

## Supporting Information

### Selective inhibition of *Helicobacter pylori* carbonic anhydrases by carvacrol and thymol could impair biofilm production and the release of Outer Membrane Vesicles

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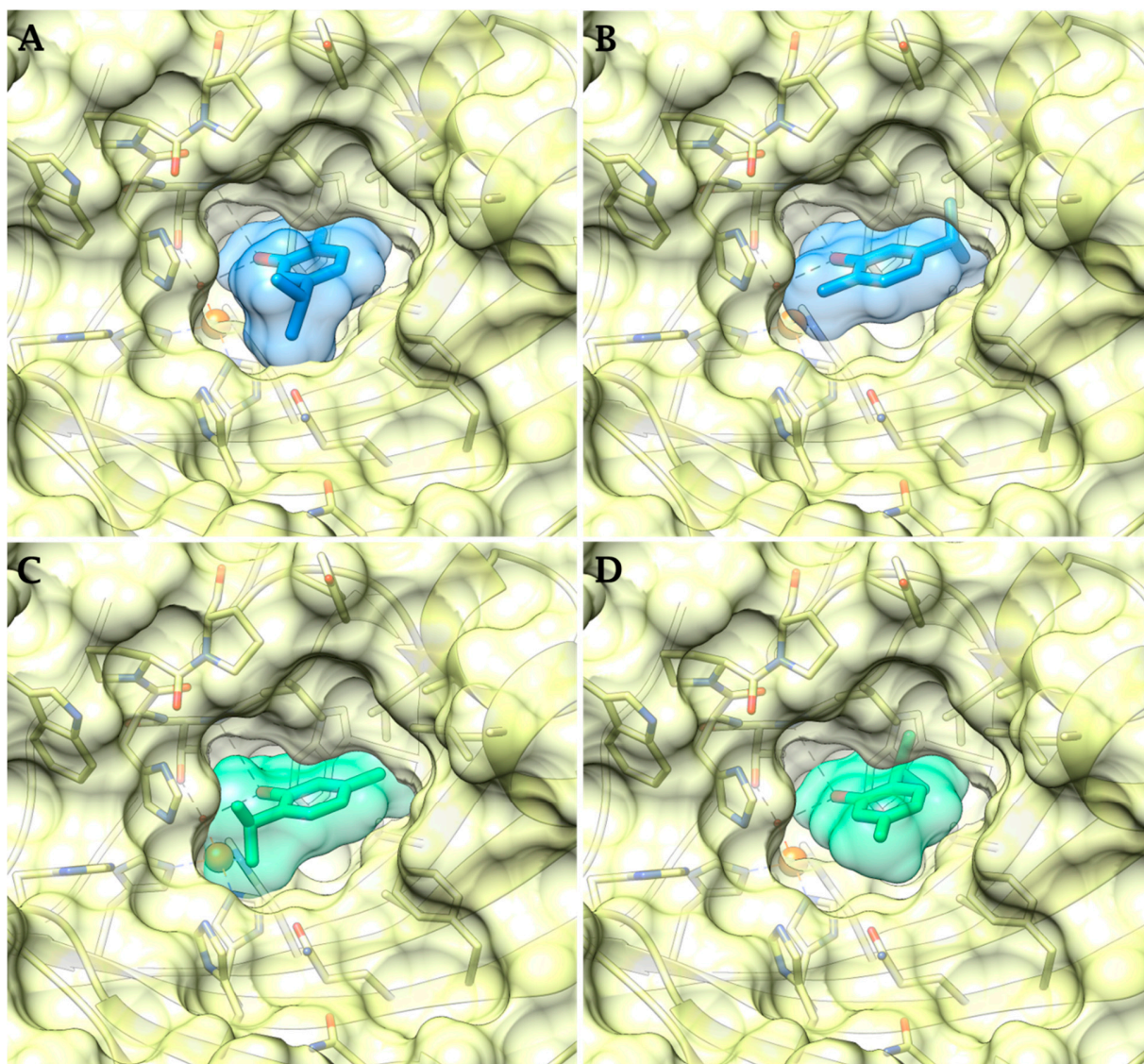
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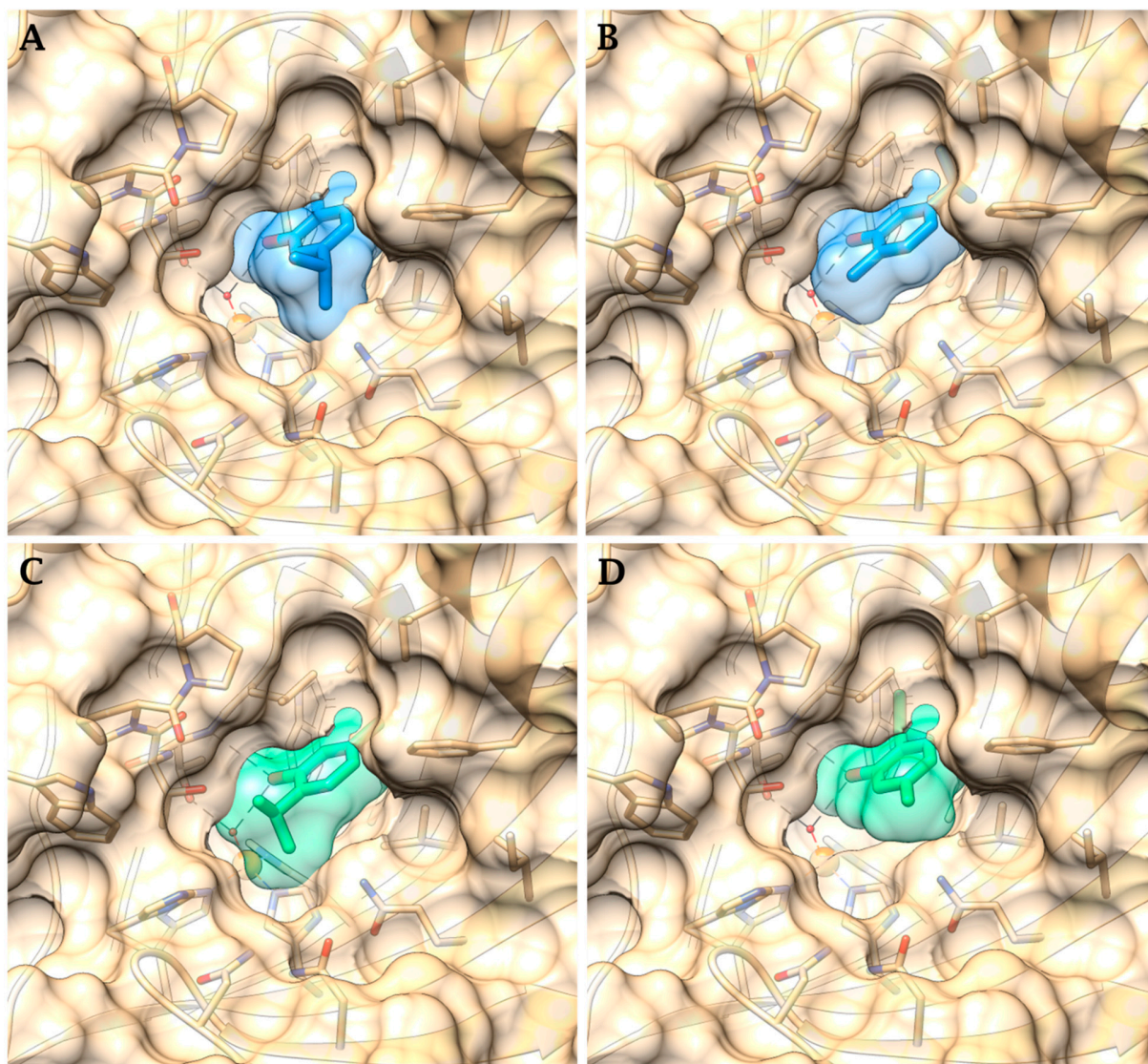
			10	20	30	40	50	60
			.... ....	.... ....	.... ....	.... ....	.... ....	.... ....
HpCA $\beta$ _A	11	-----	MKAFLGALEF	QENEYEELKE	LYESLGTKQK	PHTLFISCVD	SRVVPNLITG	
HpCA $\beta$ _B	11	-----	MKAFLGALEF	QENEYEELKE	LYESLGTKQK	PHTLFISCVD	SRVVPNLITG	
5SWC	1		SGLVPRGSHM	QRLIEGLQKF	REGYFSSHRD	LFEQLSHGQH	PRILFICCS	SRVDPNLITQ
			70	80	90	100	110	120
			.... ....	.... ....	.... ....	.... ....	.... ....	.... ....
HpCA $\beta$ _A	61		TKPGELYVIR	NMGNVIPPKT	SHKESLSTMA	SIEYAIVHVG	VQNLIICGHS	DCGACGSTHL
