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Comparative genomics of *Photobacterium* species from terrestrial and marine habitats

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ABSTRACT

Photobacterium (*P.*) is a genus widely studied in regards to its association with and ubiquitous presence in marine environments. However, certain species (*P. phosphoreum, P. carnosum, P. iliopiscarium*) have been recently described to colonize and spoil raw meats without a marine link. We have studied 27 strains from meat as well as 26 strains from marine environments in order to probe for intraspecies marine/terrestrial subpopulations and identify distinct genomic features acquired by environmental adaptation. We have conducted phylogenetic analysis (MLSA, ANI, *fur*, codon usage), search of plasmids (plasmidSPADES), phages (PHASTER), CRISPR-cas operons (CRISPR-finder) and secondary metabolites gene clusters (antiSMASH, BAGEL), in addition to a targeted gene search for specific pathways (e.g. TCA cycle, pentose phosphate, respiratory chain) and elements relevant for growth, adaptation and competition (substrate utilization, motility, bioluminescence, sodium and iron transport). *P. carnosum* appears as a conserved single clade, with one isolate from MAP fish clustering apart that doesn't, however, show distinct features that could indicate different adaptation. The species harbors genes for a wide carbon source utilization (glycogen/starch, maltose, pullulan, fucose) for colonization of diverse niches in its genome. *P. phosphoreum* is represented by two different clades on the phylogenetic analyses not correlating to their origin or distribution of other features analyzed that can be divided into two novel subspecies based on genome-wide values. A more diverse antimicrobial activity (sactipeptides, microcins), production of secondary metabolites (siderophores and arylpolyenes), stress response and adaptation (bioluminescence, sodium transporters, catalase, high affinity for oxygen cytochrome cbb3 oxidase, DMSO reductase and proton translocating NADH dehydrogenase) is predicted compared to the other species. *P. iliopiscarium* was divided into two clades based on source of isolation correlating with phylogeny and distribution of several traits. The species shows traits common to the other two species, similar carbon utilization/transport gene conservation as *P. carnosum* for the meat-isolated strains, and predicted utilization of marine-common DMSO and flagellar cluster for the sea-isolated strains. Results additionally suggest that photobacteria are highly prone to horizontal acquisition/loss of genetic material and genetic transduction, and that it might be a strategy for increasing the frequency of strain- or species-specific features that offers a growth/competition advantage.

Introduction

Photobacterium has originally been a genus closely related to marine environments, with members ranging from fish and seafood spoilers (e.g. *P. phosphoreum* and *P. iliopiscarium*) (Ast and Dunlap, 2005; Dalgaard et al., 1997; Takahashi et al., 2015), pathogens (e.g. *P. damselae*), symbionts (e.g. *P. kishitanii*) and free-living bacteria (e.g. *P. angustum*) (Labella et al., 2017; Urbanczyk et al., 2010). Members of the genus are gram-negative, facultatively aerobic and mostly psychro- and halophilic, and motile (Urbanczyk et al., 2010).

In the food industry, the species *P. phosphoreum* and *P. iliopiscarium* are closely monitored on fish and seafood as they represent potent spoilers and a health risk. Both are reported as common and abundant fish spoilers (Dalgaard et al., 1998) responsible for the production of foul odors and biogenic amines, such as histamine (Bjornsdottir-Butler et al., 2018; Bjornsdottir et al., 2009; Emborg et al., 2002; Torido et al., 2012), whose presence leads to scombroid fish poisoning upon consumption (Lehane and Olley, 2000).

However, their presence is not exclusive of aquatic areas, as they have also been reported on raw meat. *P. phosphoreum, P. carnosum,*

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originally isolated from poultry meat (Hilgarth et al., 2018b), and to a lesser extent *P. iliopiscarium*, are considered as common meat spoilers due to their ubiquitous presence on meat, their ability to grow to relevant numbers, and their predicted potential to spoil it (Fuertes-Perez et al., 2019; Höll et al., 2019). These species have been reported on culture indepentent studies on air and vacuum packaged beef (Pennacchia et al., 2011), modified atmosphere packaged (MAP) minced beef (Stoops et al., 2015), pork sausages (Bouju-Albert et al., 2018) and dry-fermented sausages (Pini et al., 2020). Complementary to these studies, culture depent studies have also reported on both detection and isolation of *Photobacterium* species on poultry, beef, turkey and pork, independently of type of packaging atmosphere and marinate (Fuertes-Perez et al., 2019; Hilgarth et al., 2018a, 2018b; Nieminen et al., 2016). Further studies have established that despite their widespread presence on raw meat, in several countries and in high numbers, they are not in all packages of the same batch, and might even follow some seasonal pattern (Fuertes-Perez et al., 2019), making their detection and recovery a fastidious task, even with targeted isolation methods (Fuertes-Perez et al., 2020; Hilgarth et al., 2018a).

A previous study has revealed high diversity within these *Photobacterium* species, and suggested a correlation between the source of isolation and phenotypic characteristics (Fuertes-Perez et al., 2019). High diversity within the genus was also reported by a previous study on comparative genomics of marine photobacteria where 16 of the 28 available *Photobacterium* species were included, and the authors report the presence of traits that appear linked to the lifestyle of the species (Machado and Gram, 2017).

The metabolism of *P. phosphoreum* as a model species for photobacteria has been previously described by Höll et al. (2019) in a metatranscriptomics analysis from meat. Photobacteria on meat are predicted to have little regard for the atmosphere used for packaging. The study also reported versatility for these bacteria, predicted to use a variety of carbon sources such as common sugars (e.g. glucose, ribose), amino acids and lipids (e.g. glycerol) and produce spoilage products such as biogenic amines. However, differences on species and strain level could not be resolved in that study.

Studies have been published previously on the genomics of photobacteria, either on a limited number of strains of several species of the genus (Machado and Gram, 2017; Urbanczyk et al., 2010), focused on strains of a specific species (Roslan et al., 2020; Yu et al., 2019) or certain relevant traits such as piezophilic adaptation (Allen and Bartlett, 2000; Campanaro et al., 2005; Hauschild et al., 2020), salt adaptation (Wu et al., 2006), lux-rib operon (Urbanczyk et al., 2008, 2012) and motility (Eloe et al., 2008). Since *P. phosphoreum, P. iliopisicarum* and *P. carnosum* are the first species widely found in a completely aquatic-unrelated environment, this study aimed to explore the genomic diversity based on terrestrial versus aquatic isolates and between the species.

Materials and methods

DNA extraction and sequencing

In total, 27 strains of the species *Photobacterium phosphoreum* (10 strains), *P. iliopiscarium* (2 strains) and *P. carnosum* (13 strains) were inoculated in marine broth (MB) supplemented with 3 g/L meat extract, and grown overnight at 15 °C. From each of the grown cultures, 5 ml were used to perform a DNA extraction with the E.Z.N.A. Bacterial DNA kit (OMEGA, Bio-Rad) with modifications in the protocol according to Fuertes-Perez et al. (2020).

Whole genome sequencing was performed on the obtained high quality DNA with Illumina HiSeq technology by Eurofins (Germany). Assembly of the genomes was performed by SPAdes (Bankevich et al., 2012). The DNA sequences were annotated by NCBI under Bioproject ID PRJNA590348.

Additionally, all the available genomes (as of August 2021) in NCBI database of the three species were retrieved and added to this study. Table S1 shows the strain denomination, source and country of isolation, origin of the genome sequence and the WGS accession number of all isolates of *P. carnosum* (15 strains), *P. phosphoreum* (26 strains) and *P. iliopiscarium* (6 strains) used in this study.

Annotation and gene search

The annotation was performed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Angiuoli et al., 2008) under the aforementioned Bioproject ID for those not yet uploaded. In order to increase the accuracy of the annotation, the genomes were additionally annotated with Rapid Annotation Subsystem Technology (RAST) server (Aziz et al., 2008), TIGR annotation (Ouyang et al., 2007) and the NCBI subcellular localization. The data obtained from different annotations was connected using PERSEUS (Tyanova et al., 2016), and used for the search of specific genes and metabolic pathways. Identities of the genes of interest were additionally manually curated using BLAST (https://blast.ncbi.nlm.nih.gov) (Altschul et al., 1990). Accession numbers for the genes searched in this study can be found in the table S2.

