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Identification of bacterial endophytes of interest for coffee crop in Vietnam[1]

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Introduction.

In Vietnam, about 250000 ha of plantation are covered with coffee trees over 20 years old which must be replanted by 2031. This rejuvenation is an expensive process due to the soil treatments necessary to overcome the high failure rate caused by nematodes and fungal pathogens[2,3,4]. In order to study their potential use in the rejuvenation process, we recovered eighty bacterial endophytes from *Coffea canephora* (roots and seeds) and *C. liberica* (roots). The isolates were identified (16S rDNA) and characterized *in vitro* for some plant growth promoting and biocontrol activities. Finally, their direct nematicidal effect on *Radopholus duriophilus* and *Pratylenchus coffeae*, as well as their antifungal effect on *Fusarium oxysporum* were studied with direct confrontations.

Materials and methods. See Duong et al. (2021)

Results.

The bacterial endophytes displayed several plant growth promoting and biocontrol traits including: the phosphate solubilization, the indolic compounds, siderophores, HCN, esterase, lipase, gelatinase and chitinase production. 17 isolates exhibited a nematicidal activity (**Table 1**), among which 5 were efficient on both *R. duriophilus* and *P. coffeae* (**Table 2**, **Figure 1**). Moreover, 17 isolates displayed an antifungal activity on *F. oxysporum* with a growth inhibition percentage comprised between 8 % and 50 % (**Table 3**)

Conclusion/Perspectives.

Coffee bacterial endophytes have a great potential of utilization in order to reduce agrochemical inputs consumption. Biocontrol efficacy *in planta* is yet to be demonstrated. Nevertheless, results concerning the nematode biocontrol capacity of one of the most promising isolate under coffee nursery conditions are currently submitted for publication.

Table 1: PGP and BC traits and nematicidal activity on *R. duriophilus* (RR: Mortality Relative Risk = $\%mortality_{treatment} / \%mortality_{control}$)

Isolate	Genus	Closely related species	Ind	Pho	Sid	Gel	Chi	Lip	Est	*R. durio* (RR±SE)
CCBMTR1	*Bacillus*	*timonensis*	++	-	-	-	-	-	++	17.95±1.17
CCBLR2	*Bacillus*	*timonensis*	++	-	-	-	-	-	-	16.52±1.17
CCBMTS9	*Methylobacterium*	*tardum*	++	-	-	-	-	-	-	14.22±1.17
LCBLR16	*Paenibacillus*	*cellulosilyticus*	-	-	-	-	-	++	-	13.86±1.17
LCBLR3	*Bacillus*	*wuyishanensis*	+	-	-	-	-	+	-	11.12±1.18
CCBLR15	*Bacillus*	*cereus sensu lato*	-	+	+	+	+	-	+	5.56±1.09
CCBLR14	*Bacillus*	*mycoides*	-	+	-	++	-	-	+	5.52±1.09
CCBLR1	*Bacillus*	*cereus sensu lato*	-	+	-	+	+	+	+	5.36±1.09
CCBLR13	*Bacillus*	*cereus sensu lato*	-	+	-	-	+	+	++	5.23±1.09
CCBMTR13	*Lechevalieria*	*aerocolonigenes*	-	-	++	+++	+++	+	+++	5.03±1.09
CCBMTS12	*Paracoccus*	*sanguinis*	+++	-	-	-	-	-	-	3.19±1.08
LCBLR5	*Herbaspirillum*	*frisingense*	-	-	+++	-	-	-	-	2.75±1.06
CCBLR22	*Pseudomonas*	*nitroreducens*	-	-	+++	-	-	-	-	2.51±1.06
CCBMTR6	*Streptomyces*	*mobaraensis*	-	-	-	-	+	-	+	2.45±1.21
CCBMTR4	*Bacillus*	*cereus sensu lato*	-	+	+	+	+	+	++	2.38±1.06
LCBLR12	*Arthrobacter*	*phenanthrenivorans*	++	-	-	-	-	-	-	2.36±1.06
LCBLR6	*Enterobacter*	*cancerogenus*	+++	+++	+	-	-	-	++	2.32±1.05

NB Tables 1 and 3
Ind: Indolic compounds; Pho: Phosphate solubilization; Sid : Siderophores; Gel: Gelatinases; Chi: Chitinases; Lip: Lipases; Est: Estrase

Figure 1: Logistic regression of the *R. duriophilus* mortality in function of the bacterial concentration

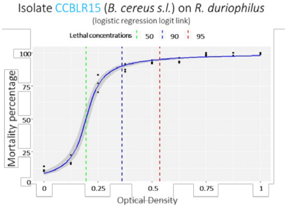

Table 3: PGP and BC traits and antifungal activity on *F. oxysporum* (inh % : percentage of growth inhibition)