HpCA $\beta$ _B	61		TKPGELYVIR	NMGNVIPPKT	SHKESLSTMA	SIEYAIVHVG	VQNLIICGHS	DCGACGSTHL
5SWC	61		SEVGDLFVIR	NAGNIIPPYG	AANG--GEGA	AMEYALVALE	INQIIVCGHS	HCGAMKGLLK
			130	140	150	160	170	180
			.... ....	.... ....	.... ....	.... ....	.... ....	.... ....
HpCA $\beta$ _A	121		INDGITKAKT	PYIADWIQFL	EPIK-EELKN	HPQFSNHFAK	RSWLTERLNV	RLQINNLLSY
HpCA $\beta$ _B	121		INDGITKAKT	PYIADWIQFL	EPIK-EELKN	HPQFSNHFAK	RSWLTERLNV	RLQINNLLSY
5SWC	119		LNS--LQEK	PLVYDWLKHT	EATRRVLVDN	YSHLEGEDLI	EVAVAE--NI	LTQLKNLQTY
			190	200	210	220	230	
			.... ....	.... ....	.... ....	.... ....	.... ....	..
HpCA $\beta$ _A	180		DFIQERVVNN	ELKIFGWHYI	IETGRIYNNY	FESHFFIEPIE	ETIKQRKSHE	NF
HpCA $\beta$ _B	180		DFIQERVVNN	ELKIFGWHYI	IETGRIYNNY	FESHFFIEPIE	ETIKQRKSHE	NF
5SWC	175		PAHSRLHRG	DLSLHGWIYR	IEEGEVLAYD	GVLHDFVAP		