Extraction of the pan-, core- and accessory genome of each species was achieved with the BlAst Diagnostic Gene findEr (BADGE) (Behr et al., 2016), with default settings, but adjusting the "megablast percent identity cut" and "megablast within group qscov" to 95/0.95 for analysis within a species, and 85/0.85 for the analysis between species. Its output was used to obtain the graphic representation by species with Blast Ring Image Generator (BRIG) (Alikhan et al., 2011), using the annotated ORFs of the pan-genome of each species as reference.

General genome statistics

The shotgun whole genome sequences of the strains used in this study were analyzed with CMG biotools (Vesth et al., 2013). General statistics such as genome size, GC content and codon usage were obtained using the aforementioned tools. They were additionally used for the generation of pan- and core-genome graphs included in this study.

Phylogenetic analysis

Based on the annotation performed as detailed in the previous section, several housekeeping gene sequences were extracted to construct an MLSA based tree. Used genes include the DNA gyrase subunit B (*gyr*B), RNA polymerase sigma factor (*rpo*D), protein recombinase A (*rec*A), DNA-directed RNA polymerase subunit alpha (*rpo*A), cell division protein (*fts*Z) and cell-shape determining protein (*mre*B). The accession number of the housekeeping genes used are displayed in table S3. Alignments were performed by ClustalW (Thompson et al., 1994) and dendrograms constructed using the maximum likelihood algorithm (Felsenstein, 1981) and Tamura-Nei model (Tamura and Nei, 1993), using MEGA v7 software (Kumar et al., 2016) and tested with 1000 bootstrap replications. Additionally, we also constructed a phylogenetic tree based on the *fur* gene, with the maximum likelihood and Tamura-Nei model, and tested with 1000 bootstrap replications.

The software JSpecies (Richter and Rossello-Mora, 2009) was used to calculate the average nucleotide identity (ANI) of all the strains of each species, with pairwise genome comparison of the whole genome shotgun sequences by means of the ANIb algorithm (Goris et al., 2007).

The Genome-to-Genome Distance Calculator by DSMZ (Meier--Kolthoff et al., 2013, 2014) was used to determine and evaluate presence of possible subspecies within each of the three species of photobacteria included in this study.

Plasmid search

Plasmid presence was predicted for genomes of self-isolated strains with the plasmidSPAdes algorithm (Antipov et al., 2016). The sequences

were compared and annotation was extracted to determine if specific additional functions were granted by presence of said plasmids.

In addition, previously described *Photobacterium* plasmids were blasted against all the contigs of the strains included in this study using CLC Main Workbench (QIAGEN). Compared plasmids include: pPHDD1 (FN597600.2), pAQU1 (AB571865.1), pP99–018 (AB277723.1), pP91278 (AB277724.1), pPHDP60 (KC344732.1), pPHDP10 (DQ069059.1), pPHDP70 (KP100338.1), pP9014 (AB453229.1), pPH1 (AY789019.1), pPBPR1 (CR377818.1), and Gung47 (KC687076.1) from *P. gaetbulicola*, as previously performed for several species by Machado and Gram (2017).

Additional online tools

Secondary metabolites were identified by submitting the entire genomes to the online tool antiSMASH 5.0 (Blin et al., 2019; Medema et al., 2011), and the resulting sequences and clusters were compared to each other with BLAST.

In addition to antiSMASH, that also allows the detection of bacteriocin related gene clusters, the genomes were submitted to BAGEL4 (de Jong et al., 2006) in order to identify bacteriocins-related gene clusters and ribosomally synthesized and post-translationaly modified peptides (RiPPs).

PHASTER (Arndt et al., 2016; Zhou et al., 2011) was utilized to search and identify prophages in all the genomes included in this study. The resulting sequences were also compared using BLAST and grouped by similarity with a cuttoff of 70/70 identity/coverage percentage.

The contigs of each of the strains of the three species of photobacteria were analyzed with CRISPRFinder (Grissa et al., 2007). The direct repeats (DR) and protospacers of each of the CRISPR-Cas systems identified were compared using BLAST to each other and to the sequences of identified prophages. Identical spacers were grouped together in order to establish phylogenetic relationships between strains.

Results

Phylogenic and taxonomic analyses

We have used an MLSA based analysis of six housekeeping genes up to 4952 bp of total concatenated length (Fig. 1A) and the average nucleotide identity (ANI) (Fig. 1B), for comparison of the phylogenetic relationships.

One available genome of *P. iliopiscarium* (NCIMB 13355) and six of *P. phosphoreum* (JCM 21184, FS-2.3, FS-6.2, FS-6.3, GCSL-P60, GCSL-P64) were found to be clones of other available genomes in the database (>99.9% ANI similarity) and therefore removed from the analysis. Accession numbers for those genomes are also included in table S1.

Results for the MLSA and ANI values appear to be similar (pairwise ANI values comparison can be found in table S4). Both approaches show a clear differentiation of the three species. Whitin the *P. phosphoreum* and *P. iliopiscarium* species, there is a separation in two main cluster that remain constant through the analyses. Although not by source of isolation, strains AK-4, AK-5, AK-8, FS-6.1 and FS-3.2, all isolated from marine environment, from *P. phosphoreum* are consistently separated from the rest of the strains of the species with ANI values of ~95–96% to the rest of the strains, close to the species demarcation values. *In silico* DDH values obtained from the DMSZ genome-to-genome distance calculator lie between 68 and 76%, below the proposed threshold for delineation of a subspecies (<79%). Therefore, the five strains of *P. phosphoreum* forming an external cluster appear to constitute a new subspecies within the species of photobacteria.

