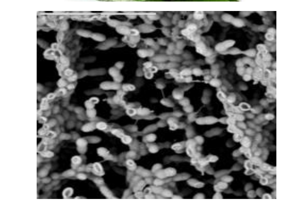
WINE POLYPHENOLS AND PROBIOTICS MIGHT RECIPROCALLY ENHANCE THEIR BENEFITS AT INTESTINAL LEVEL



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INTRODUCTION:

Wine polyphenols seems to exert an impact on intestinal microbiota growth and functionality [1]. Polyphenols are minimally absorbed at the small intestine but they are extensively metabolized at the large intestine by microbiota, giving rise to numerous low molecular weight metabolites (benzoic acids, cinnamic acids, phenylacetic acids, phenylpropionic acids, valerolactones, among others). It is to these metabolites -more than the original forms present in foods- that the biological activity and health effects associated to dietary polyphenols are attributed to. Consumption of specific probiotic strains might improve the metabolism and bioavailability of polyphenols and, in turn, enhances the health effects attributed to them [2]. On the other hand, wine polyphenols might enhance the growth and beneficial properties of probiotics in relation to intestinal health.

OBJECTIVE:

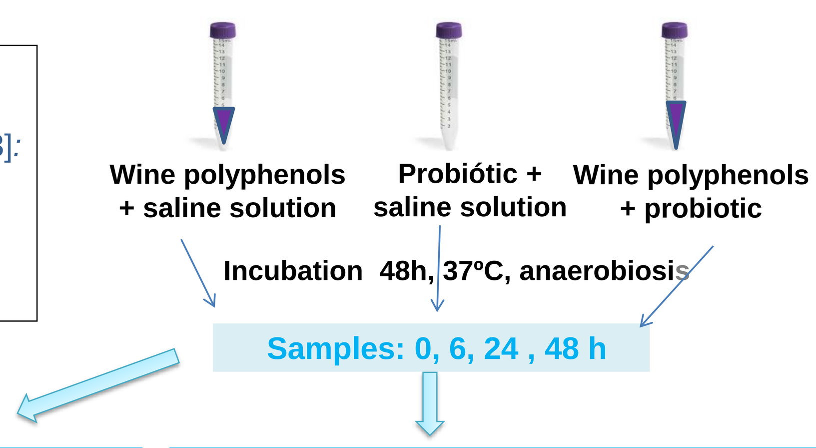
The aim of this study was to explore if the combination "polyphenols+probiotics" could act synergistically favouring, on one hand, the metabolism and bioavailability of polyphenols by the action of specific probiotic strains, and on another hand, the growth and beneficial properties of probiotics by the action of polyphenols. To achieve this, we have carried out different *in vitro* experiments to assess the metabolism of wine polyphenols by probiotics, and to evaluate the effect of wine polyphenols in probiotic viability and in probiotic capacity to inhibit the adhesion of potential pathogens (i.e., *E. coli*) to intestinal cells.

MATERIALS and METHODS

1. Metabolism of wine polyphenols by probiotic preparations and lactic acid bacteria (LAB) strains

Probiotics:
- *Commercial probiotic preparations (n=8)*
- Oenological LAB strains (García-Ruiz *et al.*[3]):
  - *P. pentosaceus* CIAL-86
  - *L. plantarum* CIAL-121
- Probiotic strains:
  - *L. plantarum* CLC 17

Wine polyphenols
- *Provinols ™ Extract*



1.1. Analysis of the phenolic compounds by UPLC-MS

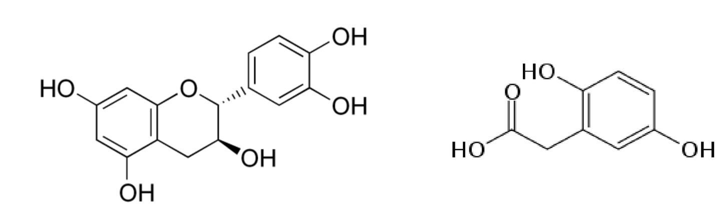
(Jiménez-Girón et al. [4]