**Figure S1.** Sequence alignment of HpCA $\beta$  (target) and type I  $\beta$ -CA from *Synechocystis* sp. (pdb 5SWC, template).

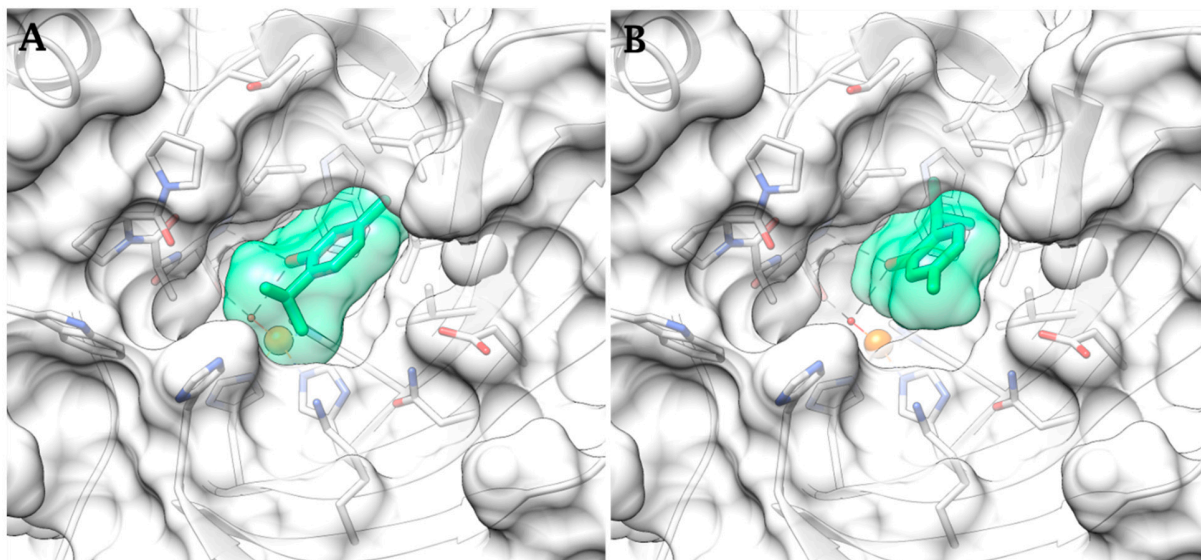


**Figure S2.** Two possible conformations of (A and B) carvacrol (blue), and (C and D) thymol (green) in hCA I active site, after superimposition with the carvacrol-HpCA $\alpha$  binding mode.

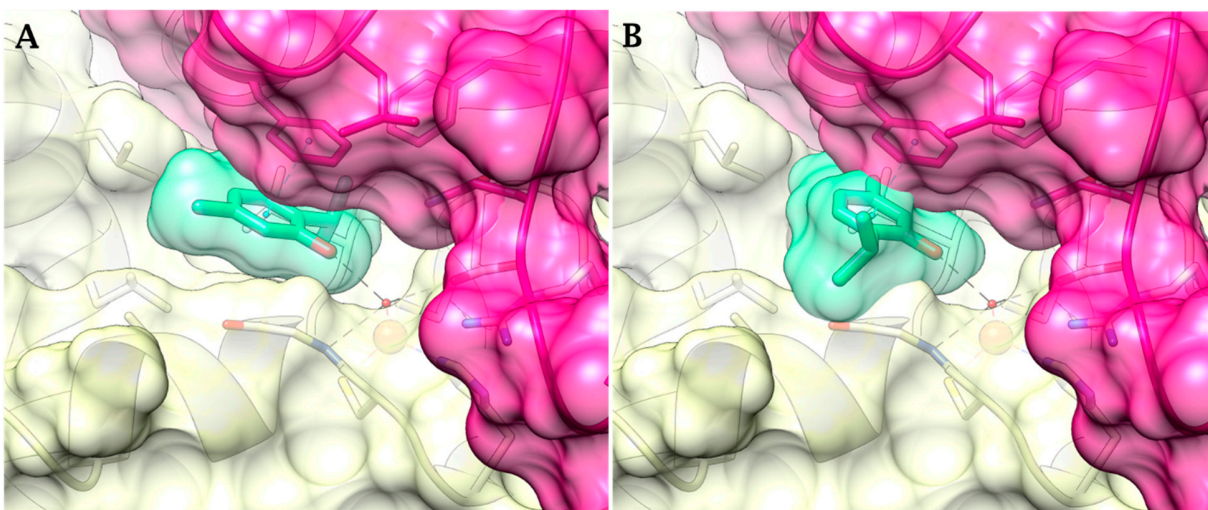




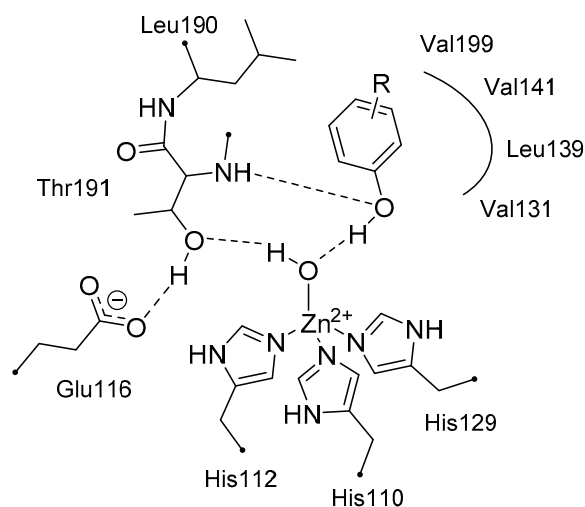
**Figure S3.** Two possible conformations of (A and B) carvacrol (blue), and (C and D) thymol (green) in hCA II active site, after superimposition with the carvacrol-HpCA $\alpha$  binding mode.



