

Primers for LAB laccases amplification

ENOLAB code	LAB species	Strain	Primer	Nucleotide sequence	Tm	Restriction enzymes
4314	<i>Lactobacillus paracasei</i>	Lb446L	F R	GATGCTAGCATGAAAACCTATACGGACTATTTTC CCGGGATCCTTACATTTTCATTCCCATTTTT	55°C	NheI and BamHI
3909	<i>Pediococcus parvulus</i>	R211A	F R	GATGCTAGCATGGCAAAGCAAGTTTATAACGA CCGGAATTCTTACATCTTCATGCC	58°C	NheI and EcoRI
5298	<i>Lactococcus lactis</i>	M16	F R	GATGCTAGCATGTTAAAATTCTTGTCAGAG CCGGAATTCTTACATTTTCATTCCCATTTTTTC	58°C	NheI and EcoRI

Laccase gene sequences

L. lactis

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L. paracasei

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P. parvulus

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SDS protein gels and protein yields of LAB laccases

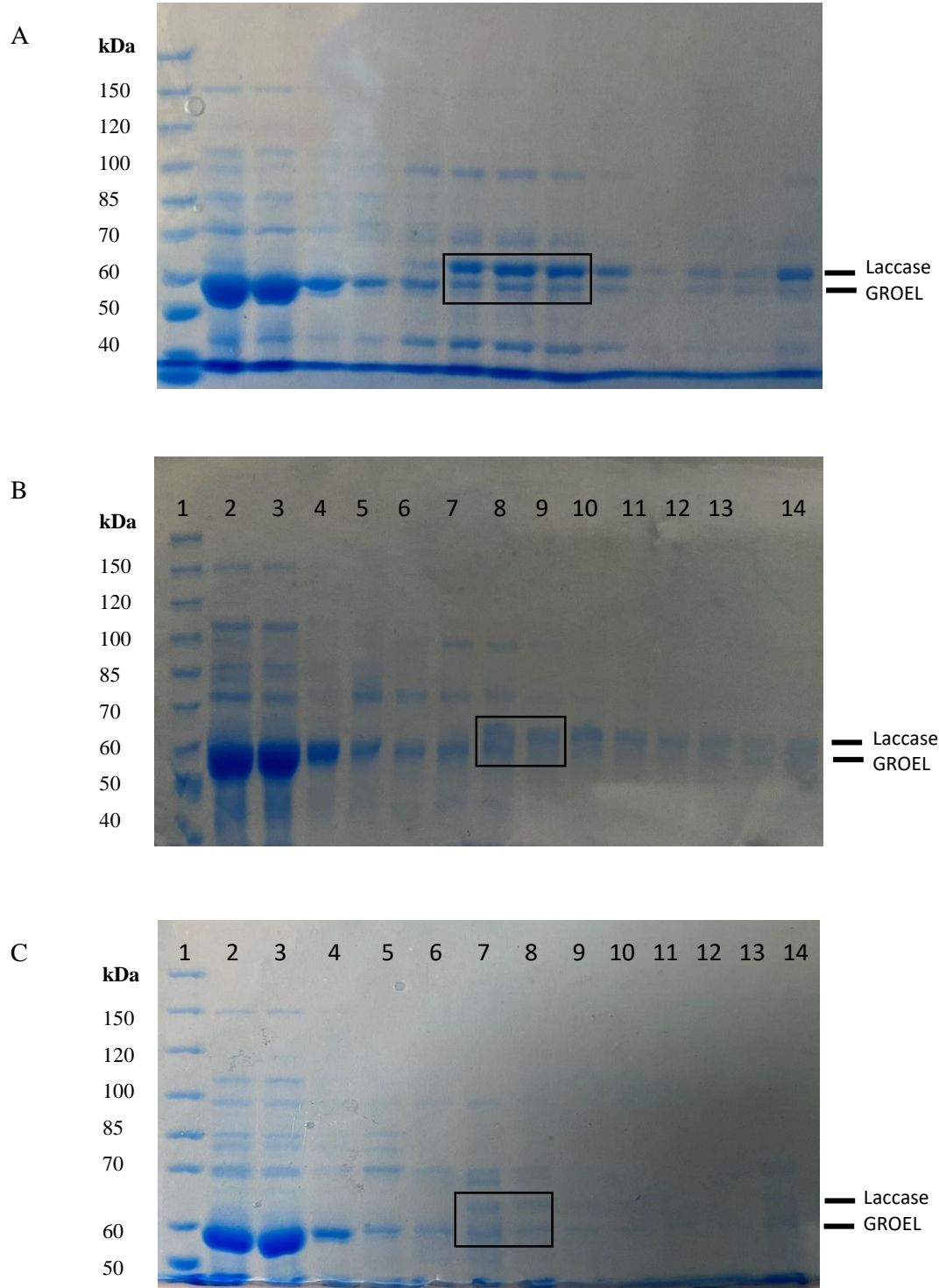


Figure 1. PageBlue stained 7.5% SDS-polyacrylamide gel electrophoresis of the four laccases. A. *P. parvulus* laccase, B. *L. paracasei* laccase, C. *L. lactis* laccase. Aliquots of the fractions from the different purification steps are shown in lanes. Lane 1, Page Ruler unstained; lane 2, crude cell extract, corresponding