In the case of *P. iliopiscarium*, however, the separation of strains TMW

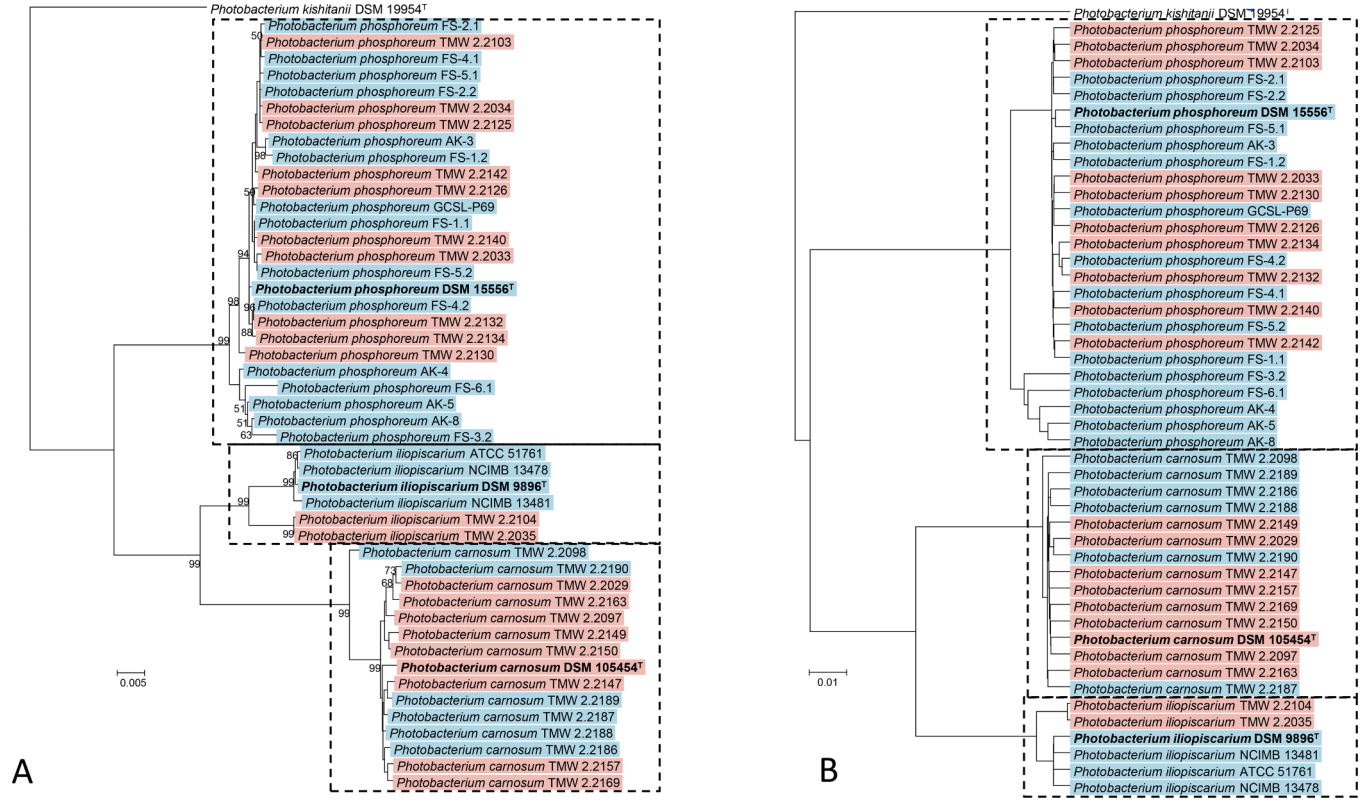

Fig. 1. A. MLSA phylogenetic tree (=4952 bp) based on the Maximum likelihood algorithm and Tamura-Nei model tested with 1000 bootstrap replications. Bootstrap values equal to or above 50 are shown. Concatenated genes include *gyr*B (=1209 bp), *rpo*D (=855 bp), *rec*A (=585 bp), *rpo*A (=927 bp), *fts*Z (=619 bp), *mre*B (=757 bp) in that order. B. ANI pairwise values based dendrogram. Both clusterings show differentiation by species marked by discontinuous line, and distinction between meat-isolated (red) and fish-isolated (blue) strains of each species. Type strains of each species are shown in bold letters and marked with a ^{T}.

2.2104 and TMW 2.2035 does correlate to the distinct source of isolation based on meat or fish/aquatic origin. Although no separation of isolates is observed in *P. carnosum*, one single salmon-isolated strain (TMW 2.2098) does cluster appart from the rest in both analysis.

We additionally tested the validity of the *fur* gene as identification marker for the three species of photobacteria (Figure S1), proposed by Machado and Gram (2015). The resulting phylogenetic tree is consistent with the clustering obtained from the MLSA and ANI values, supporting the subpopulations observed for *P. phosphoreum* and *P. iliopiscarium*, but not *P. carnosum* outlier strain.

Finally, we clustered the isolates based on codon usage (Figure S2). Differences in codon usage are low, and although delineation of species is conserved, clustering of strains is not supported by any of the other analysis. *P. phosphoreum* and *P. carnosum* show each two distinct clusters containing strains of mixed sources of isolation, while *P. iliopiscarium* shows one single strain isolated from meat (TMW 2.2035) as outlier.

Genome characteristics

We calculated an average genome size and GC content of 4.24 Mbp and 38.65%, 4.33 Mbp and 39.01%, 4.66 Mbp and 39.46% for *P. carnosum, P. iliopiscarium* and *P. phosphoreum* species, respectively. The representation of GC% content and genome size for all isolates is displayed in Figure S3.

Statistical analysis ($p < 0.05$) revealed significant variability between the GC content of the three species, and between the genome size of *P. phosphoreum* to the other two species. Additionally, we found significant differences between *P. phosphoreum* strains isolated from fish (4.58 Mbp and 39.53% GC), and those isolated from meat (4.79 Mbp and 39.35% GC), but not for the other two species.

The resulting pan-genome of the species varies between 12,699 annotated genes and 11.6 Mbp for *P. phosphoreum*, 6158 annotated genes and 5.7 Mbp from *P. iliopiscarium* and 8410 annotated genes and 7.7 Mbp from *P. carnosum*. The statistics for each of the genomes analyzed can be found in table S5.

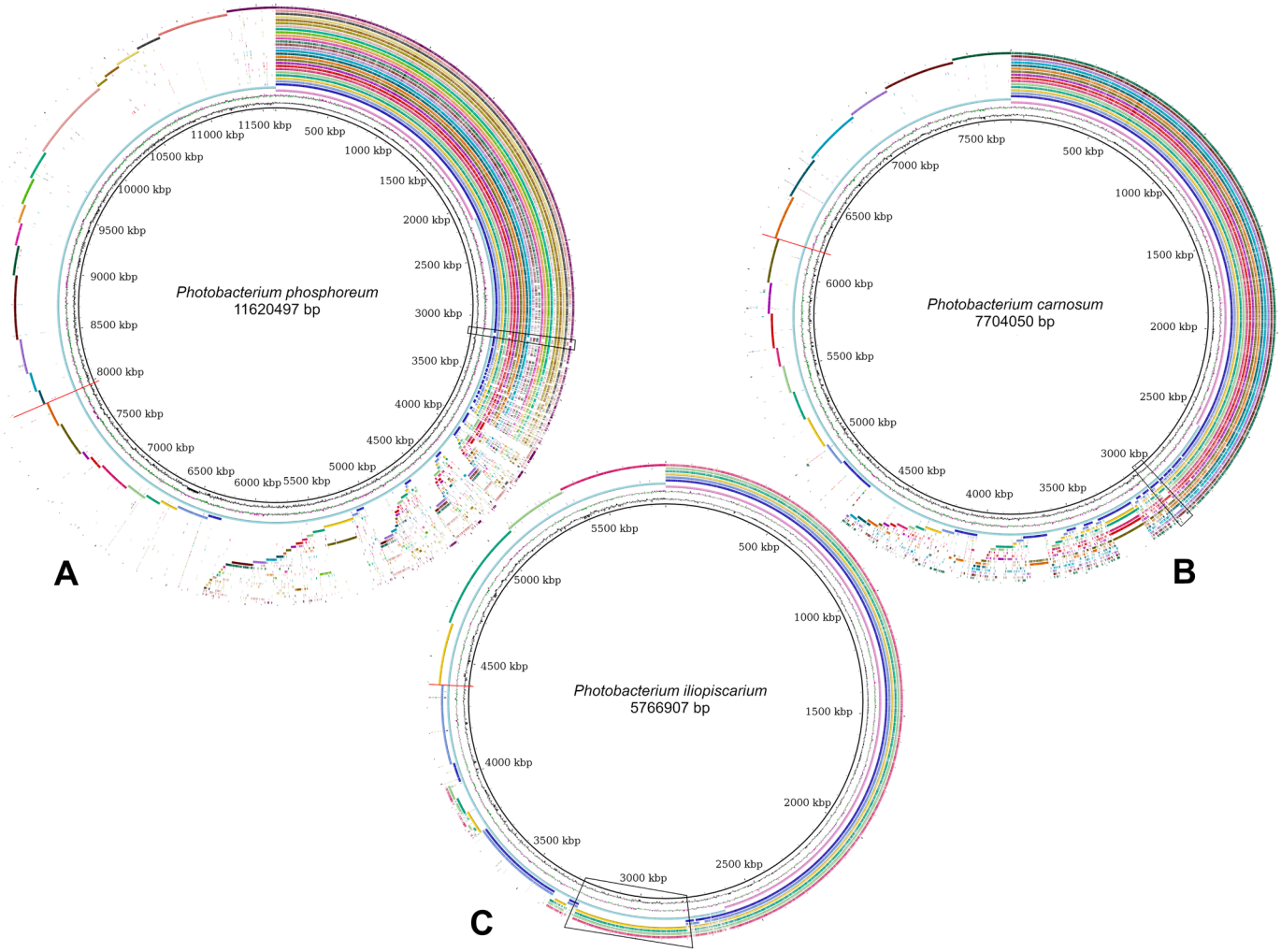

Fig. 2. Representation of the genomes of all strains of each species using the pan-genome of the species as reference, created with BRIG and using a cutoff of 90/90 identity/coverage. Inner pink line represents the core genome of the species, while the following blue line represents the accessory genome of each species. The genome of each strain is represented by a differently colored ring following the former two. **A** *P. phosphoreum*: ■ core genome, ■ accessory genome; and strains: ■ TMW 2.2033; ■ TMW 2.2034; ■ TMW 2.2103; ■ TMW 2.2125; ■ TMW 2.2126; ■ TMW 2.2130; ■ TMW 2.2132; ■ TMW 2.2134; ■ TMW 2.2140; ■ TMW 2.2142; ■ **DSM 15556^{T}**; ■ AK-3; ■ AK-4; ■ AK-5; ■ AK-8; ■ FS-1.1; ■ FS-1.2; ■ FS-2.1; ■ FS-2.2; ■ FS-3.2; ■ FS-4.1; ■ FS-4.2; ■ FS-5.1; ■ FS-5.2; ■ FS-6.1; ■ GCSL-P69. **B** *P. carnosum*: ■ core genome, ■ accessory genome; and strains: ■ **DSM 105454^{T}**; ■ TMW 2.2029; ■ TMW 2.2097; ■ TMW 2.2147; ■ TMW 2.2149; ■ TMW 2.2150; ■ TMW 2.2157; ■ TMW 2.2163; ■ TMW 2.2169; ■ TMW 2.2098; ■ TMW 2.2186; ■ TMW 2.2187; ■ TMW 2.2188; ■ TMW 2.2189; ■ TMW 2.2190. **C** *P. iliopiscarium*: ■ core genome, ■ accessory genome; and strains: ■ TMW 2.2035; ■ TMW 2.2104; ■ **DSM 9896^{T}**; ■ ATCC 51761; ■ NCIMB 13478; ■ NCIMB 13481. The red line divides for each species the strain-specific genome of the meat-isolated (before the line) strains and the fish-isolated strains (after the line). The black box represents the cluster of genes extracted with known function.