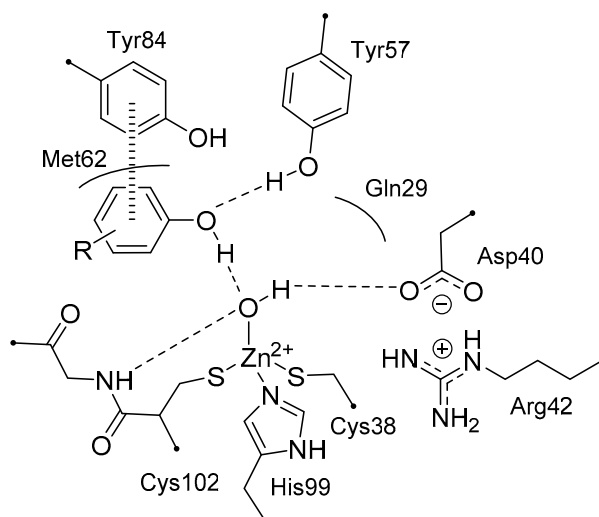
**Figure S4.** Two possible conformations of (A and B) (green) in HpCA $\alpha$  active site, after superimposition with the carvacrol-HpCA $\alpha$  binding mode.



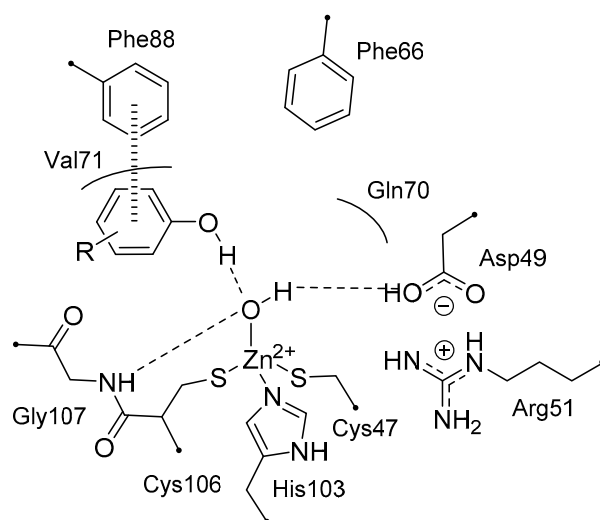
**Figure S5.** Two possible conformations of (A and B) (green) in MgCA active site, after superimposition with the carvacrol-MgCA binding mode.



**Figure S6.** 2D schematic representation of HpCA $\alpha$  residues involved in the interaction with phenols hypothesized by the *in silico* studies.



**Figure S7.** 2D schematic representation of HpCA $\beta$  residues involved in the interaction with phenols hypothesized by the *in silico* studies.



**Figure S8.** 2D schematic representation of MgCA residues involved in the interaction with phenols hypothesized by the *in silico* studies.

**Table S1.** Docking and MM-GBSA scores for carvacrol and thymol in hCA I, hCA II, HpCA $\alpha$ , HpCA $\beta$ , and MgCA active site.

Target	Ligand	Glide emodel	Docking score	MM-GBSA $\Delta G$ binding
hCA I	Carvacrol	–	–	–
	Thymol	–	–	–
hCA II	Carvacrol	–	–	–
	Thymol	–	–	–
HpCA $\alpha$	Carvacrol	-27.982	-4.727	-24.85
	Thymol	–	–	–
HpCA $\beta$	Carvacrol	-28.491	-5.654	-19.15
	Thymol	-41.021	-7.311	-23.75
MgCA	Carvacrol	-36.839	-6.478	-14.42
	Thymol	–	–	–