to the loaded sample on the column; lane 3, unbound proteins collected in the flowthrough; lanes 4-5, column washing fraction, lanes 6-13, consecutive fractions obtained after elution of the bound proteins from the Ni²⁺-NTA agarose matrix; and lane 14, pooled and dialyzed fraction. Squares highlight laccase bands with highest intensity.

Protein concentration and yield of the different bacterial laccases

Laccase	Protein concentration (mg/mL)	Protein yield (mg)
<i>P. parvulus</i>	2.51	2.01
<i>L. paracasei</i>	13.64	10.91
<i>L. lactis</i>	1.56	1.88

Biochemical characterization

Optimum pH	<i>P. parvulus</i>		<i>L. paracasei</i>		<i>L. lactis</i>	
pH	Mean	std	Mean	std	Mean	std
1	0,60	0,28	0,00	0,00	1,99	0,04
1,5	0,81	0,34	0,03	0,14	1,78	0,49
2	2,45	0,70	0,61	0,38	2,56	0,46
2,5	100,00	0,00	13,32	1,05	32,70	1,44
3	64,79	3,94	70,82	3,17	68,44	1,82
3,5	53,43	2,24	100,00	0,00	100,00	0,00
4	29,22	0,02	54,46	3,04	77,22	1,33
4,5	0,84	0,52	2,57	0,32	15,39	0,22
5	0,17	0,16	0,39	0,20	3,31	0,50
5,5	0,20	0,18	0,29	0,26	1,34	0,26
6	0,37	0,22	0,00	0,00	0,00	0,00
6,5	0,00	0,00	0,77	0,71	0,00	0,00
7	0,86	0,14	0,00	0,00	3,47	3,40

