



Yeasts from the Peruvian native cacao (*Theobroma cacao* L.) phyllosphere and their biocontrol potential against *Moniliophthora roreri*

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Abstract

Moniliophthora roreri is the causal agent of frosty pod rot, one of the most yield-reducing diseases of cacao (*Theobroma cacao* L.) in Latin America. However, there is currently no effective control method for this disease. The objective of this research was to isolate and identify the natural diversity of yeasts present in the phyllosphere of native cacao and to evaluate these isolates for potential antagonistic interactions against *M. roreri*. Fruits and leaves from Peruvian native cacao were collected and epiphytic yeasts were isolated and purified. Single-colony isolates were characterized by macro and micro-morphological characteristics, and molecularly based on sequences of the D1/D2 and ITS1-5.8S-ITS2 regions of the rRNA gene. A total of thirty species distributed in sixteen genera were found. Moreover, the yeasts *Wickerhamomyces anomalus* KLG-014 and *Candida sorboxylosa* KLG-104 showed high biocontrol potential against *M. roreri* as revealed by antibiosis in vitro assays. These results demonstrate, for the first time, the high diversity of yeasts present in the phyllosphere of native Peruvian cacao, and the discovery of species with potential for biocontrol of *M. roreri*.

Keywords Amazonian microorganisms · Chocolate · Levaduras · Moniliasis · *Saccharomycetales* · Tropical phytopathogens

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Introduction

Cacao (*Theobroma cacao* L.) is the source of chocolate and thus is of great economic importance worldwide. Peru is the second largest producer and marketer of fine organic cocoa and the ninth largest producer of cocoa beans in the world (López et al. 2020). Cacao originated and was originally domesticated in the Upper Amazon region shared by Peru and Ecuador (Zarrillo et al. 2018). However, one of the main diseases cacao faces is frosty pod rot (FPR), caused by the *Basidiomycota* fungus *Moniliophthora roreri* (Aime and Phillips-Mora 2005), which can cause losses of up to 100% if proper phytosanitary measures are not applied (Ram et al. 2004). *Moniliophthora roreri* only infects the fruit (the cacao pods) at any stage of development, and the successful dissemination of the pathogen relies on its high sporulation rate on mummified fruits (Gramacho et al. 2018).

FPR is distributed only in tropical America and some Caribbean countries (Johnson et al. 2017; Phillips-Mora et al. 2007), being an imminent threat to cacao farmers who carry out multiple activities to prevent and mitigate this disease

(Díaz-Valderrama et al. 2022). The main chemical alternative for control and prevention is the application of copper- and sulfate-based fungicides, which can be expensive and have negative environmental and public health problems. Biological control strategies have primarily focused on the application of antagonistic fungi such as *Trichoderma* spp. (Niveiro et al. 2020). Although some strains have demonstrated efficacy in reducing FPR incidence under specific conditions, their performance in field applications has been inconsistent, often influenced by environmental factors and formulation methods (Crozier et al. 2015). Therefore, it is necessary to look for other sustainable biological control agents (BCAs), such as epiphytic yeasts (Bruce et al. 2003; Zhang et al. 2007).

Yeasts are single-celled fungi that reproduce mainly by budding (Kurtzman et al. 2011). They can be classified in the phyla *Ascomycota* and *Basidiomycota* (Aime et al. 2014), and are widely known for their role in fermentation of various foods and production of bioethanol (Ho et al. 2014; Lopes et al. 2015; De Vuyst and Leroy 2020). However, there is an increasing body of literature highlighting yeasts in both, *Ascomycota* and *Basidiomycota*, are key members of the phylloplane communities, performing important epiphytic ecological roles on plant surfaces in a wide range of ecosystems (Fonseca and Inácio 2006; Kemler et al. 2017; Limtong and Nasanit 2017). This fungal diversity is beneficial to the host because epiphytic yeasts can act as BCAs of phytopathogens by limiting space and nutrients, inhibiting their growth by releasing volatile compounds, or secreting enzymes that degrade the cell wall of plant pathogens before infection (Zhang et al. 2007; Lu et al. 2014; Freimoser et al. 2019). These interactions include mechanisms of competition and antagonism, via mycoginogeny, i.e., the production of mycogins or killer toxins, one of the main antagonistic strategies against competitors that yeasts have (Klassen et al. 2017), or through the production of secondary metabolites, such as volatile organic compounds (VOCs) (Contarino et al. 2019; Kowalska et al. 2022).