Isolate	Genus	Closely related species	Ind	Pho	Sid	Hcn	Gel	Chi	Lip	Est	*F.oxy* (inh%±SE)
CLBLR18	*Burkholderia*	*cenocepacia*	-	+++	+++	-	++	+	+++	++	49.77±0.08
CCBLR24	*Bacillus*	*subtilis*	-	-	-	-	+++	-	+	++	40.77±0.06
CCBMTR6	*Streptomyces*	*mobaraensis*	-	-	-	-	-	+	-	+	40.76±0.04
CCBLR23	*Bacillus*	*subtilis*	-	-	+++	-	+++	-	+	-	30.52±0.01
CLBLR6	*Enterobacter*	*cancerogenus*	+++	+++	+	-	-	-	-	++	29.17±0.07
CLBLR13	*Burkholderia*	*seminalis*	-	+	+++	-	-	-	+++	++	26.99±0.01
CCBLR25	*Burkholderia*	*cenocepacia*	-	+	+++	-	-	-	+++	+++	26.50±0.01
CCBLR22	*Pseudomonas*	*nitroreducens*	-	-	+++	-	-	-	-	-	22.18±0.04
CLBLR8	*Bacillus*	*altitudinis*	-	-	-	-	+++	-	-	++	18.43±0.15
CCBMTS5	*Curtobacterium*	*oceanosedimentum*	-	+	-	-	-	+	-	-	17.68±0.06
CCBMTR3	*Kitasatospora*	*phosalacinea*	-	-	-	-	-	+++	++	+++	13.05±0.05
CLBLR7	*Enterobacter*	*asburiae*	++	++	+	-	-	-	-	-	12.65±0.06
CCBMTS7	*Curtobacterium*	*citreum*	-	+	-	-	-	-	+++	+	12.64±0.03
CCBLR21	*Pseudomonas*	*putida*	-	+++	++	+	+++	-	+++	++	11.30±0.04
CCBMTS8	*Brachybacterium*	*squillarum*	+	+	-	-	++	-	-	-	10.26±0.09
CCBMTR5	*Kitasatospora*	*arboriphila*	-	-	-	-	-	++	-	-	9.00±0.04
CCBLR5	*Luteibacter*	*yeojuensis*	+++	++	+	-	++	-	+	+	8.62±0.02

Table 2: Lethal concentrations on *R. duriophilus* and *P. coffeae* (24 h exposure, NE : Non Efficient, ND : Non Determined)

Isolate	Genus	Closely related species	*R. durio* (RR±SE)	Lethal concentrations (LC) *R. duriophilus* (OD_{600nm}, 24 h)			Lethal concentrations (LC) *P. coffeae* (OD_{600nm}, 24 h)			CFU/mL
				LC_{50}±SE	LC_{90}±SE	LC_{95}±SE	LC_{50}±SE	LC_{90}±SE	LC_{95}±SE	
CCBMTR1	*Bacillus*	*timonensis*	17.95±1.17	0.53±0.02	0.78±0.02	0.83±0.02	NE	NE	NE	10^4-10^5
CCBLR2	*Bacillus*	*timonensis*	16.52±1.17	NE	NE	NE	ND	ND	ND	10^5-10^6
CCBLR15	*Bacillus*	*cereus sensu lato*	5.56±1.09	0.20±0.01	0.36±0.02	0.54±0.03	0.40±0.01	0.52±0.01	0.56±0.02	10^6-10^7
CCBLR14	*Bacillus*	*mycoides*	5.52±1.09	0.31±0.01	0.48±0.01	0.54±0.01	0.39±0.00	0.47±0.01	0.48±0.01	10^6-10^7
CCBLR1	*Bacillus*	*cereus sensu lato*	5.36±1.09	0.61±0.02	0.83±0.02	0.88±0.02	0.55±0.01	0.72±0.01	0.78±0.02	10^6-10^7
CCBLR13	*Bacillus*	*cereus sensu lato*	5.23±1.09	0.18±0.01	0.35±0.01	0.53±0.03	0.50±0.01	0.63±0.02	0.66±0.02	10^6-10^7
LCBLR5	*Herbaspirillum*	*frisingense*	2.75±1.06	0.83±0.02	1.38±0.07*	1.96±0.14*	NE	NE	NE	10^7-10^8
CCBLR22	*Pseudomonas*	*nitroreducens*	2.51±1.06	NE	NE	NE	ND	ND	ND	10^7-10^8
CCBMTR4	*Bacillus*	*cereus sensu lato*	2.38±1.06	0.54±0.01	0.75±0.03	0.97±0.05	0.35±0.01	0.49±0.01	0.53±0.01	10^6-10^7
LCBLR12	*Arthrobacter*	*phenanthrenivorans*	2.36±1.06	0.23±0.02	0.48 ±0.03	0.55 ±0.04	NE	NE	NE	10^8-10^9
LCBLR6	*Enterobacter*	*cancerogenus*	2.32±1.05	NE	NE	NE	ND	ND	ND	10^7-10^8

References: [1] Duong et al. (2021). *Microbiological Research* 242, 126613. https://doi.org/10.1016/j.micres.2020.126613; [2] Truong (2018). *The Western Highlands Agriculture & Forestry Science Institute (WASI)*, http://wasi.org.vn/en/thach-thuc-cua-san-xuat-ca-phe-o-viet-nam/; [3] Tran (2002). *Hanoi Agriculture University*, DS thesis. [4]Chapman (2014). *The World Bank*.