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THE PROBIOTIC STRAIN *Shewanella putrefaciens* Pdp11 STRONGLY MODULATES GENE EXPRESSION OF THE FISH PATHOGEN *Vibrio harveyi*

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Introduction

Shewanella putrefaciens Pdp11 was isolated from the skin of healthy gilthead seabream (*Sparus aurata*) and has been studied as a probiotic in the culture of some fish species, such as *S. aurata* and *Solea senegalensis*. These are two relevant species cultured in the Mediterranean area, but the pathogens that affect them represent a serious problem for the commercial development of their farming industry (Cámara-Ruiz *et al*., 2020). *Vibrio harveyi* is a significant pathogen of cultured marine fish, especially in southern Europe, and causes a wide variety of pathologies in its hosts (Zhang, He and Austin, 2020). Studies on SpPdp11 probiotic showed antagonistic activity against *V. harveyi in vivo* (Cámara-Ruiz *et al*., 2020), so there is an interest in understanding the mechanisms underlying the observed effects in order to enhance them. In this work, the interaction between SpPdp11 and *V. harveyi* was studied by RNA-seq to understand how SpPdp11 interferes with the pathogen through bioinformatics analysis.

Material and methods

Three types of cultures were performed for this study: SpPdp11 alone (code: Pdp11), *V. harveyi* alone (code: Vibrio) and SpPdp11 and *V. harveyi* together (code: PV). Aquafeed medium was used for all the cultures, which is composed of 160 g/L feed + M3 minimal saline medium. Liquid cultures of SpPdp11 and *V. harveyi* were brought to a known concentration of equal numbers of CFU/mL and 100 µL of the source flasks were added to solid plates of aquafeed medium. For the interaction, a 1:1000 concentration of *V. harveyi*:SpPdp11 was assumed based on previous studies of their growth curves. All cultures were incubated for 24h at 23ºC and samples were stored in TRIsure™ until processing. RNA extraction was performed with the GeneJET purification kit (Thermo Scientific™) following the manufacturer's instructions.

Sequencing libraries (paired-end, 2x75 bp) were constructed at the Ultrasequencing Service of the University of Malaga and sequencing was performed on the Illumina NextSeq™ 550 platform. Six samples of each experimental group (Pdp11, Vibrio and PV) were sequenced. Raw reads were processed using a bioinformatic pipeline including pre-processing with SeqTrimBB (v2.1.8), mapping with BWA (v0.7.5), differential gene expression with DEgenesHunter (v1.0) and functional enrichment of GO terms with in-house R scripts. GO enrichment was performed for the three ontologies: Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). For the processing of Vibrio data, in addition, a de novo assembly of the genome and its functional annotation were performed, and predicted genes were searched for in the core genome of Vibrionales order (results not shown). Data processing was carried out with the computational resources of the Andalusian Bioinformatics Platform. To find out the effects of the interaction in both species, two gene expression comparisons were carried out: PV vs Pdp11 and PV vs Vibrio. Genes were considered as differentially expressed (DEG) if fold change > 2 and adjusted p-value < 0.05. A network analysis was performed for the most relevant functional enrichment results using Gephi (v0.9).

Results

When comparing PV vs Pdp11 through differential expression analysis, 66 DEGs were obtained, of which 31 were upregulated and 35 were downregulated. Significant GO terms (p-value < 0.05) were obtained in BP and MF ontologies, but not in CC. Nevertheless, due to the low number of DEGs obtained, few genes were annotated in the enriched terms and no further analysis was performed.

When comparing PV vs Vibrio, 2370 DEGs were obtained, of which 1113 were upregulated and 1257 were downregulated. Significant GO terms were obtained in the three ontologies for both upregulated and downregulated genes, but the enrichment yielded higher significance values in the case of the upregulated genes than of downregulated genes. The most relevant results were obtained in BP ontology, so a network analysis was performed in order to study the distribution of up and downregulated genes and enriched terms. Briefly, upregulation of expression was observed in genes related to motility, localization, organization and peptide metabolism, while downregulation was observed in genes related to stress response, signal transduction, transcription, transport and metallic clusters assembly.

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Discussion

A quick glance at the results shows that the number of deregulated genes as a result of the interaction is much higher in *V. harveyi* than in SpPdp11. This suggests that the presence of SpPdp11 affects *V. harveyi* to a greater extent than *V. harveyi* affects SpPdp11. Considering that *V. harveyi* is a pathogenic strain and SpPdp11 is a probiotic strain, this may be positive for its probiotic capacity, as it not only maintains its functionality almost intact, but also produces a huge imbalance in that of *V. harveyi*. Although the pathogenicity mechanisms of *V. harveyi* remain to be properly resolved, many virulence factors have been identified, as hemolysins, proteases, lipopolysaccharide (LPS), the capacity to bind iron, interaction with bacteriophages, biofilm formation and quorum sensing (Zhang, He and Austin, 2020). Interestingly, we found that genes related to metallic clusters assembly were downregulated in *V. harveyi* in the presence of SpPdp11, which could be modulating this virulence factor. Moreover, this has been found to be important for pathogenicity in fish, but not in invertebrates (Owens, Austin and Austin, 1996). As for other groups of downregulated genes, such as those related to stress response or transcription, it would be necessary to elucidate how they are related to known virulence factors. Overexpressed genes are mostly related to two major functionalities, protein biosynthesis and motility. SpPdp11 is clearly affecting motility and organization of *V. harveyi*, but the consequences of this deregulation need to be elucidated. Regarding protein biosynthesis and translation, it is well described that *V. harveyi* uses many proteins as virulence factors (Zhang, He and Austin, 2020), so an experimental study on the expression of known virulence factors under conditions of interaction with SpPdp11 would be desirable. Based on these results, new interaction experiments will be conducted to observe the behaviour of the two species at the interaction front and metabolomics and proteomics studies to explore further effects of the deregulation of gene expression.

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