Killer toxins interact with the surface of the target microorganisms, and trigger its killing through target-specific modes of action, such as membrane permeabilization, glucanase or exo-chitinase activity, or tRNA fragmentation (Golubev 2006; Mannazzu et al. 2019). The killer toxin activity of yeasts has been demonstrated against several plant pathogens, such as *Penicillium digitatum* and *Botrytis cinerea* (Masih and Paul 2002; Platania et al. 2012). Moreover, the production of bioactive VOCs is also another important yeast antagonistic strategy (Contarino et al. 2019). Several VOCs with inhibitory growth effects have been identified in various yeast species against plant pathogens, such as *Aspergillus flavus*, *Fusarium cereale*, *F. poae*, *B. cinerea*, and others (Choińska et al. 2020; Di Francesco et al. 2020). Therefore, epiphytic yeasts recovered from the phyllosphere

of cacao have the biotechnological potential, through multiple mechanisms, to be BCAs against cacao pathogens such as *M. royeri*.

In the last years, there has been a growing number of studies of epiphytic yeasts on cacao (Neto et al. 2021; Junior et al. 2022), including the recent discovery of the yeast *Hannaella theobromatis* (Llanos-Gómez et al. 2024). However, in Peru the potential of epiphytic yeasts as BCAs has not been evaluated. Therefore, the objective of this study was to identify culturable epiphytic yeast species from the surface of native cacao leaves and fruits from Amazonas, Peru, and to find the best candidates as BCAs against *M. royeri*.

Material and methods

Area of study and sampling

The samples were collected in Bagua, Utcubamba and Condorcanqui, the main cacao-producing provinces in the department of Amazonas, Peru (Oliva Cruz 2020). Samples of leaves and fruits in good phytosanitary condition were taken from eighteen plots, with a distribution of six plots per province (Table 1). Each plot had an area of approx. one hectare and were distributed at different altitudes in meters above sea level (m a.s.l.), such as low (0 to 400 m a.s.l.), medium (400 to 800 m a.s.l.) and high (800 to 1200 m a.s.l.). Each collection point was georeferenced. Within each plot, a zig-zag path was followed to sample five random cacao trees. From each tree, we collected four leaves and four fruits.

Isolation of yeasts

We washed cacao fruits in the field with 50 mL of ultrapure water and collected the solution in Falcon tubes. In the lab, we suspended the washed-off solution in 250 mL of sterile saline solution (0.85% NaCl; 50 mg L⁻¹ chloramphenicol). The suspension was shaken for one hour, and then serial dilutions (10⁻², 10⁻³, and 10⁻⁴) were prepared. Aliquots of 0.1 mL from each dilution were spread on Petri plates containing yeast extract peptone dextrose agar medium (YEPD; 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 15 g/L agar, and 50 mg L⁻¹ chloramphenicol), and the plates were incubated for 5 days at 26–28 °C. To separate yeast strains, we looked for single colonies of various colors, such as whitish cream, yellowish cream, olive brown, pale pink to dark pink, among others, and textures, such as rough, mucilaginous, butyric, flattened and mucoid, with entire and wavy margins. Single yeast colonies were sub cultured on potato dextrose agar medium (PDA; 4 g/L potato extract, 20 g/L dextrose, 15 g/L agar, and chloramphenicol 50 mg L⁻¹). Filamentous

Table 1 Geographical data for the eighteen plots sampled in this study

Province	District	Locality	Plot #	Coordinates		Altitude (m.a.s.l.)
				Latitude (S)	Longitude (W)	
Utcubamba	Cajaruro	San José Bajo	1	05°42'16.5"	078°24'22.5"	648
	Cajaruro	Diamante Bajo	2	05°43'1.1"	078°21'1.0"	855
	Cajaruro	El Limoncito	3	05°43'36.1"	078°21'0.7"	762
	Cajaruro	Naranjos Alto	4	05°44'37.0"	078°21'37.0"	656
	Cajaruro	Alto Amazonas	5	05°43'5.5"	078°17'1.7"	1195
	Cajaruro	Santa Isabel	6	05°43'45.4"	078°20'28.1"	740
Bagua	Copallín	San Roque	7	05°40'14.7"	078°27'20.8"	480
	Copallín	Palo Grande	8	05°40'20.4"	078°24'38.2"	948
	Copallín	La Yuguana	9	05°40'47.9"	078°24'20.2"	894
	La Peca	Cerca al Pueblo	10	05°36'42.6"	078°26'50.5"	782
	Aramango	El Muyo	11	05°24'39.6"	078°27'31.1"	380
	Imaza	Villa Rica	12	04°58'29.4"	078°13'29.8"	412
Condorcanqui	Nieva	Fe y Alegría	13	04°35'21.3"	083°51'1.6"	242
	Nieva	El Bosque	14	04°35'42.9"	083°52'5.9"	202
	Río Santiago	Puerto Galilea	15	04°01'38.8"	083°45'25.6"	219
	Río Santiago	Villa Gonzalo	16	03°56'4.8"	083°44'41.8"	186
	El Cenepa	Quebrada Huampami	17	04°26'30.1"	078°09'13.5"	232
	El Cenepa	Quebrada Huampami	18	04°26'26.1"	078°09'15.0"	238