Figure S4 and Fig. 2 show the variation of pan- and core-genome sizes with each new strain included in the analysis and the BRIG overlapping representation of the pan-, core- and accesory genome of each species, respectively. The size of the core-genome is stabilized at ~3 Mbp and already 2 to 3 strains appear enough to cover its diversity within each species.

Out of all the annotated genes of each species, 17.7% (2248 genes) in *P. phosphoreum*, 44.3% (2731 genes) in *P. iliopiscarium* and 32.0% (2694 genes) in *P. carnosum* represent the core genome of the species respectively. Between 30 and 50% of the total genes of a species are grouped within the strain-specific part of the accessory genome.

The annotation reveals that in each case the 33–48% of the total genes in the genome are only predicted proteins with no assigned or unknown function (Figure S5). These hypothetical proteins appear mainly distributed across the strain specific genome of each strain (47–57% of the total strain specific gene pool). The three species also accumulate multiple mobile elements (e.g. transposases) that can sum up to 2–3% of the total genome content.

BADGE and BRIG based untargeted gene search

Using the BRIG representation as reference, we identified clusters of genes only shared by a fraction of the strains of each species. The flagellar cluster was the main function present in those regions (black box in Fig. 2). Its distribution correlates to the source of isolation and phylogenetic analysis in the case of *P. iliopiscarium*, being only present in fish-borne strains. Presence of the flagellar cluster in the other two species is not tied to source of isolation or clades identified in the MLSA analysis, and it appears mostly random. All members of the proposed subspecies of *P. phosphoreum* contain the intact flagellar cluster.

We additionally found that fish/marine strains of *P. iliopiscarium* contain in their genome several genes belonging to a type IV secretion system (black box Fig. 2C), not present in any of the meat-borne strains. Remaining regions of group specific genes are mostly hypothetical proteins or mobile elements, but we identified no additional growth, survival or adaptation predicted advantages.

Targeted gene search

The summary of present/absent pathways and genes is displayed in Fig. 3 and table S6 and focused on the main differences between species and strains since overall metabolism of the three species has already been described by Hilgarth (2018).

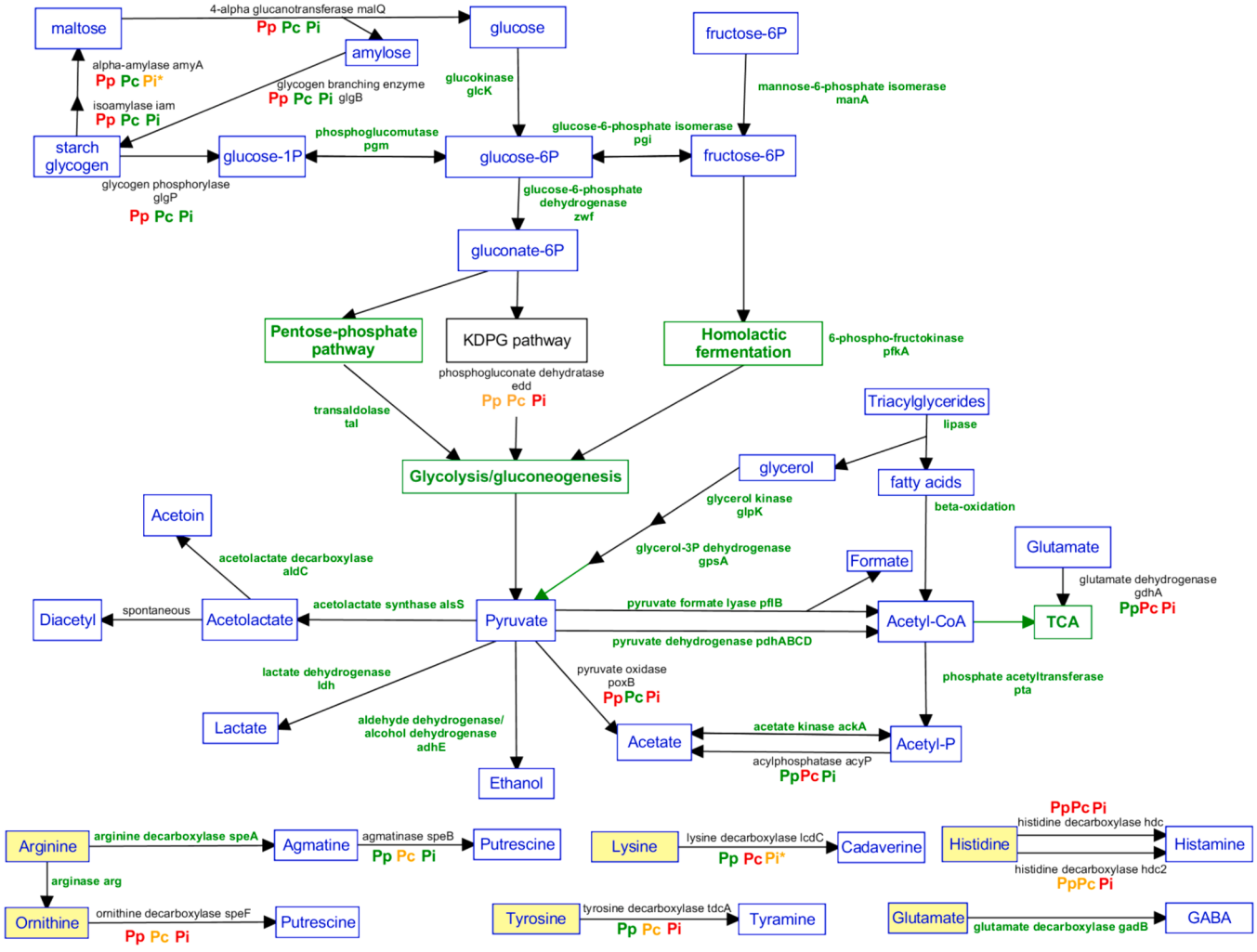

Fig. 3. Representation of pathways and genes present in the three species of photobacteria. Genes in green were found in all strains screened. Genes in black were found in only some of the isolates and have a color code for each species where: Pp = *P. phosphoreum*, Pi = *P. iliopiscarium*, Pc = *P. carnosum*; green=present in all strains of a species, red=absent in all strains of the species, orange=present in some strains of the species. An asterisk indicates that there is a source-of-isolation based distribution of the gene.

- **Carbohydrate metabolism**

All strains share the required set of genes for the glycolysis/gluconeogenesis, pentose phosphate pathway, and homolactic fermentation, but not the heterolactic due to missing xylulose-5-phosphate phosphoketolase gene (*xpk*A). The Entner-Doudoroff route is only complete in one *P. carnosum* strain (TMW 2.2186) and two *P. phosphoreum* strains (FS-5.1, FS-6.1), all marine, with only the phosphogluconate dehydratase gene (*ed*D) missing from the rest.

In addition to glucose and fructose, all strains are predicted to use ribose and mannose, although we could not find presence of specific transporters for the uptake of ribose, and the PTS mannose specific transporter (subunit A) is absent in some *P. carnosum* strains and meat-isolated *P. iliopiscarium* strains.

We found the ribonucleotide reductase subunits genes in all strains (*nrd*AB), needed for the conversion of nucleosides to deoxy-nucleosides. Its assembly subunit was found in all strains of *P. phosphoreum*, but only two fish-isolated strains of *P. carnosum* (TMW 2.2098, TMW 2.2186) and no *P. iliopiscarium*.