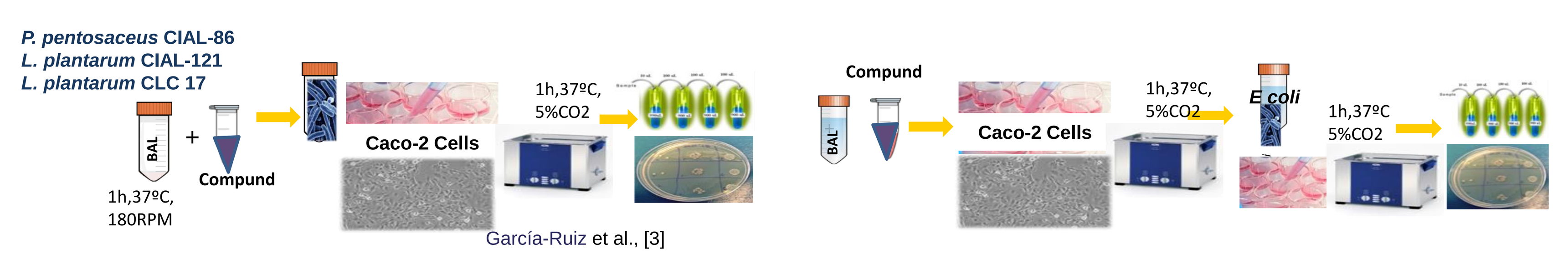
1.2. Effect of the phenolic compounds in the viability of the LAB strains

Microbial counts

2. Effect of phenolic compounds and probiotics in the capacity of the probiotics to inhibit *E. coli* adherence

Probiotics:
- *Oenological LAB strains:*
  - *P. pentosaceus* CIAL-86
  - *L. plantarum* CIAL-121
- *Probiotic strains*
  - *L. plantarum* CLC 17





LAB Adherence (1 h, 37ºC, 5%CO2) | **Inhibition *E. coli*** (1 h,, 37ºC, 5%CO2) + *E. coli* (1 h, 37ºC, 5%CO2)

RESULTS

1. Metabolism of wine polyphenols by probiotic preparations and LAB strains

1.1. Analysis of the phenolic compounds by UPLC-MS

Table 1 summarizes the changes in the concentration of the 15 phenolic metabolites quantified during the incubations of probiotics with the wine extract.

Table 1. Changes (% in respect to t=0) in the concentration of phenolic compounds during incubations of the wine extract with bacteria.