fungal growth was discarded. A similar procedure was used to isolate yeasts from leaves, weighing two grams of sample and suspending it in 250 mL of saline solution. In addition, small leaf segments ($\sim 1.5 \times 1.5 \text{ cm}^2$) were cut and fixed with petroleum jelly inside the lids of Petri dishes containing YEPD medium. The plates were carefully sealed and incubated in non-inverted position. This procedure was performed to isolate ballistosporic yeasts, following Abu et al. (2015).

Morphological characterization and preservation of yeasts

Single-colony derived yeast cultures were grown on PDA. From these cultures, we analyzed the macro characteristics such as color, texture (rough, mucilaginous, butyric, flattened or mucoid), margin (entire or wavy) and elevation (pronounced or superficial) of the colony, and presence of pseudohyphae. We also evaluated micromorphological characteristics such as color and size of cells, budding, presence of bipartition, and presence of pseudohyphae, and other microstructures of interest. All characteristics were analyzed by culturing the isolates at 28 °C for 5 to 14 days. Pure isolates were mid-term stored at 4 °C in test tubes in PDA medium slants, and long-term stored in 2 ml cryogenic tubes with 30% glycerol at -80 °C (Kurtzman et al. 2011).

DNA extraction and molecular identification of yeasts

DNA extraction was performed from pure yeast cultures, using the Wizard® genomic DNA purification kit (Promega, Wisconsin, EE. UU.) following the manufacturer's instructions. Quantification of the DNA obtained was performed with the BioSpectrometer® Basic (Eppendorf, New Jersey, USA) by loading 2 µl of sample into a 1-mm cuvette. The DNA concentrations obtained were adjusted to a concentration of 0.5 ng/µl before PCR. We then amplified the Internal Transcribed Spacer region (ITS) encompassing the ITS1, 5.8S and ITS2 subregions, and the Large Sub-Unit (LSU) region of the rRNA gene using ITS1/ITS4, and NL1/NL4 primers pairs, respectively (White et al. 1990; Fell et al. 2000). The combination of both regions provides good identification results in most cases (Kurtzman and Robnett 1998; Scorzetti et al. 2002). PCR amplification cycles and thermocycling conditions were performed as specified by White et al. (1990) and Kurtzman and Robnett (1998). PCR amplicons were purified with the Wizard® PCR Clean-up System purification kit (Promega, Wisconsin, USA) and Sanger sequenced by Macrogen Inc. (Seoul, South Korea). Raw sequences were trimmed, curated and assembled using Sequencher v.5.4 software (Gene Codes, USA). ITS and LSU curated sequences were subjected to BLASTn searches (Camacho et al. 2009) in the non-redundant database of the National Center for Biotechnology Information (NCBI).

These searches allowed us to have a first approximation to the species identity of isolates. With this information, relevant literature was searched and the sequences of type and reference strains of related strains and species were retrieved, including sequences of basal groups that served to delimit the root of the phylogenetic trees. In other words, to identify isolated yeasts, we applied the phylogenetic species concept (Boekhout et al. 2021), using the obtained ITS and LSU sequences, and properly labelled sequences from yeast type specimens. All these sequences were aligned with MUSCLE (Edgar 2004) implemented in the MEGA-X software (Kumar et al. 2018). ITS and LSU sequence alignments were concatenated with SeaView 4.7 (Gouy et al. 2010) and phylogenetic inference was processed with RxBML (Stamatakis et al. 2008). The phylogenetic trees were visualized and edited in the FigTree program (Rambaut 2009). We determined the species identity of an isolate by examining the monophyletic nature of the clade it forms with the type specimen of the species in question, and comparing sequence similarity values among them. Also, we inspected the validity of the name of the identified species in Index Fungorum (<https://www.indexfungorum.org/>) and Mycobank (<https://www.mycobank.org/>). If a strain was identified belonging to a species for which its name was invalidly published, we added quotation marks to the full name (genus and epithet).