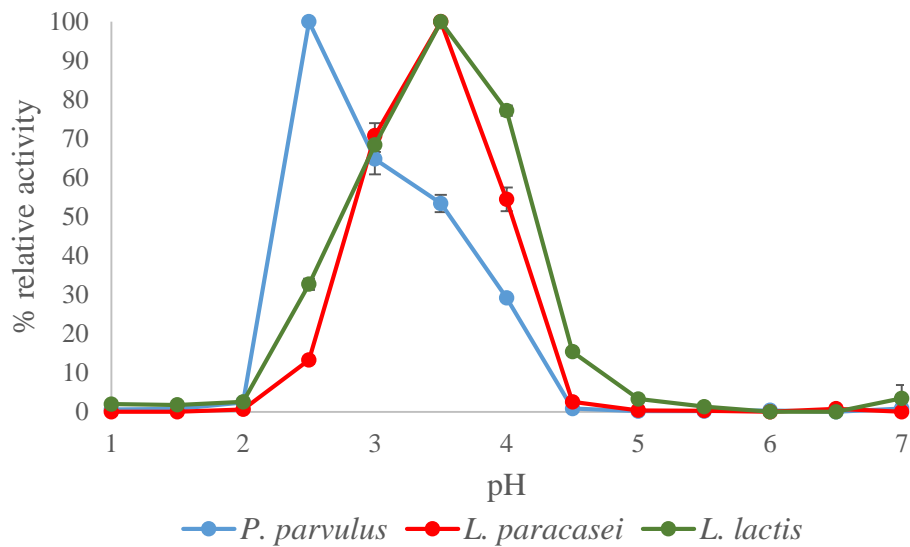


Figure 2. Percentage of relative activity on ABTS of the LAB laccases at several pH values.

Optimum temperature							
	<i>P. parvulus</i>		<i>L. paracasei</i>		<i>L. lactis</i>		
T	Mean	std	Mean	std	Mean	std	
4,0	86,39	4,63	51,40	3,26	78,13	3,25	
15,0	94,62	4,31	60,56	4,79	89,97	4,54	
28,0	100,00	0,00	100,00	0,00	100,00	0,00	
37,0	72,68	2,44	80,39	5,80	73,98	5,05	
45,0	31,64	1,41	63,47	5,12	57,43	2,85	
55,0	8,19	0,68	37,65	3,95	40,22	2,25	
65,0	4,14	0,94	21,44	2,91	26,14	3,53	
75,0	5,81	0,45	12,20	1,22	20,61	1,50	
85,0	3,45	0,19	11,87	3,27	19,73	1,73	
99,0	1,17	0,61	1,99	0,42	3,02	1,53	

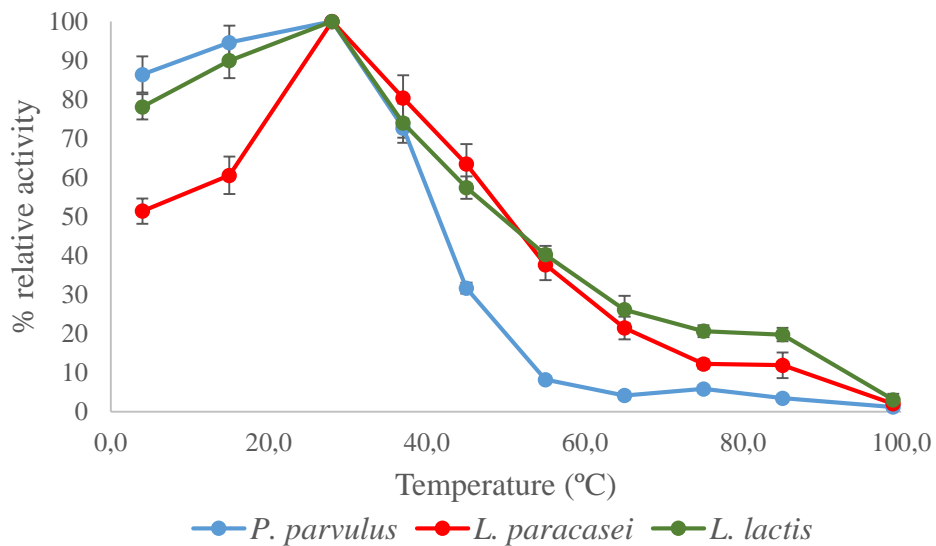


Figure 3. Percentage of relative activity on ABTS of the LAB laccases at several temperature values.