Samples	Time (h)	Gallic acid	Syringic acid	3-O-methyl gallic acid	Vanillic acid	Protocate-chuic acid	Salicylic acid	4-Hydroxy benzoic acid	Phthalic acid	Ferulic acid	Caffeic acid	p-Coumaric acid	(+)-Catechin	(-)-Epicatechin	B1	B2
Wine extract	6	103	104	105	106	101	101	100	98	103	101	104	102	107	105	102
	24	103	99	102	110	100	102	99	95	114	109	111	101	103	106	107
Probiotic #1 + Wine extract	6	95	100	85	108	90	89	98	101	87	96	101	98	95	90	101
	24	94	87	86	102	85	89	84	90	102	89	107	82	87	88	92
Probiotic #2 + Wine extract	6	104	86	103	91	98	-	98	103	87	100	100	95	92	102	92
	24	112	86	110	109	101	83	110	102	109	111	114	94	97	102	100
Probiotic #3 + Wine extract	6	103	85	101	97	101	109	115	110	103	101	114	109	98	106	111
	24	105	87	103	99	109	95	111	104	118	100	98	118	89	99	107
Probiotic #4 + Wine extract	6	93	93	96	87	93	83	94	89	89	95	98	88	94	87	98
	24	88	110	88	82	97	93	88	91	84	113	106	85	104	84	89
Probiotic #5 + Wine extract	6	102	88	102	97	88	123	111	111	107	107	110	106	107	105	101
	24	99	112	102	106	124	113	113	117	86	98	99	115	110	134	93
Probiotic #6 + Wine extract	6	107	115	96	104	103	119	104	117	161**	124*	153**	121*	128*	122*	130*
	24	122	140*	100	110	113	106	105	99	164**	136*	181**	125	126*	121*	134*
Probiotic #7 + Wine extract	6	106	108	119	93	109	90	94	100	118	99	93	99	109	107	106
	24	120	127	118	101	117	96	113	102	152*	112	111	114	116	121	124*
Probiotic #8 + Wine extract	6	96	104	92	96	101	106	93	114	88	108	98	98	101	100	114
	24	101	109	95	93	99	106	96	110	114	95	105	96	97	101	112
L. plantarum CLC 17 + Wine extract	6	89	90	102	101	97	91	96	94	89	98	94	92	98	100	106
	24	125*	130**	134	141*	130*	139*	132**	134	111	119	123*	138*	130*	151**	141*
P. pentosaceus CIAL 86 + Wine extract	6	94	97	96	83	90	95	89	91	96	96	87	103	95	101	96
	24	98	110	101	97	95	94	89	90	101	93	92	105	98	103	104
L. plantarum CIAL-121 + Wine extract	6	89	91	91	92	84	96	95	91	92	83	84	89	96	99	98
	24	97	94	106	104	101	91	99	102	99	95	91	107	108	104	102

* Mean significantly different from 100 (p<0.05) using paired-sample t-test. ** Mean significantly different from 100 (p<0.01) using paired-sample t-test.

1.2. Effect of the phenolic compounds in the viability of the BAL strains

Bacteria viability (CFU/mL) during incubation of probiotics in the absence or in the presence of the wine extract was carried out for all the probiotics. Figure 1 displays the data for those probiotics that were found capable to metabolize wine polyphenols (Table 1), this is to say, preparations #6 and #7, and *L. plantarum* CLC 17 strain.