Antagonist activity of yeast strains against *Moniliophthora roreri*

Antagonistic tests were performed on PDA medium, which has been widely used in antagonism tests of epiphytic yeasts against multiple plant pathogens (Elkhairy et al. 2023; Barda et al. 2015; Kheireddine et al. 2018), including *M. roreri* (Junior et al. 2022) and the other major cacao pathogen *M. perniciosa* (Neto et al. 2021). We recognize the limitations of using this medium as it does not accurately represent what happens on the surface of cacao fruits upon *M. roreri* infection. However, these in vitro tests are a good first approximation before moving on to the field tests.

We worked with three strains of *M. roreri* (K-399, ELA-001, MBG-01 that were isolated from FPR-mummified cacao fruits from three different geographic areas: the Loreto region (Iquitos), Cusco (La Convención) and Amazonas (Bagua), respectively. All three areas have high incidence of *M. roreri* and encompass most of the genetic diversity of the pathogen present in Peru (Díaz-Valderrama et al. 2022). Cultures of *M. roreri* were grown on PDA medium, and their growth rate was evaluated at 7 and 15 days. Integrity of cultures was corroborated based on the analysis of macro characteristics, such as color and shape of the colony, and micro characteristics, such as color and cell size, as reported in Díaz-Valderrama and Aime (2016a), in addition to successful molecular genotyping with *M. roreri*-specific

microsatellite markers (unpublished data), as in Díaz-Valderrama and Aime (2016b). Also, the strain MBG-01 of *M. roreri* was previously used in other antagonistic tests (Leiva et al. 2022).

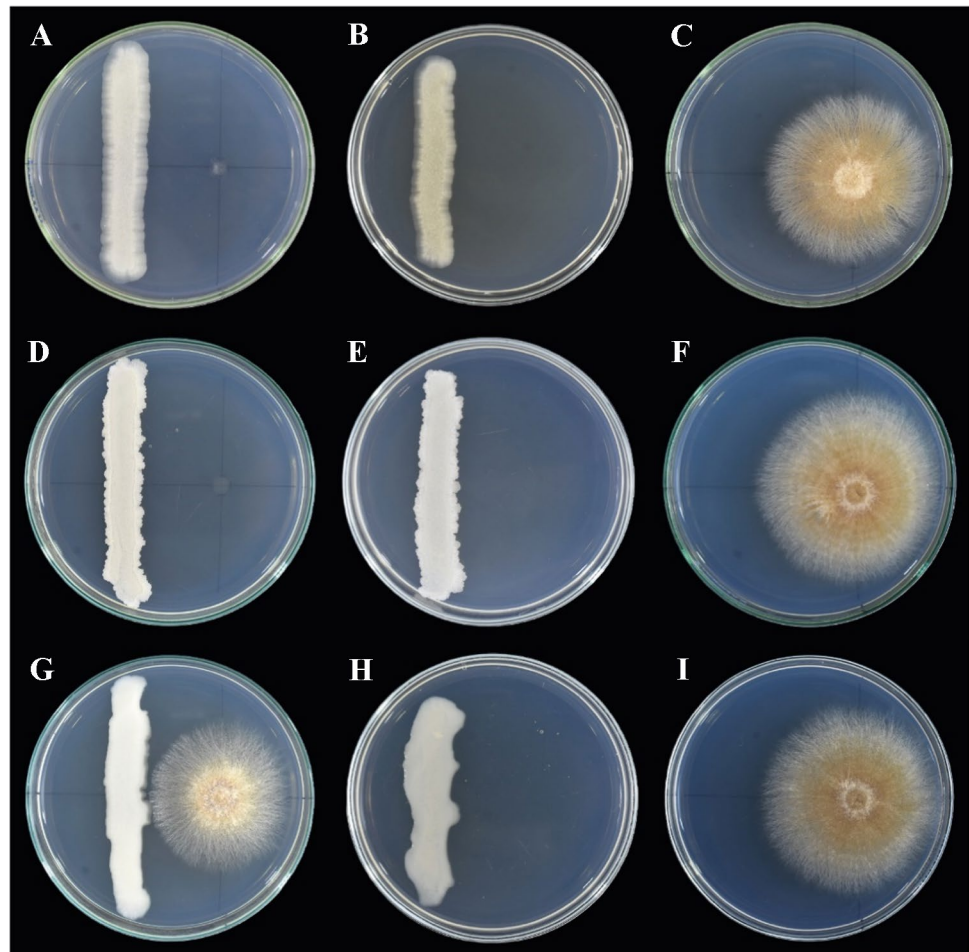
Twenty yeast strains with biocontrol potential were selected based on literature. The antagonistic activity of isolated yeasts against *M. roreri* was evaluated as described by Neto et al. (2021) and Junior et al. (2022), with some modifications. A small portion of the yeast biomass was transferred to test tubes with 5 ml of Sabouraud Dextrose Broth medium (Peptone 10 g/L, Dextrose 40 g/L; pH 5.6). These tubes were incubated in an MRC shaker model TOU-120 (Flexible Assembly Systems Inc., San Diego, California, USA) for 24 h at 120 rpm at a temperature of 28 °C. Optical density was measured on a BioSpectrometer® Basic (Eppendorf, Hamburg, Germany) at 600 nm, and adjusted to an optical density of 0.5. Similarly, we measured the optical density of *M. roreri* spores. We scraped the mycelium of fifteen-day-old cultures and transferred it to eppendorf tubes containing 500 ml of sterile distilled water for optical density measurement. The concentration obtained was adjusted to an absorbance of 0.5 for the antagonism assays. A 20 µl aliquot of yeast dilution was linearly plated 3 cm from the edge of the 9 cm Petri dish containing PDA. Next, 10 µL of the *M. roreri* spore dilution was plated 3 cm away from the plated yeast line (Fig. 1). The plates were incubated for 15 days at 26–28 °C, as in Junior et al. (2022). Each confrontation experiment had four repetitions with two controls: the pathogen alone, and the yeast alone.