The degradation of glycogen and starch (glycogen phosphorylase *glg*P and glycogen debranching enzyme *glg*X), together with maltose/maltodextrin transport (*mal*F, *mal*G, *mal*K) is only predicted for *P. carnosum* and *P. iliopiscarium*. However, we found no enzymes for the synthesis of glycogen/starch. Subunit *mal*E of the maltose/maltodextrin transport and α-amylase coding gene were only found in *P. carnosum* and meat-isolated strains of *P. iliopiscarium*.

The α-galactosidase was ubiquitous in both species, but only randomly present in some strains of *P. phosphoreum*. The α-mannosidase was found also in all strains of *P. iliopiscarium*, random strains of *P. carnosum* and one single fish-borne strain of *P. phosphoreum*. We found a galctose/methyl-galactoside transporter randomly distributed in two of the species, but only meat-borne strains for *P. iliopiscarium*.

- **Pyruvate metabolism**

All screened strains are predicted producers of acetate, ethanol, lactate, formate, acetolactate (and ultimately acetoin). The enzyme pyruvate oxidase (*pox*B), responsible for the formation of acetate, carbon dioxide and hydrogen peroxide from pyruvate) was found in all strains of *P. carnosum* but none of the strains of the other two species, while the enzyme acylphosphatase (*acyl*P, acetyl-P to acetate) was only present in *P. phosphoreum* and *P. iliopiscarium*.

- **Tricarboxylic acid cycle (TCA)**

The complete set of genes for the TCA and the glyoxylate cycle are present in the genomes of all strains and species analyzed. Anaplerotic routes related genes initiated from amino acids are mostly present in the genomes of all strains, with the exception of the glutamate dehydrogenase (*gdh*A) to incorporate glutamate in the form of α-ketoglutarate into the TCA cycle, only present in all strains of *P. phosphoreum*.

- **Triacylglyceride metabolism**

Genes required for the cleavage of lipids, transport and degradation of glycerol and fatty acids (β-oxidation, both aerobic and anaerobic) are also equally present in all strains.

- **Amino acids**

Amino acid degrading genes are present in all strains, including the complete ADI pathway with production of ammonia and carbon dioxide (arginine deiminase *arc*A, ornithine transcarbamoylase *arc*B, carbamate kinase *arc*C), degradation of aspartate to iminosuccinate via aspartate oxidase (*nad*B) releasing hydrogen peroxide, conversion of serine to pyruvate via serine dehydratase (*sda*AB), conversion of aspartate and oxoglutarate to glutamate and pyruvate via aspartate aminotransferase (aspB) and oxaloacetate-decarboxylating malate dehydrogenase (*mdh*).

All strains of the three species contain a minimum of 2 and up to 7 loci of the arginine decarboxylase (*spe*A) coding gene, producing carbon dioxide and agmatine from arginine, and one loci of the glutamate decarboxylase (*gad*B), responsible for the production of gamma-aminoburyric acid (GABA). Further degradation of agmatine into putrescine and urea via agmatinase (*spe*B) is predicted in all strains except the one divergent fish-isolated *P. carnosum* strain TMW 2.2098. Conversion of arginine into ornithine and urea via arginase gene (*arg*) was predicted in all strains, but further conversion of ornithine into putrescine and carbon dioxide via ornithine decarboxylase (*spe*F) was exclusively predicted for two strains of *P. carnosum* (meat-isolated TMW 2.2029 and fish-isolated TMW 2.2189).

Decarboxylation of lysine to cadaverine (and carbon dioxide) via lysine decarboxylase (*lcd*C) was only predicted for *P. phosphoreum* strains, and fish-isolated *P. iliopiscarium* strains. Production of tyramine from tyrosine by the tyrosine decarboxylase (*tdc*A) was predicted for all *P. phosphoreum* strains and two meat-isolated *P. carnosum* strains (TMW 2.2147 and TMW 2.2157). We did not find the histidine decarboxylase gene (*hdc*) in any of the screened genomes. However, we detected a histidine-histamine antiporter and a proposed alternative histidine decarboxylase (*hdc*2) in one fish-isolated *P. carnosum* strain (TMW 2.2187), and 11 *P phosphoreum* strains (random distribution).

- **Respiration**

All strains are predicted to have a functional respiratory chain (Figure S6) and synthesize heme. All strains have two NADH dehydrogenase loci (*ndh*) and one copy of the sodium transporting NADH:ubiquinone reductase complex (*nqr*A-F). Only subunit *nuo*B from the proton transporting NADH:quinone oxidoreductase is present in all strains, and additionally all *P. phosphoreum* strains (except AK-8, FS-3.2, FS-6.1) contain an entire set of all its subunits (*nuo*A-N). All strains contained complete cytochrome c and bd oxidases (*cox*ABC, *cyd*ABX). Additionally, the cytochrome cbb3-type oxidase (*cco*NOP), was present in *P. phosphoreum* and *P. iliopiscarium* but missing in all strains of *P. carnosum*.

All strains are predicted to use fumarate (fumarate reductase, *frd*ABCD), trimethylamine-N-oxide (TMAO reductase, *tor*A, producing trimethyl–amine TMA), nitrate (nitrate reductase, *nap*AB), nitrite (nitrite reductase, producing ammonia, *nir*BD) and sulfate (sulfate denylyltransferase *cys*DN, adenylyl-sulfate kinase *cys*C, phosphoadenylyl-sulfate reductase *cys*H, assimilatory sulfite reductase *cys*JI, releasing H_2S) as alternative electron acceptors. Only marine-strains of *P. iliopiscarium* and randomly distributed strains of *P. phosphoreum* contain the DMSO reductase (*dms*ABC, producing dimethyl sulfide).

- **Environmental adaptation and stress response**

As previously studied by Hauschild et al. (2020), we have investigated the genomes of all strains for the presence of genes facilitating salt, pressure and oxidative stress response as well as life-style related traits such as motility, bioluminescence, sodium intake and iron accumulation.

Pressure response related genes are all present in all strains and species with the exception of porin-like protein *omp*L, randomly distributed in *P. carnosum* and *P. phosphoreum*, and only present in meat-isolated *P. iliopiscarium* strains. Salt response relevant genes were found in all strains with the exception of outer membrane proteins *omp*W/*omp*V and porin *omp*C/*omp*F, absent in all genomes. All strains have the superoxide dismutase (2 copies, also hydrogen peroxide producing) and catalase/peroxidase enzymes genes against oxidative stress. Additionally, all *P. phosphoreum* strains and *P. iliopiscarium* NCIMB 13481 contain an additional copy of the superoxide dismutase and catalase in their genome.

The flagellar gene cluster is complete only in randomly distributed strains of *P. carnosum* and *P. phosphoreum*, and only in marine-isolated strains of *P. iliopiscarium*. *P. phosphoreum* is the only species containing the entire lux-rib operon (all strains).

We searched for all sodium and sodium-dependent transporters's genes available. Their abundance only differed at the species level, with *P. phosphoreum* having 4–5 more loci on average. No differences were observed based on source of isolation/clades. The same applies to iron transports and unspecific stress proteins (cold-, heat-, phage-shock proteins and universal stress proteins).

Reintjes et al. (2019) utilize in their work laminarin, xylan, chondroitin sulfate, arabinogalactan, fucoidan and pullulan as common substrates in marine habitats and/or substrates whose hydrolyzing enzymes are widely distributed on marine bacteria. We found genes for the hydrolysis of arabinogalactan (beta-galactosidase) in all strains. Additionally we found xylose transporters exclusive for *P. phosphoreum* strains, and an endoxylanase ubiquitous for said species, randomly distributed in *P. carnosum*, and tied to marine-isolated *P. iliopiscarium* strains. The hydrolysis of pullulan, on the other hand, is exclusive and common in *P. iliopiscarium* and *P. carnosum*, but absent in *P. phosphoreum*. Although all strains checked contained an L-fucose symporter, only *P. carnosum* meat-borne strain TMW 2.2163 has the rest of the enzymes needed to utilize fucose, and therefore fucoidan.

- **Plasmids and Virulence Genes**

We found no match in any of the strains for the virulence genes identified in *P. damselae* phospholipase-D damselysin gene (*dly*) and the pore-forming toxin gene (*hlyA*), or the common plasmids identified in the genus *Photobacterium*.

PlasmidSPAdes identified 35 contigs that conform circular DNA segments in 15 of the 27 screened strains, distributed in the three species of photobacteria. Many of the predicted plasmids had a low coding density. Associated functions refer to conjugation proteins, mobile elements, secretion systems and toxin/antitoxin systems, and do not appear to offer any evolutionary or physiological advantage to their respective strains.