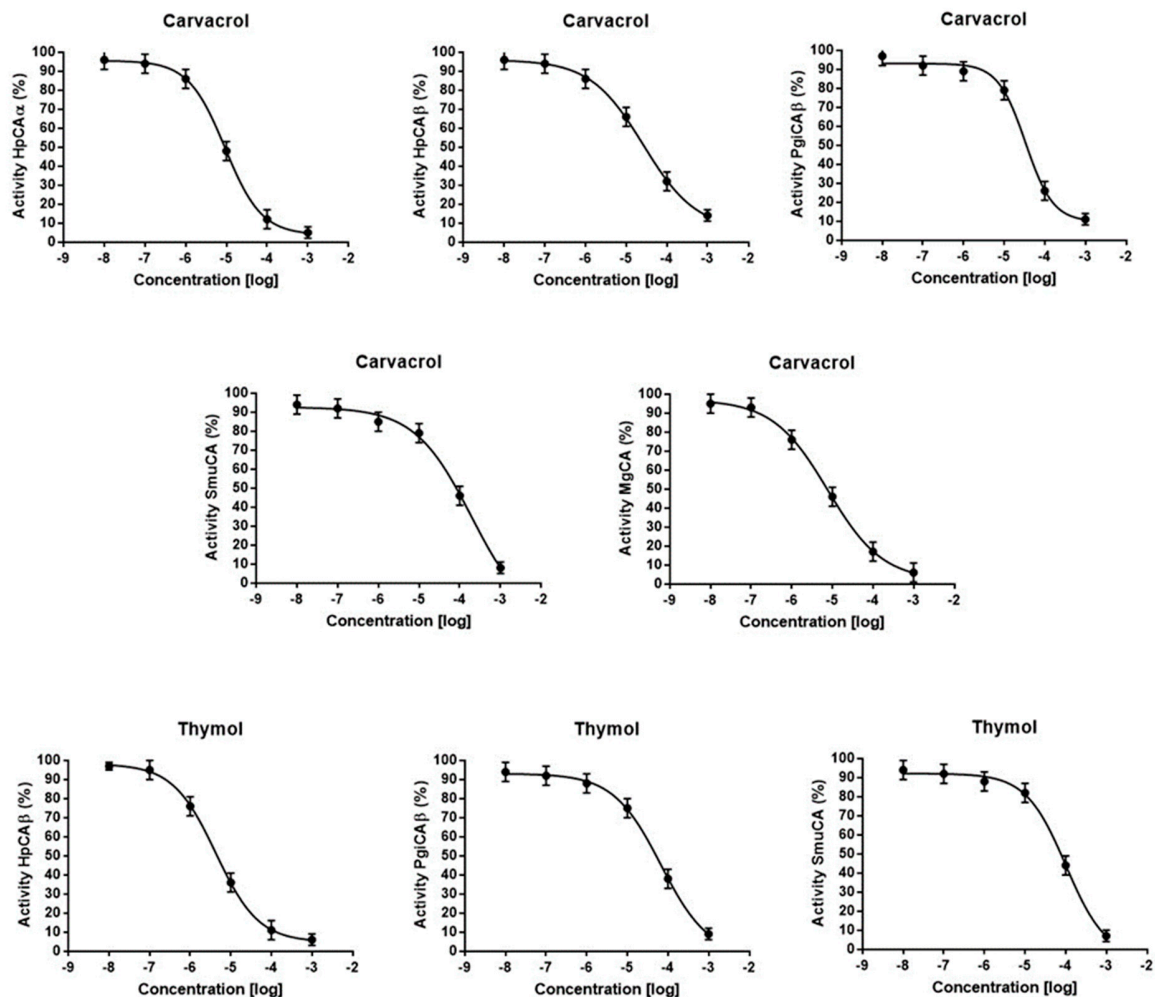


**Table S2.** Organisms, CA classes, acronyms and accession numbers of the amino acid sequences used in the phylogenetic analysis.

<b>Organism</b>	<b>CA class</b>	<b>Acronym</b>	<b>Accession number</b>
<i>Helicobacter pylori</i>	$\alpha$	HpylCA_alpha	WP_010882609.1
<i>Sulfurihydrogenibium</i> sp. YO3AOP1	$\alpha$	SspCA_alpha	WP_012459296.1
<i>Neisseria gonorrhoeae</i>	$\alpha$	NgonCA_alpha	WP_003688976.1
<i>Streptococcus salivarius</i>	$\alpha$	SsalCA_alpha	WP_002888224.1
<i>Lacticaseibacillus paracasei</i>	$\alpha$	Lpar_alpha_pro	WP_003573881.1
<i>Lacticaseibacillus rhamnosus</i>	$\alpha$	Lrha_alpha_pro	WP_005704914.1
<i>Lactobacillus acidophilus</i> ( <i>Lactobacillus plantarum</i> )	$\alpha$	Laci_alpha_pro	VDH11415.1
<i>Escherichia coli</i>	$\beta$	EcoCA_beta	WP_047081292.1
<i>Legionella pneumophila</i>	$\beta$	LpnCA_beta	WP_011946835.1
<i>Helicobacter pylori</i>	$\beta$	HpyCA_beta	WP_000642991.1
<i>Burkholderia thailandensis</i>	$\beta$	BthCA_beta	WP_009893276.1
<i>Brucella suis</i>	$\beta$	BsuCA_beta	AAN33967.1
<i>Methanothermobacter</i> <i>thermautotrophicus</i>	$\beta$	Cab_beta	WP_048061095.1
<i>Myroides injeensis</i>	$\beta$	MinCA_beta	WP_026038855.1
<i>Porphyromonas gingivalis</i>	$\beta$	PgiCA_beta	WP_012458351.1
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BLC1	$\beta$	Bani_beta_pro	AEN75835.1
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BB-12	$\beta$	Bbb12_beta_pro	ADC85071.1
<i>Bifidobacterium longum</i> W11	$\beta$	Blon_beta_pro	WP_071475326.1

## Raw data section

Carbonic Anhydrase inhibitory activity:



Microbiological assays:

Cristal violet assay for MBIC determination of **amoxicillin** (AMX) *versus Helicobacter pylori*

First experiment	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
	2,0420	2,1027	0,6297	0,6175
	1,7917	1,6029	0,4828	0,5998
	1,8848	1,1908	0,4802	0,6132
	1,4891	1,1937	0,3262	0,6212
	1,8742	1,1313	0,5031	0,4522
	1,5423	1,2948	0,3912	0,3670
	1,7235	1,6220	0,4114	0,3941
	1,5109	1,5093	0,6297	0,3863
	1,5797	1,3237	0,4828	0,6175
	1,6886	1,2467	0,4802	0,5998

	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
<b>Mean</b>	1,7127	1,4218	0,4817	0,5269
<b>subtraction of whites</b>	1,2310	0,8949		
	Positive CTRL	AMX Treated		
<b>% Biofilm formation</b>	100	72,70238434		