We took measurements of the diameter of the pathogen colony after 7 and 15 days of inoculation. With the diameter, we estimated the approximate area of growth of the pathogen. We also counted the spores produced in culture at 15 days of growth. For this, a 0.5-mm-diameter disc with mycelium and spores of *M. roreri* was taken from the confrontation and control plates and transferred to Falcon tubes with 4 ml of sterile distilled water. These tubes were vortexed, and we extracted an aliquot of 100 µl of solution. We counted the spores in this suspension in a Neubauer chamber under a microscope and estimated the total number of spores produced within the area of growth recorded.

Statistical analysis

Before applying any statistical tests, we assessed the assumptions of normality and homoscedasticity using the Shapiro–Wilk and Bartlett’s tests, respectively, implemented in the ‘nortest’ package (Gross and Ligges 2015). Both tests yielded highly significant results, indicating violations of normality and homoscedasticity (Supplementary Table S1). Consequently, we applied the non-parametric Kruskal–Wallis test to compare the medians of *M. roreri* growth and spore production in response to the confronting yeast among the

Fig. 1 Yeast - *Moniliophthora roreri* confrontation tests on PDA medium for 15 days at 26–28 °C: (A) Isolate KLG-104 confronted with *M. roreri*; (B) Isolate KLG-104 control alone; (C) *M. roreri* control alone; (D) Isolate KLG-014 confronted with *M. roreri*; (E) Isolate KLG-014 control alone; (F) *M. roreri* control alone; (G) Isolate KLG-068 confronted with *M. roreri*; (H) Isolate KLG-068 control alone; (I) *M. roreri* control alone



departments of Amazonas, Cusco and Loreto. To identify significant pairwise differences, we conducted a post hoc Conover test (Conover and Iman 1979; Conover 1999) using the ‘PMCMRplus’ package (Pohlert 2023). All statistical analyses were conducted in R software version 4.4.1, with a significance level of $p < 0.05$.