Secondary metabolism and additional features

Results for the identified features of each strain are summarized in Fig. 4.

- **Prophages and CRISPR**

PHASTER identified 32 intact phages in 26 strains of the three species. Two of them, in the genome of *P. carnosum* TMW 2.2098 and

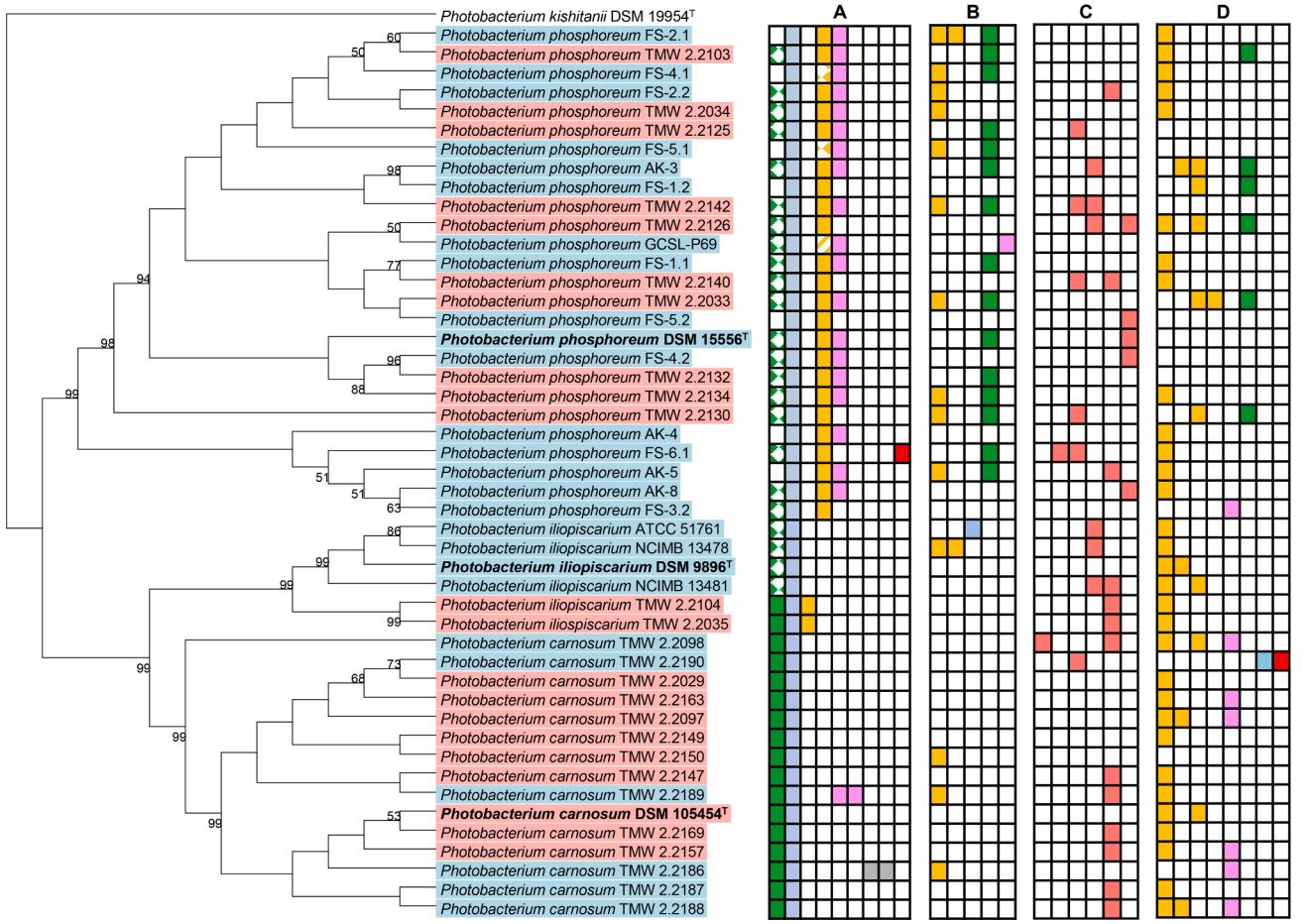

Fig. 4. Summary figure of results from online tools search. The tree is based on the MLSA phylogenetic tree. Type strains of each species are marked in bold and with a T. Source of isolation of each strain is marked by a color code: red for meat, blue for fish. Results for each online tool is displayed in a different grid, marked with different colors for the different types of metabolites, and separated in different colors for a different identity fo the same type of metabolite. Additionally, different patterns in the same column indicate that, despire being the same cluster, they differ in the organization of the genes. **A** Identified secondary metabolites with antiSMASH: betalactones (■), bacteriocins (■), arylpolyenes (■), siderophores (■), butyrolactones (■), type III polyketide synthase (■). **B** Identified bacteriocins with BAGEL: sactipeptides (■), colicin (■), microcin (■), penisin II (■). **C** Intact bacteriophages are displayed in different columns for different sequences (■). **D** CRISPR-cas clusters are displayed in different colors for different types of cas genes, and grouped in columns by gene architecture and direct repeat sequence: type I-F (■), type I-C (■), type I-D (■), type I-E (■), type III-D (■).

P. phosphoreum FS-6.1 respectively are unique, while the rest of them are repetitions of 4 unknown phages, only one of them species specific (*P. phosphoreum*). Three of the common phages appear to code for endolysins, required for the lytic cycle.

We additionally queried the genomes for presence of CRISPR-Cas systems, that appeared frequent among the species of photobacteria. Type I-F appears to be the most common but we also found evidence of type I-C and type I-D in some of the strains from both *P. carnosum* and *P. phosphoreum*, and genes identified as type III-D or type I-E in *P. carnosum* TMW 2.2190.

Protospacers and direct repeats were specific for each type of cas-operon and ranging from 4 to 64 in numbers. Some sequences are duplicated in the same strain or are present in more than one strain with random distribution, but most are unique.

The unique prophage of *P. carnosum* strain TMW 2.2098 was identical to unique protospacers of *P. phosphoreum* strains TMW 2.2034, TMW 2.2103, TMW 2.2140 and *P. carnosum* TMW 2.2163. The unique prophage from *P. phosphoreum* FS-6.1 strain, was identical to two unique protospacers of *P. phosphoreum* AK-4 strain. Since protospacers can offer an overview on previous phage attacks, and considering these events were only observed for two unique phages, it is possible they represent tools for competition even against other species/strains of photobacteria.

- **Secondary metabolites**

We identified with antiSMASH the presence of beta-lactone producing gene clusters, uniquitous for *P. carnosum* and *P. iliopiscarium,* and random in *P. phosphoreum*, with two different architectures.

One bacteriocin production gene cluster detected via antiSMASH was identical in all screened strains. However, BAGEL predicted bacteriocin production only in random strains of the three species. *P. phosphoreum* (10), *P. carnosum* (3) and *P. iliopiscarium* (1) strains are predicted to produce sactipeptides (one or two types). *P. phosphoreum* is also predicted to produce microcin (15 strains). Additionally, one *P. phosphoreum* strain (GCSL-P69) and one *P. iliopiscarium* strain (ATCC 51761) are predicted to be penisin II and colicin producers, respectively.

Production of arylpolyenes was predicted on all strains of *P. phosphoreum*. In addition, the two meat-isolated strains of *P. iliopiscarium* have been predicted to also produce one type of arylpolyene, different from that present on *P. phosphoreum* strains. The distribution of a siderophore-production cluster was observed in most strains of *P. phosphoreum* with a random distribution and one single salmon-isolated strain of *P. carnosum*, TMW 2.2189, also predicted to produce a second type of siderophore. Production of butyrolactones was only predicted in a different fish-isolated strain of *P. carnosum* (TMW 2.2186), and production of type III polyketide synthase (T3PKS) was reserved to a single marine-related *P. phosphoreum* strain (FS-6.1). These last traits appear as only specific for unique strains.

