

# TRANSCRIPTOMIC-BASED ANALYSIS OF THE *LACTOBACILLUS PLANTARUM* WCFS1 RESPONSE TO OLEUROPEIN

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## ABSTRACT

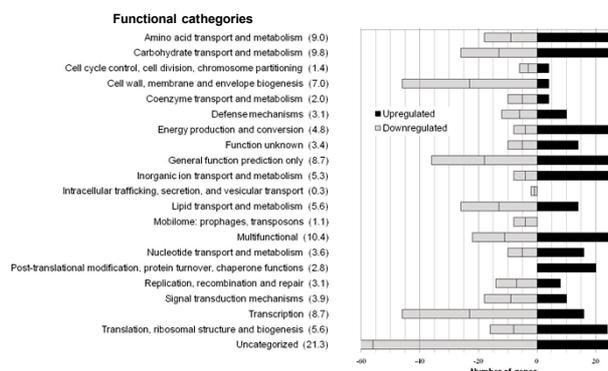
The beneficial effects of virgin olive oil have been ascribed to the content of polyphenols, which exert antioxidant, anti-inflammatory, anti-cancer, antimicrobial, antiviral, cardiac- and neuro-protective effects (1). Oleuropein (OLEU), a secoiridoid glucoside characteristic of *Oleaceae*, is the main phenolic component of olive leaves, seed, pulp and peel of unripe fruits. During fruit maturation, OLEU undergoes hydrolysis, yielding different products, including the phenolic compound hydroxytyrosol (3,4-dihydroxyphenylethanol). Hydrolysis of OLEU is involved in olive production and is responsible for its characteristic bitter taste (2). It has been described that gut commensal microorganisms like *Lactobacillus plantarum*, tolerate toxicity of several phenolic acids and bioactivate these compounds. *L. plantarum* is among the species occurring during olive fermentation able to hydrolyze OLEU through  $\beta$ -glucosidase and esterase activity (3). However, the response of *L. plantarum* to OLEU is still not fully understood. To know the response mechanisms of the gut commensal microorganism *L. plantarum* in presence of OLEU by a whole genome transcriptional study.

## MATERIAL AND METHODS

Four separate batches of *L. plantarum* WCFS1 cultures (in triplicate) were cultured in MRS until an OD<sub>600nm</sub> of 0.8-0.9 and induced or not with oleuropein (15 mM) for 10 min. The cells were centrifuged and resuspended in buffer for RNA extraction. Copy DNA was synthesized, marked and, coupled to Cy3 and Hyper5 for further hybridization. A microarray platform with 3066 genes was designed (Agilent Technologies). Probes and hybridization were performed according to previously described protocols (Agilent, G4140-90051). Data were normalized to "loess" LIMMA. Differential expression of genes was evaluated by a t moderate test. False Discovery Rate (FDR) less than 5% was established. The impact of this phenolic compound on the transcriptomic profile of *L. plantarum* WCFS1 was evaluated by sorting all genes whose transcript level showed changes of at least  $\pm 1.5$ . The observed results were validated by qRT-PCR.

## RESULTS

The microarray data revealed that approximately 12% of the genes displayed differential expression. Overall, 355 transcripts were affected, among which 155 were upregulated and 200 downregulated. The differentially expressed genes belong to 17 different functional categories (COGs). The largest COGs group corresponded to carbohydrate transport and metabolism (8%), amino acid transport and metabolism (7%), and cell wall, membrane and envelope biogenesis (6%) (Fig.1). The transcriptional profile reveals induction of genes involved in general stress. A specific oxidative stress response (*lp\_1424*, *lp\_1425*) was induced including genes involved in the cysteine metabolism (*lp\_0254*, *lp\_0255*, *lp\_0256*) (Table I). Similar to that observed for *p*-coumaric acid, the expression of some genes involved in the biosynthesis of chorismate was detected.



**Figure 1.** Number of *L. plantarum* WCFS1 genes by functional categories that were upregulated and downregulated in the presence of oleuropein 15 mM.

**Table I.** Top ten upward regulated genes and induction levels in the presence of oleuropein 15 mM

Gen ID	Locus	Fold Change	Description	COGs	Subcellular Localization
<i>lp_1424</i>	-	6,08	NADPH-dependent FMN reductase family protein	General function prediction only	Intracellular
<i>lp_1425</i>	-	5,81	fumarate reductase/succinate dehydrogenase, FAD-binding flavoprotein; NADPH-dependent	General function prediction only	Intracellular
<i>lp_0254</i>	<i>cysE</i>	4,57	serine O-acetyltransferase	Amino acid transport and metabolism	Intracellular
<i>lp_0255</i>	<i>metC1</i>	3,94	cystathionine beta-lyase / cystathionine gamma-lyase	Amino acid transport and metabolism	Intracellular
<i>lp_2638</i>	-	3,65	membrane protein	General function prediction only	Multi-transmembrane
<i>lp_0256</i>	<i>cysK</i>	3,57	cystathionine beta-synthase	Amino acid transport and metabolism	Intracellular
<i>lp_1729</i>	<i>malT</i>	3,35	carbohydrate/proton transporter	General function prediction only	Multi-transmembrane
<i>lp_3368</i>	-	2,96	multidrug transport protein, major facilitator super family (MFS), EmrB/CacA subfamily, N-terminal domain	Inorganic ion transport and metabolism	No information
<i>lp_1816</i>	<i>ispD</i>	2,89	3-2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	Lipid transport and metabolism	Intracellular
<i>lp_1817</i>	-	2,84	tarJ ribitol-5-phosphate 2-dehydrogenase	Amino acid transport and metabolism	Intracellular

## CONCLUSIONS

The results indicated that OLEU exposure a complex response in *L. plantarum* which could improve its ability to survive and perform olive fermentations.

## References

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