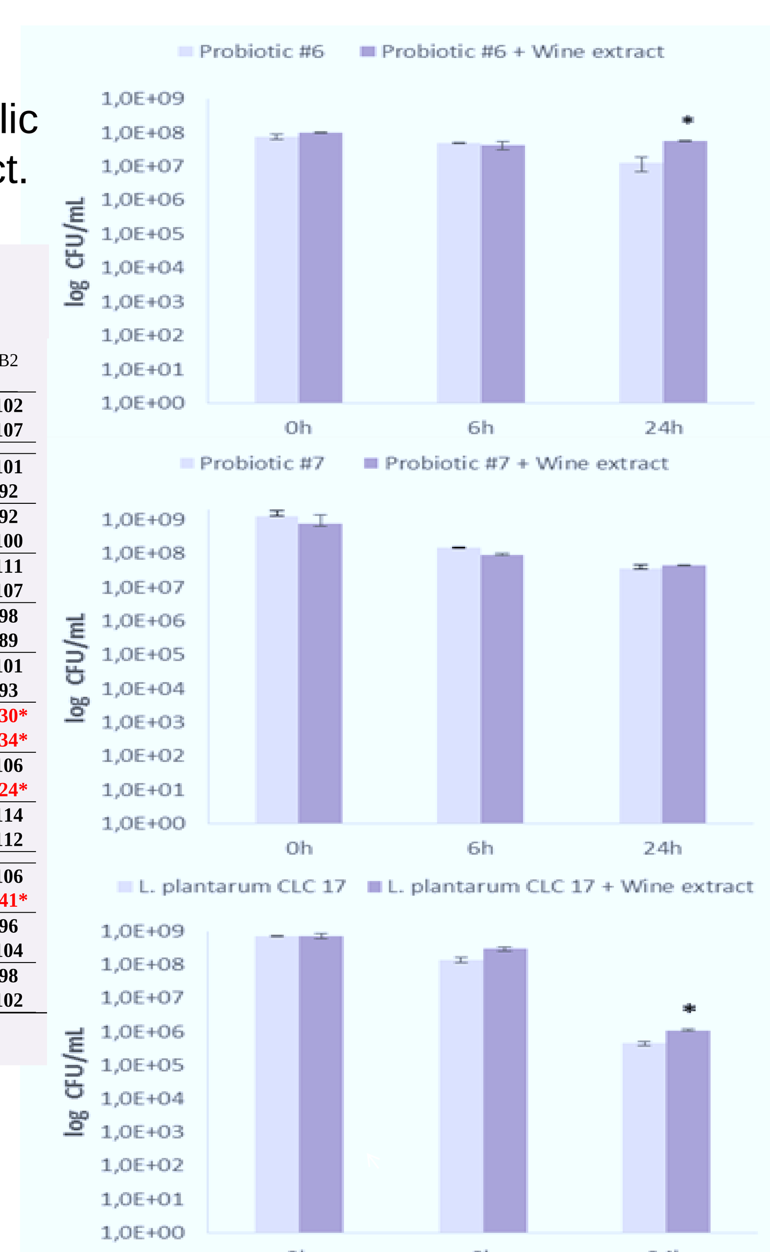


Fig. 1- Bacteria viability (CFU/mL) during the incubations of the wine extract with probiotic bacteria. Results are shown as media ±SD. * Significant differences (p < 0.05) from incubation of bacteria on their own.

2. Effect of phenolic compounds and probiotics in the capacity of the probiotics to inhibit the E. coli adherence

Fig. 2 shows the adherence (%) of LAB strains to Caco-2 cells in the absence and presence of (+)-catechin and 3,4-dihydroxypheylacetic acid. In addition, *E. coli* adherence (%) in presence of probiotics and phenolics is shown in Fig 3.

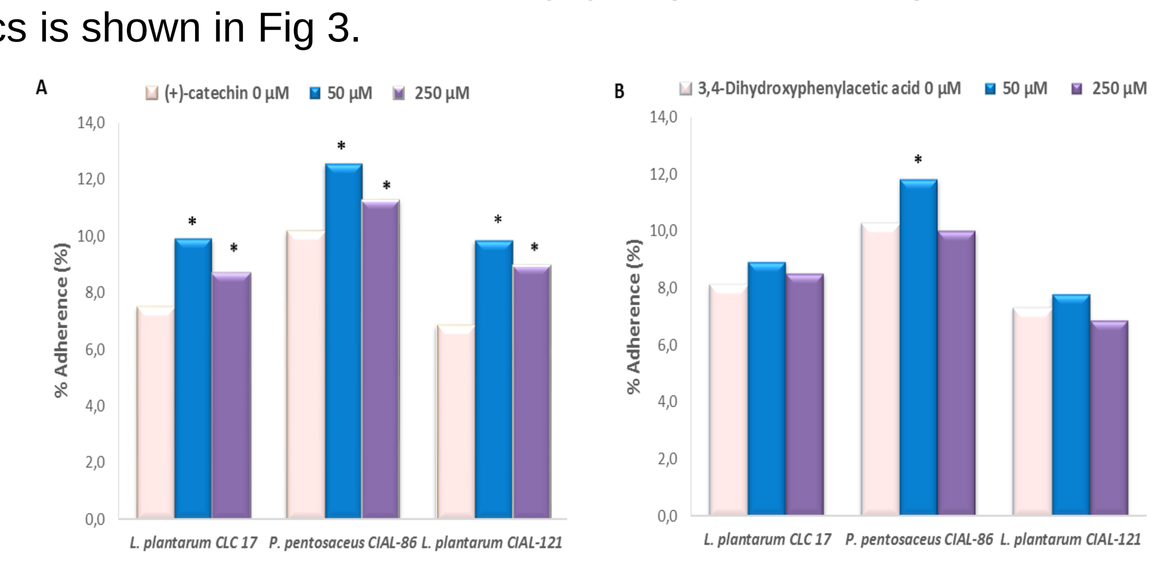


Fig. 2. Adherence (%) of *L. plantarum* CLC 17, *P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121 strains to Caco-2 cells in the absence and presence of (+)-catechin and 3,4-dihydroxypheylacetic acid. * Mean significantly different (p < 0.05) from assay in the absence of phenolic compound.