Thermostability							
	<i>P. parvulus</i>		<i>L. paracasei</i>		<i>L. lactis</i>		
T	Mean	std	Mean	std	Mean	std	
28,0	100,00	0,00	78,48	4,76	100,00	0,00	
37,0	62,30	5,20	100,00	0,00	92,10	4,40	
45,0	27,51	1,86	69,70	4,94	55,33	4,44	
55,0	8,05	0,84	30,68	0,77	26,04	4,79	
65,0	4,09	0,80	14,85	3,98	19,49	4,49	
75,0	2,64	2,09	8,51	0,55	5,52	1,52	
85,0	1,21	1,13	2,82	1,95	7,41	0,35	
99,0	0,57	0,10	0,75	0,27	2,79	0,81	

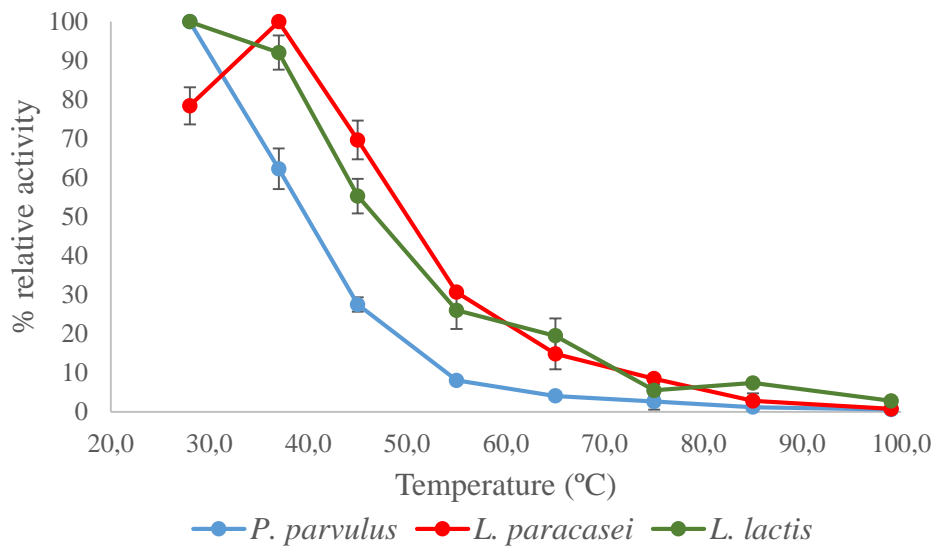


Figure 4. Percentage of relative activity on ABTS of the LAB laccases at several temperature values after 10 minute's incubation of enzymes.

Inhibitors	<i>P. parvulus</i>		<i>L. paracasei</i>		<i>L. lactis</i>	
	Mean	std	Mean	std	Mean	std
Control	100,0	0,00	100,00	0,00	100,00	0,00
ZnCl ₂	68,5	7,23	71,17	6,96	55,35	7,00
Bipy.	96,3	6,72	88,00	3,84	44,77	6,85
EDTA	99,9	4,64	97,62	2,58	98,74	2,19
Thio. Ac.	5,5	1,46	1,05	0,24	2,46	2,62
Cys	3,2	0,89	0,19	0,28	2,26	1,98
Na. Az.	29,7	4,69	36,35	2,60	17,63	5,44
Semicarb.	4,4	0,64	2,71	1,11	5,42	1,45
Rasag.	67,8	6,76	84,56	6,56	100,00	0,00
Phenan.	39,6	1,82	29,83	6,97	99,53	0,82
Parg.	57,2	4,92	70,78	6,34	94,24	5,47
EDC	54,7	3,06	100,00	0,00	96,13	5,71
Deprenyl	28,3	2,26	62,76	4,26	84,98	2,84
Cyclop.	41,5	2,13	85,80	8,13	98,95	1,83
Clorgyl.	37,0	9,44	46,73	3,66	55,04	7,64
NaCl	33,6	1,55	62,09	2,50	92,92	0,83
NaF	8,3	0,26	26,45	3,54	42,99	5,99

