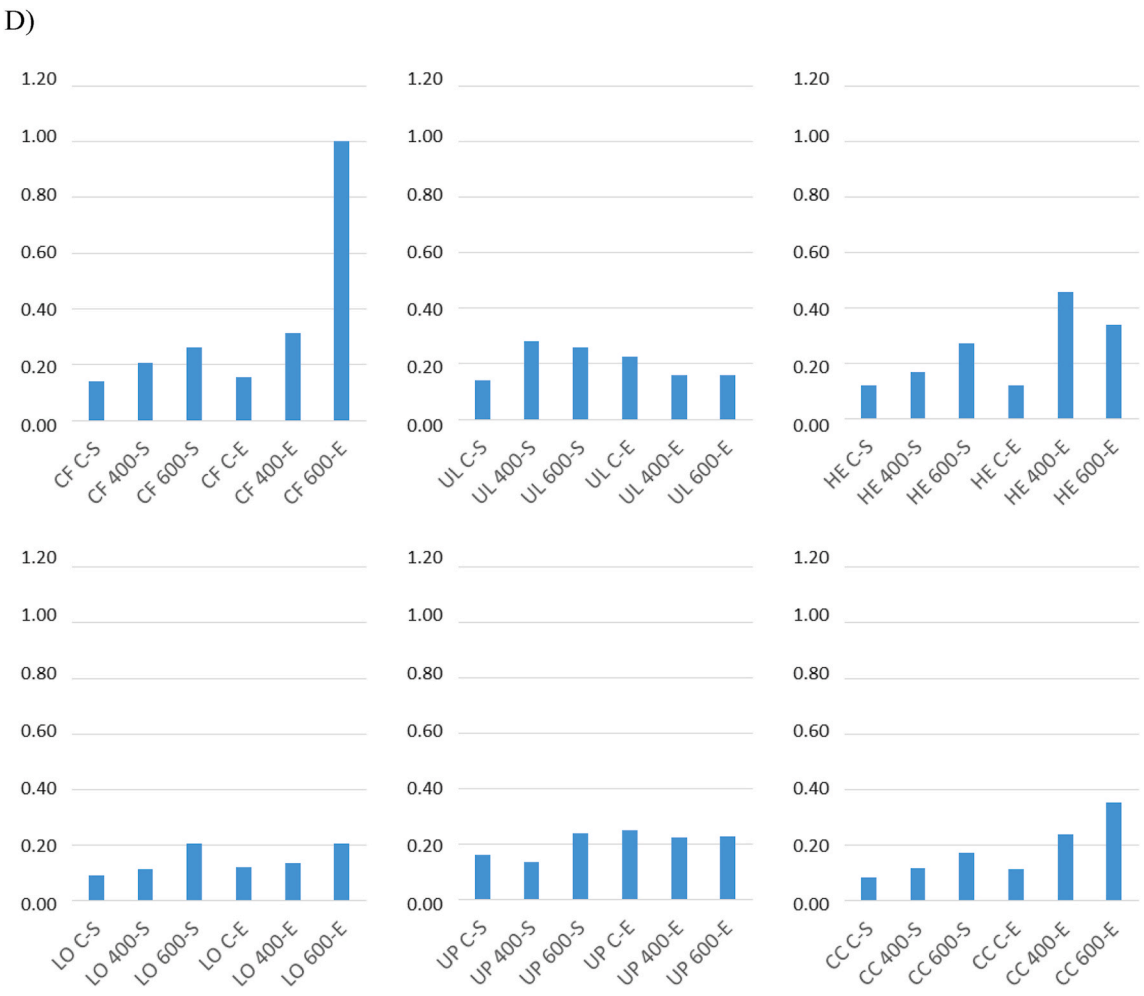

Fig. 2. (*continued*).

1.33 for UP to 2.14 for CC at the start of storage, and from 1.06 for UP to 1.86 for CC at the end of storage. Menhinick index values for seaweed microbiota were higher than those reported for table olives microbiota during fermentation, which ranged from 0.65 to 1.10 (Lucena-Padrós et al., 2014). Species richness decreased through refrigerated storage for all seaweeds. HPP treatments also decreased species richness, the reduction being more intense for the 600 MPa treatment.

Bacterial diversity in untreated seaweeds, evaluated through the Shannon-Weaver index (Fig. 2B), ranged from 1.95 for UP to 2.64 for LO at the start of storage, and from 1.04 for UP to 2.24 for CC at the end of storage. These values were higher than the 0.60–0.90 values recorded for table olives microbiota (Lucena-Padrós et al., 2014), but lower than the 5.14 and 5.67 values obtained for two brown seaweeds of the genus *Lobophora* by high troughput sequencing (Vieira et al., 2016). Bacterial diversity decreased from the start to the end of refrigerated storage. HPP treatments also lowered the bacterial diversity, although an increase in bacterial diversity at the end of storage was observed for UL and UP, with levels higher than in the respective untreated samples.

Evenness in the distribution of species in untreated seaweeds, measured by Pielou index (Fig. 2C), was very similar for all seaweeds at both time points. Values ranged from 0.92 for CF to 0.97 for UL at the start of storage, and from 0.91 for UL to 0.98 for HE at the end of storage. Pielou index values for seaweeds were higher than the 0.50–0.80 values obtained for table olives microbiota (Lucena-Padrós et al., 2014). In HPP-treated seaweeds, values in most cases increased with respect to untreated seaweeds at the start of the storage, but lower values were recorded for CF and HE.

Dominance, measured by Simpson reciprocal index (Fig. 2D), ranged from 0.09 for LO to 0.16 for UP at the start of storage of untreated seaweeds, considerably lower than the 1.60–2.30 values reported for table olives microbiota (Lucena-Padrós et al., 2014). At the end of storage, dominance increased for all untreated seaweeds to values ranging from 0.11 to 0.38, with no change observed in the order of seaweeds. HPP treatments increased dominance values for most of the seaweeds, with more marked increases at the end than at the start of storage.

4. Conclusions

High bacterial diversity was observed among the 1,029 isolates obtained from the six species of edible seaweeds considered in the present study. Isolates from untreated seaweeds belonged to 18 orders, 35 families, 71 genera and 135 species. Many of those species had not been previously reported as forming part of seaweed microbiota. HPP treatments favoured the relative abundance of Firmicutes and decreased the relative abundance of other three phyla, resulting in a considerable reduction of seaweed bacterial diversity. The number of orders, families, genera and species represented by bacterial isolates was lowered by HPP treatments with respect to untreated seaweeds. Refrigerated storage of both untreated and HPP-treated seaweeds also reduced their bacterial diversity at the order, family, genus and species level. Biodiversity analysis by means of different indexes highlighted the richness in bacterial species found in edible seaweeds.

Funding

This study was supported by project AGL 2013-42911-R (Ministerio de Economía y Competitividad, Spain).

Declaration of competing interest

Antonia Picon, on behalf of all authors of the manuscript 'Bacterial diversity in six especies of fresh edible seaweeds submitted to high pressure processing and long-term refrigerated storage', declares that there is no conflict of interest.

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