Second experiment	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
	1,457	1,503	0,675	0,329
	1,693	1,075	0,675	0,348
	1,164	1,077	0,366	0,348
	1,359	1,347	0,318	0,503
	1,227	0,901	0,318	0,639
	1,145	0,738	0,503	0,524
	1,037	1,346	0,634	0,504
	1,571	1,200	0,466	0,5998
	0,969	1,078	0,675	0,639
	0,986	1,020	0,634	0,524

	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
<b>Mean</b>	1,2609	1,1285	0,5265	0,4958
<b>subtraction of whites</b>	0,7344	0,6326		

	Positive CTRL	AMX Treated
% Biofilm formation	100	86,14613688

AlamarBlue assay for MBIC determination of **amoxicillin** (AMX) *versus Helicobacter pylori*

First experiment 570 nm	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
	0,5972	0,5745	0,4365	0,4028
	0,5977	0,6295	0,4369	0,4074
	0,6014	0,633	0,3937	0,4205
	0,6108	0,6125	0,3971	0,4432
600 nm	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
	0,2101	0,1995	0,5241	0,4968
	0,1914	0,1859	0,5338	0,5007
	0,2044	0,2001	0,4867	0,5167
	0,2066	0,1983	0,492	0,5373
(570 nm)	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
	0,6018	0,6124	0,4161	0,4185
(600 nm)	0,203125	0,19595	0,50915	0,512875
	Positive CTRL	AMX Treated		
MBIC	74,03425314	75,95753515		

Second experiment 570 nm	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
	0,688	0,644	0,42	0,426
	0,69	0,685	0,43	0,436
	0,644	0,694	0,415	0,445
	0,681	0,7	0,387	0,434
600 nm	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
	0,182	0,203	0,513	0,522
	0,171	0,166	0,528	0,535
	0,168	0,169	0,473	0,541

	0,176	0,174	0,522	0,53
	Positive CTRL	AMX Treated	Medium Sterility CTRL	AMX Sterility CTRL
(570 nm)	0,6758	0,6808	0,4130	0,4353
(600 nm)	0,17425	0,178	0,509	0,532
	Positive CTRL	AMX Treated		
MBIC	89,03962295	85,6201168		

CFU count for MBIC determination of **amoxicillin** (AMX) *versus Helicobacter pylori*

	Non treated (CFU/mL)	AMX Treated (CFU/mL)
First experiment	1,40E+06	8,10E+06
	3,80E+06	6,70E+06
Second experiment	1,51E+07	1,93E+07
	4,60E+07	4,25E+07



Cristal violet assay for MBIC determination of **carvacrol (CAR)** versus *Helicobacter pylori*

First experiment	Positive CTRL	CAR Treated	Medium Sterility CTRL	CAR Sterility CTRL
	0,6433	0,2253	0,2343	0,2004
	0,84	0,1881	0,2144	0,1896
	0,9323	0,1874	0,1857	0,1815
	0,7468	0,198	0,2017	0,1807
	0,7606	0,1795	0,2162	0,2358
	0,6639	0,1976	0,1881	0,1846
	0,7905	0,1969	0,1886	0,1807
	0,7726	0,2031	0,2192	0,2004
	0,893	0,1961	0,1881	0,2198
	0,7718	0,1912	0,1886	0,1846

	Positive CTRL	CAR Treated	Medium Sterility CTRL	CAR Sterility CTRL
Mean	0,78148	0,19632	0,20249	0,19581
subtraction of whites	0,57899	0,00051		
	Positive CTRL	CAR Treated		
% Biofilm formation	100	0,088084423		

Second experiment	Positive CTRL	CAR Treated	Medium Sterility CTRL	CAR Sterility CTRL
	0,9526	0,2969	0,2062	0,1986
	0,9861	0,1836	0,2681	0,246
	0,9518	0,1663	0,2437	0,2462
	0,8247	0,1645	0,2603	0,2406
	0,7595	0,1848	0,2482	0,1627
	0,7277	0,1664	0,2385	0,2361
	0,9844	0,2243	0,1764	0,1627
	0,9453	0,2743	0,2441	0,1555
	0,9797	0,1724	0,1764	0,1627
	0,9356	0,1916	0,2441	0,1555

	Positive CTRL	CAR Treated	Medium Sterility CTRL	CAR Sterility CTRL
Mean	0,90474	0,20251	0,2306	0,19666
subtraction of whites	0,67414	0,00585		
	Positive CTRL	CAR Treated		

<b>% Biofilm formation</b>	<b>100</b>	<b>0,867772273</b>
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AlamarBlue assay for MBIC determination of **carvacrol (CAR)** versus *Helicobacter pylori*

<b>First experiment</b> 570 nm	<b>Positive CTRL</b>	<b>CAR Treated</b>	<b>Medium Sterility CTRL</b>	<b>CAR Sterility CTRL</b>
	0,423	0,2863	0,3491	0,3404
	0,4491	0,3105	0,3376	0,3453
	0,4594	0,3462	0,3366	0,3378
	0,4646	0,3539	0,3352	0,3221
670 nm	<b>Positive CTRL</b>	<b>CAR Treated</b>	<b>Medium Sterility CTRL</b>	<b>CAR Sterility CTRL</b>
	0,2101	0,3294	0,3914	0,3861
	0,202	0,3538	0,3816	0,389
	0,2072	0,3933	0,3824	0,3809
	0,2305	0,4041	0,3806	0,3695
(570 nm)	<b>Positive CTRL</b>	<b>CAR Treated</b>	<b>Medium Sterility CTRL</b>	<b>CAR Sterility CTRL</b>
	0,449025	0,324225	0,339625	0,3364
(600 nm)	0,21245	0,37015	0,384	0,381375
	<b>Positive CTRL</b>	<b>CAR Treated</b>		
<b>MBIC</b>	64,79921108	15,01670066		