Discussion

Phylogeny and taxonomy

16S rRNA gene sequences have limited discriminatory power, and the group comprising *P. phosphoreum, P. iliopiscarium, P. carnosum* and *P. kishitanii* share identical 16S rRNA gene sequences (Hilgarth et al., 2018b; Sawabe et al., 2007). A previous study on the *Vibrionaceae* family proposes the use of the ferric uptake regulator (*fur*) as identification marker (Machado and Gram, 2015). We found that *fur* could be effectively used for clustering of the three species we analyzed, and results were consistent with other analysis. However, it appears to lose discriminatory power between strains of a single species and we conclude for that purpose that the use of several concatenated sequences (MLSA) or alternative genes would still offer a more realistic representation of phylogenetic relationships.

Phylogeny based on ANIb values and MLSA suggest that *P. carnosum* species constitutes one single clade whose strains, with the exception of strain TMW 2.2098 form MAP salmon, which appears to be a cross contamination from processing, are closely related to each other. On the other hand, *P. phosphoreum* and *P. iliopiscarium* both appear to split into two clades, environmentally-driven based on the source of isolation in the case of the latter. This distribution of the three species in either one or two conserved clades/subgroups is in agreement with previous work by (Fuertes-Perez et al., 2019) on their phenotypic characterization. Based on ANIb values (95–6%) and in silico DDH values (<79%), the two different clades within *P. phosphoreum* constitute two subspecies, which should be proposed in a future taxonomic publication.

Genome variability

Machado and Gram (2017) reported a genome size and GC content of 4.2 - 6.4 Mbp and 38.7 - 50.9% respectively for 16 of the 35 species of photobacteria used in their work, including *P. phosphoreum* and *P. iliopiscarium*. This findings are in accordance to the results of our analysis for the three species. According to (Musto et al., 2004, 2005, 2006), the GC content of the genome has a direct correlation to the optimum growth temperature of the species. The significant differences on the GC content of the genomes of all three of the species are in accordance with the higher optimum temperatures at which *P. phosphoreum* and *P. iliopiscarium* are able to grow (15 - 25 °C, ~39% GC) in comparison to *P. carnosum* (10 – 15 °C, ~38% GC) (Hilgarth et al., 2018b).

The pan-genome of the three species includes a large repertoire of accessory genes, many of which occur in only a single isolate, suggesting that these organisms are prone to horizonal gene transfer. The low variability in codon usage between strains and species suggests that acquired genes might come from bacteria with similar GC% content, maybe even other photobacteria. Despite an enrichment of these regions in elements devoid of known function, the high genome variability and exchange observed in photobacteria might be an advantageous strategy to acquisition of new capabilities for adaptation, stress response, competition and new niche colonization.

Distribution of multiple features on photobacteria appear not linked to isolation source or phylogenetic grouping (e.g. flagellar cluster for *P. carnosum* and *P. phosphoreum*, KDPG pathway, amino acid decarboxylases, bacteriophages, CRISPR-cas systems, bacteriocins and siderophores), as pointed out before by Machado and Gram (2017) in an analysis of 16 *Photobacterium* species (35 strains, including 2 marine-strains of both *P. iliopiscarium* and *P. phosphoreum*, but none of *P. carnosum*, and no terrestrial strains of any species).

The number of transposases suggests that these species might be prone to transposon-mediated exchange of genes, although less than their high-pressure adapted counterpart *P. profundum* (Aziz et al., 2010; Machado and Gram, 2017). Additionally, the high amount of protospacers per strain found in each CRISPR-cas locus, described as the prokariotic defense mechanism against external attacks (Pourcel et al., 2005) and the common presence of bacteriophages in their genome suggests that these species of photobacteria are also prone to phage infections.

Adaptation and competitiveness

The results suggest different levels of environmental adaptation not only at the species level, as is the case of *P. carnosum*, but also based on observed phylogenetic clades in the case of *P. iliopiscarium*.

- **Substrate utilization**

Utilization of a variety of carbohydrates appears to be the most diversified section of the genome of the photobacteria. Predicted utilization of common sugars (e.g. ribose, glucose, fructose and mannose) is

consistent with results of acid production from different substrates reported by Fuertes-Perez et al. (2019). These four substrates constitute the main components of the carbohydrates present on meat (Eskin and Shahidi, 2012; Koutsidis et al., 2008; Lawrie and Ledward, 2006; Nychas et al., 2007). However, glucose, fructose and mannose are also highly abundant in fish (Tarr, 1966) and therefore their utilization does not necessarily represent an environmental adaptation, but rather a statement of their ability to grow on both meat and fish.

Both *P. carnosum* and *P. iliopiscariu*m have the glycogen phosphorylase (*glg*P) and glycogen debranching enzyme genes, and only *P. carnosum* and meat-borne strains of *P. iliopiscarium* have the α-amylase gene, that breaks down alpha-linked polyssacharides such as glycogen and starch. Glycogen can be found both on meat and fish, but while it can be highly abundant in muscle meat, up to 1.8% (Immonen and Puolanne, 2000; Immonen et al., 2000; Ninios et al., 2014; Pethick et al., 1995; Trowbridge and Francis, 1910) even after 3 weeks (Koutsidis et al., 2008), it is reported to fluctuate in the 40 – 200 mg/100 g range in fish (Guillaume et al., 2001; Tarr, 1966). Since this gene is also present in other marine photobacteria such as *P. kishitanii* (PSV18756.1), *P. damselae* (TMX76883.1), *P. lutimaris* (PSU35927.1), *P. proteolyticum* (OLQ70040.1) it is unlikely that this is an acquired environmental advantage, but rather loss of the genes on *P. phosphoreum* in adaptation to more parasitic/symbiontic behavior as established by Henrissat et al. (2002). On the other hand, availability of additional substrates would allow *P. carnosum* and *P. iliopiscarium*, slower growers (Fuertes-Perez et al., 2019), to be more competitive in the meat environment.

We found in addition that photobacteria are able to degrade other polyssacharides that were previously reported as common in marine environments (Reintjes et al., 2019). While predicted degradation of pullulan and maltose import appears *P. carnosum* and *P. iliopiscarium* specific (and more so the meat-born strains of the latter), xylose degradation and transport is predicted as more common for *P. phosphoreum* and fish-strains of *P. iliopiscarium*. Additionally, one single strain of *P. carnosum* (TMW 2.2163) has the genes required for the degradation of L-fucose and fucoidan, and is able to express them according to results of acid production from carbohydrates reported by Fuertes-Perez et al., 2019. The strain-specificity to these genes suggests that they were acquired horizontally, although all strains appear to contain one L-fucose:proton symporter. Fucose is mostly present in plants and algae, and also in mammals as part of different types of glycans (Becker and Lowe, 2003), but there are no reports of its significant presence on either raw fish or meat. The three cases might represent and advantage in other niches not meat or fish related, but rather plant based (marine or terrestrial), supporting the idea that photobacteria might be more widespread that known. Yet, *P. iliopiscarium* strains display substrate preferences based on strain origin and clade distribution, with meat-borne strains closer to *P. carnosum* (sugar transports, α-amylase), while fish-borne strains are closer to *P. phosphoreum* (xylose utilization). Xylanases also appear to have quite a relevance in the industry (e.g. paper industry) (Qeshmi et al., 2020).

In addition to the glycolysis and pentose phosphate pathway, we could also find one fish-borne strain of *P. carnosum* and two fish-borne strains of *P. phosphoreum* with both key enzymes for the alternative Entner-Doudoroff pathway. Flamholz et al. (2013) suggests advantage in using this alternative pathway by utilizing far fewer enzymes as a trade-off with the lower energy yield, and it additionally allows the utilization of gluconate as energy source (Vegge et al., 2016).

- **Respiration**

All isolates are predicted to carry out aerobic and anerobic respiration. The sodium-translocating NADH:quinone oxidoreductase found in all strains, an analog to Complex I of the respiratory chain that pumps Na+ instead of *H*+ (Verkhovsky and Bogachev, 2010), is consistent with the sodium requirement of the three photobacterium species (Fuertes--Perez et al., 2019; Hauschild et al., 2020; Hilgarth et al., 2018a, 2018b). However, presence of high-oxygen affinity cytochrome cbb3-type oxidase (Pitcher and Watmough, 2004) in *P. phosphoreum* and *P. iliopiscarium*, and the full copy of the proton-translocating Complex I (*nuo*A-N) only present in *P. phosphoreum* strains might offer advantages in low anoxic/microaerobic and low sodium environments, respectively. The three *P. phosphoreum* strains missing the proton-transporting complex might indicate that the sodium-translocating analog is still the commonly used by these species of photobacteria.