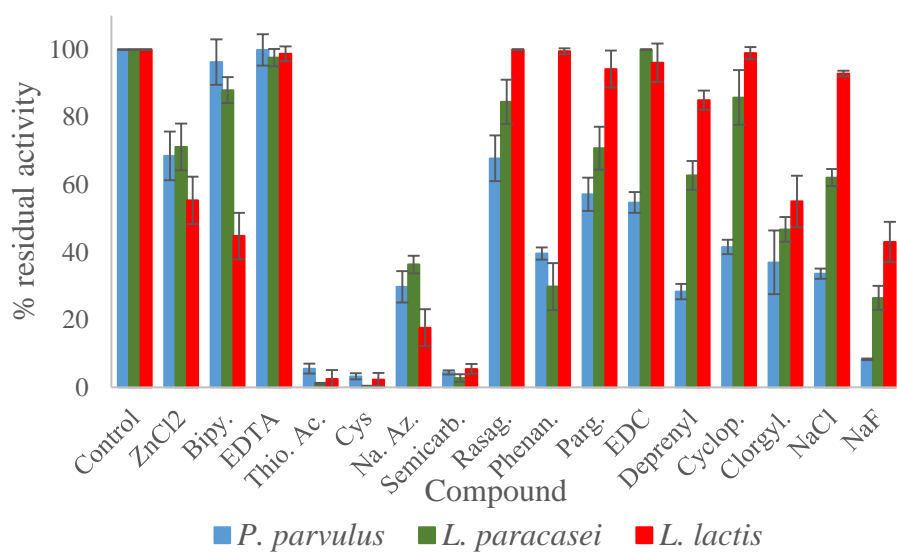


Figure 5. Effect of putative inhibitors on *P. parvulus* (blue), *L. paracasei* (green), and *L. lactis* (red) laccases. EDC: N-(3-dimethyl aminopropyl)-N'-ethyl carbodiimide; ZnCl₂: zinc chloride; Bipy.: 2,20-bipyridyl; EDTA: ethylenediaminetetraacetic acid; Thio. ac.: thioglycolic acid; Cys.: cysteine; Na Az.: sodium azide; Semicarb.: semicarbazide; Rasag.: rasagiline; Phenan.: 1,10-phenanthroline; Cyclop.: cyclopropenyl; Depren.: deprenyl; Clorgyl.: clorgyline; Parg.: pargyline; NaCl: sodium chloride; NaF: sodium fluoride. The plotted values represent means \pm standard deviations of triplicate assays.

Kinetic parameters of heterologous bacterial laccases

Laccases	$k_{0,5}$ (mM)	Specific activity (U/mg)
<i>P. parvulus</i>	0.21	2.58
<i>L. paracasei</i>	0.48	2.64
<i>L. lactis</i>	0.17	0.53

Biogenic amines degradation by LAB laccases

	Laccase	ABTS 2mM		No mediator		Epicatechin	
		Mean	std	Mean	std	Mean	std
	<i>L. paracasei</i>						
BA	His	0	0	0	0,00	3,9	2,55
	Tyr	79,95	1,48	0	0,00	2,25	0,21
	Put	0	0	0	0,00	0	0,00
	Dop	100	0	97,2	3,96	57,3	0,00
	PEA	0	0	2,95	1,48	9	0,71
	<i>P parvulus</i>						
BA	His	0	0	1,15	1,63	0,565	0,62
	Tyr	76,9	4,24	12	2,40	5,7	0,57
	Put	0	0	11,35	1,34	10,65	1,34
	Dop	100	0	100	0,00	47,65	0,49
	PEA	0	0	17,75	0,35	0	0,00
	<i>L.lactis</i>						
BA	His	0	0	0	0,00	0	0,00
	Tyr	24,75	0,21	40,75	5,59	0	0,00
	Put	0	0	0	0,00	0	0,00
	Dop	100	0	91,7	1,84	65,35	4,60
	PEA	0	0	22,1	1,84	7,45	2,90