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Shewanella putrefaciens FISH PATHOGENIC STRAINS CONTAIN PLASMIDS THAT ARE ABSENT IN THE PROBIOTIC STRAIN S. PUTREFACIENS PDP11

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Introduction

Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts. *Shewanella putrefaciens* Pdp11 has been described as a probiotic for farmed fish species such as *Solea senegalensis* and *Sparus aurata* Tapia-Paniagua et al., 2012). In contrast, other strains of *S. putrefaciens* have been described as pathogenic for other cultured fish (Esteve et al 2017)damage of the mouth, extensive skin discoloration, exophthalmia, ascites and bad odour. The S. putrefaciens group was recovered from freshwater samples taken at the L′Albufera system, along autumn–winter 2015. Its counts significantly increased in freshwater parallel to hypoxia and temperature rising. Shewanellae strains were identified as S. putrefaciens and S. xiamenensis by 16S rRNA gene sequencing. These isolates recovered from sick eels or freshwater were virulent for European eel by IP challenge (LD50 106 CFU g−1 body weight. The plasmids plays an important role in the genes transfer and insertion then there can been implicated in antibiotics resistance, degradative pathway and pathogenicity characteristics (Kornelia et al 2015). The genetic variation conducted by plasmid could induce an impact in probiotic proprieties. In this research, we searched the present or absent of plasmids in pathogenic and probiotic strains of *S. putrefaciens*. As well as, this plasmids implication in development of virulence factors.

Material and methods

Shewanella putrefaciens strain Pdp11 was isolated from skin mucosa of healthy gilthead seabream (*Sparus aurata* L.) (Chabrillón *et al.*, 2005) and *Shewanella putrefaciens* SH12, SH4, SH6, SH9, SH16 have been associated with diseases in eel (*Anguilla anguilla* L.)(Esteve, , 2017)damage of the mouth, extensive skin discoloration, exophthalmia, ascites and bad odour. The S. putrefaciens group was recovered from freshwater samples taken at the L′Albufera system, along autumn–winter 2015. Its counts significantly increased in freshwater parallel to hypoxia and temperature rising. Shewanellae strains were identified as S. putrefaciens and S. xiamenensis by 16S rRNA gene sequencing. These isolates recovered from sick eels or freshwater were virulent for European eel by IP challenge (LD50 106 CFU g−1 body weight.All *S. putrefaciens* strains were grown on 10ml of Tryptone soja broth supplemented with NaCl 1.5% (TSBs) for 24h at 23ºC. Given the possibility that, the hypothetical plasmids could be integrated into the bacterial genome, strains were cultured under different and strict growth conditions (temperature, incubation time, growth medium and freeze-thaw) to favor their excision. For this, all strains of the study were cultured in 10 mL tubes of TSBs and minimal media (M9), and incubated at 23ºC or 4ºC for 24h and 48 h on shaking at 80 rpm. Cultures inoculated in parallel with TSBs medium containing glycerol (20 %) were subjected to a freeze-thaw cycle for 24 h at -80 ºC before incubation. The positive control was *Escherichia coli* V157, a strain harbouring seven plasmids, grown on Luria Bertani broth (LB) for 24h at 37ºC under agitation at 80 rpm. The cultures were centrifuged and the pellet was used for plasmid DNA (pDNA) isolation. pDNA integrity was checked by agarose gel. Rolling circle amplification (RCA) was performed using the TempliPhi 100 amplification kit for each isolated plasmid following the manufacturer's instructions.

The Illumina sequences were obtained by Seoane et al. 2019, and Plasmid-specific Sanger sequences from pDNA isolation and RCA amplification as above. Vector removing and quality trimming steps of Sanger reads were performed with BBDuk. Two sets of vector-free Sanger reads were obtained in the quality trimming. The low stringency threshold generates longer reads used to capture plasmid-specific Illumina reads and the high stringency threshold for the final assembly. Then, plasmids were assembled *de novo* using a workflow integrating Sanger and Illumina reads. Plasmid sequences were recircularized by the MARS method, and plasmids were aligned by the Clustal method of the Seaview program to finally obtain the plasmid consensus sequences. The plasmids annotation and functional characterization were performed. The sequence analysis showed that the plasmids encoded a putative replication initiator protein of the repB family, and proteins related to plasmid stability and a toxin-antitoxin system.

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Results and Discussion

Using a workflow integrating Sanger and Illumina reads, the complete consensus sequences of the plasmids were obtained. We found that the main ORFs conserved in both plasmids pSH4 and pSH12 belong to the replication protein initiator repB superfamily, and PemK/PemI family protein. The Rep proteins are especially important as they are primarily responsible for the initial DNA binding and nicking activities (Stolz, 2014). Another major ORF identified is the PemK protein as part of toxin-antitoxin (TA). This system is found both in bacterial chromosomes and in MEGs such as plasmids and prophages (Bukowski et al., 2019).

The presence of plasmids is expected to be associated with bacterial survival and, commonly, with virulence factors. The probiotic strain *S. putrefaciens* Pdp11 did not present plasmid, which was only found in two of the five pathogenic strains. The results allowed us to discard the probiotic Pdp11 could present a pathogenic characteristic as the TA type II system as a virulence factor and its self-regulating characteristics, which may be behind its probiotic nature, making the Pdp11 strain unique in comparison to other *S. putrefaciens* strains.

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