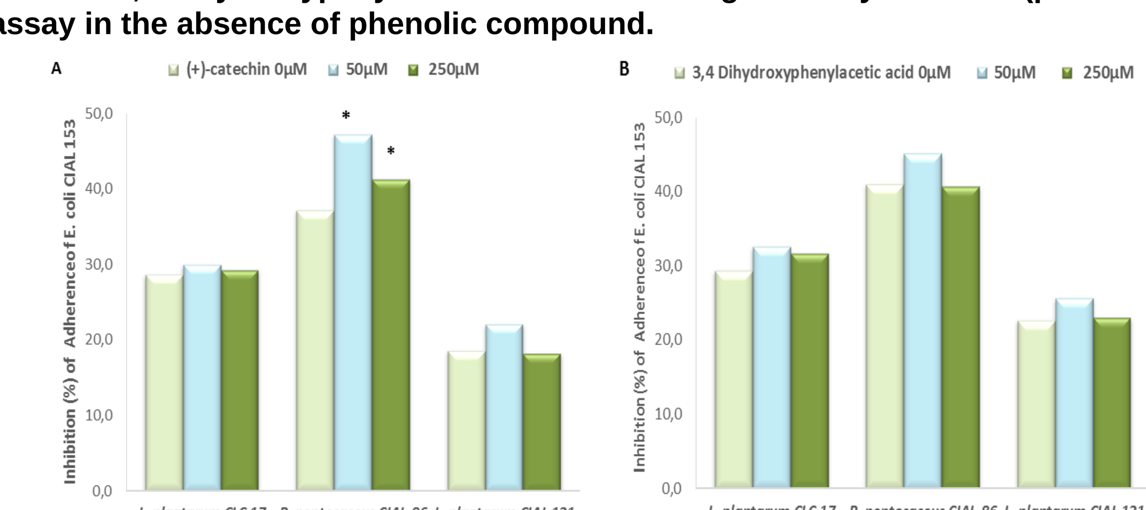


Fig. 3. Inhibition (%) of *E. coli* adherence to Caco-2 cells in the presence of *L. plantarum* CLC 17, *P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121 and in the absence and presence of (+)-catechin and 3, 4-dihydroxypheylacetic acid. * Mean significantly different (p < 0.05) from assay in the absence of phenolics

CONCLUSIONS

- ✓ Out of the eight probiotic preparations and three isolated LAB tested, two preparations (#6 & #7) and the reference strain, *L. plantarum* CLC 17, were able to release different phenolic metabolites (Table 1), that are known to be produced *in vivo* after wine consumption.
- ✓ For these three active probiotics, loss of bacteria viability was attenuated in the presence of the wine extract under nutrient-restricted culture conditions (Fig. 1).
- ✓ On the other hand, wine phenolic compounds [i.e., (+)-catechin] and wine-derived phenolic metabolites (i.e., 3,4-dihydroxypheylacetic acid), were found to enhance LAB adherence to Caco-2 cells (Fig. 2).
- ✓ Moreover, LAB strains and phenolic compounds seem to act synergistically to inhibit the adherence of *E. coli* CIAL-153 to Caco-2 (Fig.3) , which suggests that in the presence of polyphenols, probiotics could compete better with intestinal pathogens in adhering to the intestinal mucosa.

These *in vitro* results support the statement that benefits of wine polyphenols and probiotics may be enhance by their concomitant interaction at intestinal level, which could be used in future nutritional developments [5].

References

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