<b>Second experiment</b> 570 nm	<b>Positive CTRL</b>	<b>CAR Treated</b>	<b>Medium Sterility CTRL</b>	<b>CAR Sterility CTRL</b>
	0,4317	0,3092	0,207	0,3263
	0,3605	0,3299	0,3865	0,3146
	0,418	0,3081	0,3237	0,2925
	0,3749	0,3094	0,3261	0,2694
600 nm	<b>Positive CTRL</b>	<b>CAR Treated</b>	<b>Medium Sterility CTRL</b>	<b>CAR Sterility CTRL</b>
	0,1924	0,3581	0,2325	0,3681
	0,1804	0,3625	0,3129	0,3529
	0,2067	0,3093	0,3621	0,3293
	0,1844	0,3278	0,3665	0,3078

	<b>Positive CTRL</b>	<b>CAR Treated</b>	<b>Medium Sterility CTRL</b>	<b>CAR Sterility CTRL</b>
<b>(570 nm)</b>	<b>0,39628</b>	<b>0,31415</b>	<b>0,31083</b>	<b>0,30070</b>
<b>(600 nm)</b>	<b>0,19098</b>	<b>0,33943</b>	<b>0,31850</b>	<b>0,33953</b>
	<b>Positive CTRL</b>	<b>CAR Treated</b>		
<b>MBIC</b>	<b>68,97758462</b>	<b>19,54681043</b>		

CFU count for MBIC determination of **carvacrol (CAR)** *versus Helicobacter pylori*

	<b>Non treated (CFU/mL)</b>	<b>CAR Treated (CFU/mL)</b>
<b>First experiment</b>	<b>4,43E+07</b>	<b>0,00E+00</b>
	<b>2,49E+07</b>	<b>0,00E+00</b>
<b>Second experiment</b>	<b>6,60E+07</b>	<b>0,00E+00</b>
	<b>6,20E+07</b>	<b>0,00E+00</b>

Cristal violet data for MBIC determination of **thymol (THY)** versus *Helicobacter pylori*

First experiment	Positive CTRL	THY Treated	Medium Sterility CTRL	THY Sterility CTRL
	1,5161	0,8537	0,5647	0,6769
	1,5774	0,7529	0,4973	0,5269
	1,3881	0,8514	0,5434	0,7480
	1,5479	0,9176	0,5647	0,8963
	1,5107	0,6881	0,7548	0,7868
	2,5187	0,7480	0,5647	0,8918
	2,0421	0,707	0,8682	0,7794
	2,0092	0,8207	0,7548	0,8519
	1,8331	0,7771	0,4973	0,6769
	1,8954	0,7214	0,5434	0,5269

	Positive CTRL	THY Treated	Medium Sterility CTRL	THY Sterility CTRL
Mean	1,7839	0,7838	0,6153	0,7362
subtraction of whites	1,1685	0,0476		
	Positive CTRL	thy Treated		
% Biofilm formation	100	4,074314957		

Second experiment	Positive CTRL	THY Treated	Medium Sterility CTRL	THY Sterility CTRL
	2,0217	0,9475	0,7794	0,7794
	1,7013	1,0887	0,8049	1,1133
	1,6374	1,0115	0,8835	0,7882
	1,9662	1,4667	1,2155	0,9052
	1,6075	0,9475	0,8835	1,1533
	1,9513	0,7485	0,8049	0,9052
	2,4107	1,0115	0,8049	0,8049
	1,8531	0,7956	0,8835	0,7882
	1,5566	0,7625	1,2155	0,9052
	1,3254	0,8056	0,8049	1,1533

	Positive CTRL	THY Treated	Medium Sterility CTRL	THY Sterility CTRL
Mean	1,8031	0,9586	0,9081	0,9296
subtraction of whites	0,8951	0,0289		
	Positive CTRL	TIM Treated		

<b>% Biofilm formation</b>	<b>100</b>	<b>3,233266672</b>
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AlamarBlue data for MBIC determination of **thymol (THY)** versus *Helicobacter pylori*