The three species have a variety of reductases aimed at utilization of several alternative electron acceptors during anaerobic respiration. Nitrate and trimethylamine-N-oxide (TMAO) are examples of compounds they can use, but while abundant on marine environments and fish (Koike and Hattori, 1978; Yancey et al., 1982), are scarce on meat (Cho et al., 2017; Iammarino and Di Taranto, 2012). DMSO reductase is another example only prevalent in *P. phosphoreum* and fish-borne *P. iliopiscarium* strains, and an additional link of said isolates to a marine environment (Lee et al., 1999).

Siderophores are iron-chelating molecules that allow their producers an efficient way of recovering iron for respiration and redox reactions from environments with low availability (Comi, 2017; Gram et al., 2002). We observed an enrichment of siderophore producing gene clusters in *P. phosphoreum* that might suggest an advantage in low-iron availability, or high competitive microbiota environments such as meat.

- **Antimicrobial activity**

P. phosphoreum shows more predicted diversity of bacteriocin production than the other two species, although all isolates are predicted to produce at least one type of bacteriocin. Out of the four different types of bacteriocins predicted on photobacteria, microcin (only in *P. phosphoreum*) appears to be commonly produced by *Enterobacteriaceae* against other bacteria including those belonging to the same family, such as *E. coli* (Baquero et al., 2019). Colicins, one of the most studied group of bacteriocins, are usually produced by gram-negative bacteria and thoroughly studied in strains of *E. coli* (Micenkova et al., 2019) as effective agent against other strains of the same species. Finally, penisin was described first as a novel lantibiotic, product of *Paenibacillus ehimensis*, able to kill methicillin-resistant *Staphylococcus aureus* (Baindara et al., 2016). Both colicin and penisin II synthesis clusters are unique to one strain, most likely acquired by horizontal exchange. Results suggest that *P. phosphoreum* might compensate for the narrower carbon utilization of the species by producing bacteriocins against a wider selection of competitors in the same niche.

Some predicted bacteriocins might have additional external value. Sactipeptides, microcins and penisin appear valuable in the pharmaceutical industry as starting point for development of new antibiotics (Baindara et al., 2016; Himes, 2017; Severinov and Nair, 2012).

The type IV secretion system appears only on the marine-isolated strains of *P. iliopiscarium*. This type of secretion systems are mainly known for their involvement in bacterial conjugation, and their presence would contribute to the horizontal gene tansfer higher frequency (Wallden et al., 2010), but they also provide means for manipulation and removal of competing bacteria by secretion of macromolecules into other cells (Sgro et al., 2019).

- **Marine habitat-specific features**

Gene loci involved in bioluminescence, high pressure, osmotolerance have directly been linked to marine-adapted bacteria in other works (Brodl et al., 2018; Campanaro et al., 2005; Hauschild et al., 2020), although Moran et al. (2007) proposed that cultured marine bacteria and non-marine related bacteria mainly differed only in their sodium-based transport systems. These features appear as either species-specific features (bioluminescence) or as loci duplications with a higher copy frequency in *P. phosphoreum* (sodium-dependent transporters, stress and oxidative stress response) not linked to the source of

isolation. *P. phosphoreum* appears, therefore, better adapted to marine environments, and better suited for stress-responding, giving it advantage despite a narrower carbon source utilization.

The flagellar cluster has a random distribution except for *P. iliopiscarium*, correlating to source of isolation and phylogeny. Its expression, however, might require specific circumstances, as previous studies did not detect motility on strains with the complete set of genes (Fuertes-Perez et al., 2019). Motility has been defined as critical in the survival as free-living bacteria in marine-environments (Eloe et al., 2008) and therefore the absence of the flagellar cluster is likely due to loss of the genes in adaptation to the meat/fish environment where motility is not required.

- **Food safety and spoilage**

All strains of photobacteria have spoilage potential on meat and are predicted producers of H_2O_2 from pyruvate (*pox, P. carnosum*), aspartate and arginine (both highly abundant on meat (Holló et al., 2001) resulting in greening of the meat. All strains are predicted producers of acetate and lactate from pyruvate and/or acetyl-CoA contributing to a lowering of the pH and sour odors (Gram et al., 2002). In addition, all strains are predicted producers of putrescine from arginine (except one *P. carnosum* strain), with cases in some strains of tyramine (from tyrosine), cadaverine (from lysine) and putrescine (from ornithine) producers. Finally, more relevant on fish spoilage, all of them are predicted to produce trimethylamine from TMAO, more relevant in fish and responsible for foul-odors (Gram and Dalgaard, 2002).

Although *P. phosphoreum* and *P. iliopiscarium* have been largely described as histamine producers on fish (Bjornsdottir-Butler et al., 2018; Bjornsdottir et al., 2009; Kanki et al., 2004), and the histamine production of several fish-isolated strains previously measured (López Caballero et al., 2002; Morii and Kasama, 2004; Torido et al., 2012; Wang et al., 2020), we did not detect presence of the *hdc* gene in any of the screened isolates, also reported by Machado and Gram, 2017. Measured production of histamine in some of the isolates included in this study (AK-3, AK-8, FS-1.1, FS-1.2, FS-2.1, FS-4.2), reported by Bjornsdottir-Butler et al., 2018, might be due to the presence of an alternative histidine decarboxylase gene (*hdc*2) recently identified by Bjornsdottir-Butler et al., 2020. Detection of the *hdc*2 gene in one *P. carnosum* and 11 *P phosphoreum* strains suggests production of histamine and therefore health risk by their presence on meat. Regarding available *P. phosphoreum* sequences of the *hdc* gene, identification of the respective isolates was mostly performed by physiological tests (e.g. bioluminescence) and 16S rRNA sequences (BAE94284.1, BAE94283.1, BAC45246.1, AAO65983.1) (Kanki et al., 2004), insufficient criteria to differentiate close *Photobacterium* species (e.g. *P. kishitanii* and *P. phosphoreum*) (Ast and Dunlap, 2005; Machado and Gram, 2015; Sawabe et al., 2007). Results suggest that previous reports on presence of the *hdc* locus on *P. phosphoreum* were either due to misidentification of the isolates, or to its strain-specific nature (i.e. not common to the entire species, only to some strains).

Conclusion

This study reports high genomic diversity present within the three species of photobacteria known to be relevant in meat and seafood spoilage. Central pathways involving the most common carbon sources e.g. glycolysis, gluconeogenesis, pentose phosphate pathway, beta-oxidation of lipids, TCA and glyoxylate cycle, the majority of anaplerotic routes and amino acid metabolism, are generally conserved in all strains and species. However, there are differences in multiple metabolic routes between or within the species (e.g. pyruvate oxidase, KDPG pathway, respiration), utilization of less common carbon sources (e.g. maltose, glycogen), production of metabolites (e.g. biogenic amines), adaptative features (e.g. stress response) and antimicrobial activity (e.g. bacteriocins). The proposed high frequency of genetic exchange in photobacteria suggests itself as an advantageous strategy, despite accumulation of elements devoid of function, aimed at diversification and acquisition of beneficial characteristics.

P. carnosum strains form a single clade species with one divergent strain from MAP salmon that does not, however, show distinct traits or predicted adaptation to a different niche indicating cross-contamination within processing/packaging. The species appears mostly focused on diversification of carbon sources available for energy production and adapted to nutrient rich environments from meat, fish or even plant origin. On the other hand, *P. phosphoreum*, as results suggest, is represented by two clades, which appear to represent two novel subspecies within. However, this differentiation is not clearly reflected by predicted metabolic divergence or isolation origin. In contrast to *P. carnosum*, this species has a wider diversity of antimicrobial activity, predicted stronger response to stress and low availability of resources (e.g. iron, oxygen and sodium) and adaptation to more than one life-style (e.g. symbiontic), better fit to eliminate or outgrow possible competitors. Finally, *P. iliopiscarium* is divided into two clades, fish- and meat-borne, which show several examples of an evolutionary adaptative response. Meat-borne strains appear more similar to *P. carnosum* and retain similar carbon utilization and transport oriented genes, while fish-isolated strains retain the utilization of marine-common compounds (e.g. DMSO) and motility directed to a free-lifestyle.

CRediT authorship contribution statement

Sandra Fuertes-Perez: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Rudi F. Vogel:** Project administration, Funding acquisition, Conceptualization, Supervision, Writing – review & editing. **Maik Hilgarth:** Project administration, Funding acquisition, Conceptualization, Supervision, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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