<b>First experiment</b> 570 nm	<b>Positive CTRL</b>	<b>THY Treated</b>	<b>Medium Sterility CTRL</b>	<b>THY Sterility CTRL</b>
	<b>0,413</b>	<b>0,32</b>	<b>0,2269</b>	<b>0,3004</b>
	<b>0,418</b>	<b>0,3036</b>	<b>0,302</b>	<b>0,306</b>
	<b>0,3876</b>	<b>0,2837</b>	<b>0,2859</b>	<b>0,3121</b>
	<b>0,4183</b>	<b>0,2917</b>	<b>0,2889</b>	<b>0,2988</b>
600 nm	<b>Positive CTRL</b>	<b>THY Treated</b>	<b>Medium Sterility CTRL</b>	<b>THY Sterility CTRL</b>
	<b>0,1349</b>	<b>0,3755</b>	<b>0,2664</b>	<b>0,3592</b>
	<b>0,1988</b>	<b>0,3575</b>	<b>0,3599</b>	<b>0,3682</b>
	<b>0,1884</b>	<b>0,3318</b>	<b>0,3377</b>	<b>0,37940</b>
	<b>0,1444</b>	<b>0,3439</b>	<b>0,346</b>	<b>0,35900</b>
	<b>Positive CTRL</b>	<b>THY Treated</b>	<b>Medium Sterility CTRL</b>	<b>THY Sterility CTRL</b>
<b>(570 nm)</b>	<b>0,4092</b>	<b>0,2998</b>	<b>0,2759</b>	<b>0,3043</b>
<b>(600 nm)</b>	<b>0,166625</b>	<b>0,352175</b>	<b>0,3275</b>	<b>0,36645</b>
	<b>Positive CTRL</b>	<b>THY Treated</b>		
<b>MBIC</b>	<b>73,58131957</b>	<b>12,84515501</b>		

<b>Second experiment</b> 570 nm	<b>Positive CTRL</b>	<b>THY Treated</b>	<b>Medium Sterility CTRL</b>	<b>THY Sterility CTRL</b>
	<b>0,4207</b>	<b>0,3007</b>	<b>0,3068</b>	<b>0,3137</b>
	<b>0,45</b>	<b>0,3259</b>	<b>0,2743</b>	<b>0,3435</b>
	<b>0,4369</b>	<b>0,2767</b>	<b>0,2958</b>	<b>0,2983</b>
	<b>0,455</b>	<b>0,3319</b>	<b>0,2894</b>	<b>0,2822</b>
600 nm	<b>Positive CTRL</b>	<b>THY Treated</b>	<b>Medium Sterility CTRL</b>	<b>THY Sterility CTRL</b>
	<b>0,1602</b>	<b>0,3642</b>	<b>0,3713</b>	<b>0,3843</b>
	<b>0,1746</b>	<b>0,398</b>	<b>0,3345</b>	<b>0,4295</b>
	<b>0,1722</b>	<b>0,3386</b>	<b>0,3542</b>	<b>0,3661</b>
	<b>0,1664</b>	<b>0,4153</b>	<b>0,3477</b>	<b>0,342</b>



	<b>Positive CTRL</b>	<b>THY Treated</b>	<b>Medium Sterility CTRL</b>	<b>THY Sterility CTRL</b>
<b>(570 nm)</b>	<b>0,4407</b>	<b>0,3088</b>	<b>0,2916</b>	<b>0,3094</b>
<b>(600 nm)</b>	<b>0,16835</b>	<b>0,379025</b>	<b>0,351925</b>	<b>0,380475</b>
	<b>Positive CTRL</b>	<b>THY Treated</b>		
<b>MBIC</b>	<b>75,39339975</b>	<b>10,33354534</b>		

CFU count for MBIC determination of **thymol (TIM)** *versus Helicobacter pylori*

	<b>Non treated (CFU/mL)</b>	<b>THY Treated (CFU/mL)</b>
<b>First experiment</b>	<b>1,46E+07</b>	<b>0,00E+00</b>
	<b>2,97E+07</b>	<b>0,00E+00</b>
<b>Second experiment</b>	<b>6,47E+07</b>	<b>0,00E+00</b>
	<b>5,33E+07</b>	<b>0,00E+00</b>

Flow cytometry analyses:

**OMV concentrations obtained by flow cytometry analyses.**

Carvacrol			
Planktonic CTRL		Planktonic Carvacrol	
<i>Total OMVs</i>	<i>OMVs eDNA<sup>+</sup></i>	<i>Total OMVs</i>	<i>OMVs eDNA<sup>+</sup></i>
45.99	15.00	16.50	9.00
70.80	33.00	11.01	1.50
44.40	15.00	10.20	3.00

Thymol			
Planktonic CTRL		Planktonic Thymol	
<i>Total OMVs</i>	<i>OMVs eDNA<sup>+</sup></i>	<i>Total OMVs</i>	<i>OMVs eDNA<sup>+</sup></i>
248.25	209.25	60.60	3.60
212.40	139.80	38.25	3.75
232.20	162.00	20.49	3.99
163.50	65.25	115.80	58.20
111.99	65.01	48.75	31.50
90.51	52.50	47.01	29.49

Amoxicillin			
Planktonic CTRL		Planktonic Amoxicillin	
<i>Total OMVs</i>	<i>OMVs eDNA<sup>+</sup></i>	<i>Total OMVs</i>	<i>OMVs eDNA<sup>+</sup></i>
33.60	10.80	36.00	7.80
39.00	21.75	28.00	7.80
40.50	18.75	26.75	6.75
58.14	8.25	31.26	1.38
60.36	9.75	48.00	5.13
67.80	9.60	36.87	3.42
Biofilm CTRL		Biofilm Amoxicillin	
<i>Total OMVs</i>	<i>OMVs eDNA<sup>+</sup></i>	<i>Total OMVs</i>	<i>OMVs eDNA<sup>+</sup></i>
527.40	372.60	510.75	225.00
630.00	447.75	450.99	225.51
568.20	393.60	593.40	331.20
142.75	29.36	162.66	26.67
218.66	108.00	209.64	51.75
287.29	147.58	238.86	61.14