Results

Isolated yeasts and identification

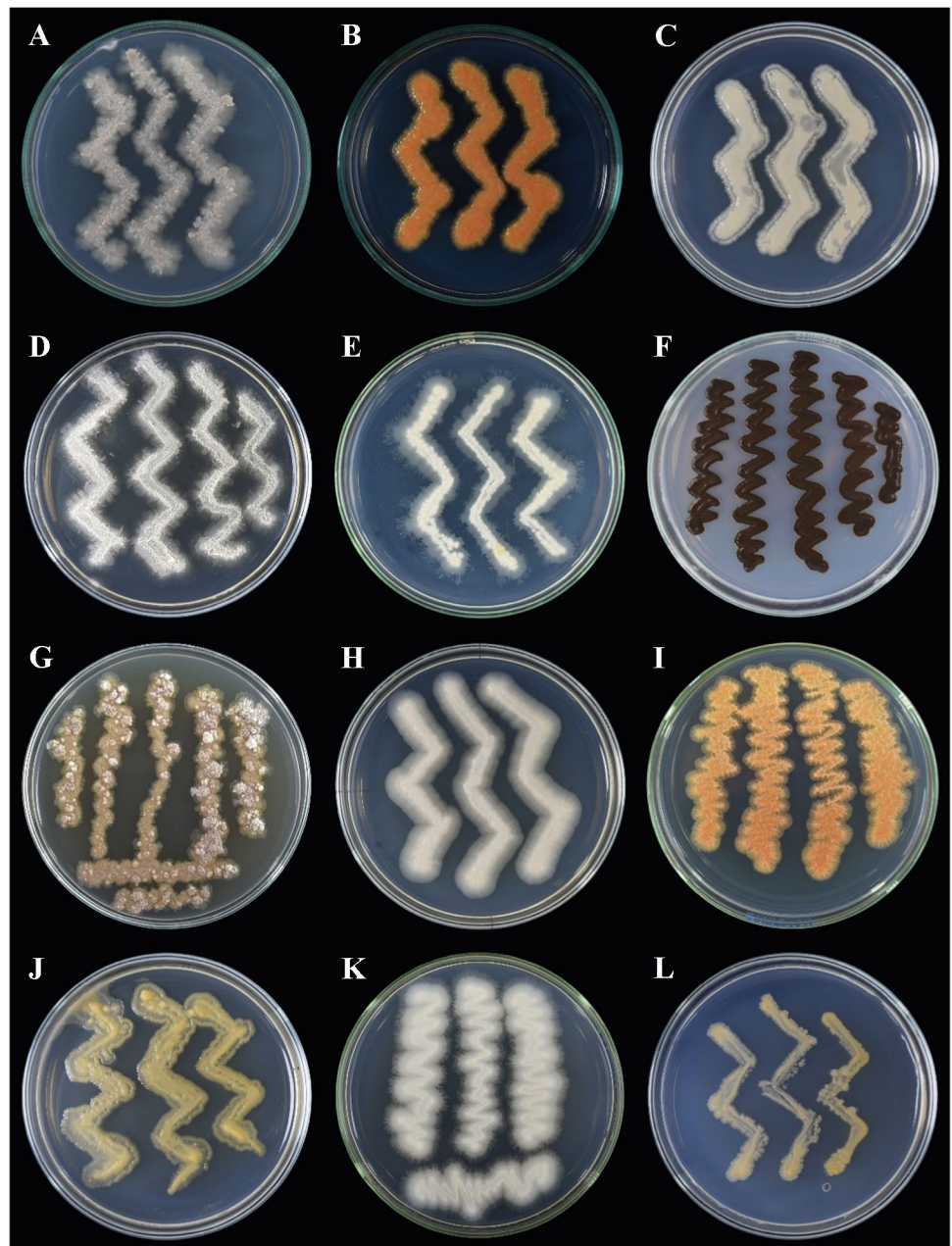
A total of 96 yeast isolates were obtained from cacao fruit and leaf surfaces in the provinces of Utcubamba (26 isolates, 14 isolated from fruits and 12 from leaf), Bagua (35 isolates, 21 from fruit and 14 from leaf) and Condorcanqui (35 isolates, 22 from fruit and 13 from leaf). All these isolates were successfully preserved, and morphologically and molecularly characterized (Fig. 2; Supplementary Table S2; Supplementary File S1). Isolates were identified as belonging to thirty species within seventeen genera in *Ascomycota* ($n = 45$ isolates) and *Basidiomycota* ($n = 51$) (Table 2;

Supplementary File S1; Supplementary Table S3). We identified thirty-three species in total, with two of them are new species in the *Hannaella* and *Pseudozyma* genera based on phylogenetic evidence (Supplementary File S1), requiring future formal description. The genera with the highest number of species were *Hannaella* and *Candida*. More isolates were collected from Condorcanqui province, but the greatest number of yeast species were isolated from Utcubamba (Supplementary Table S2). For species with more than one isolate, we identified that *C. sorboxylosa* ($n = 2$), “*Debaryomyces vindobonensis*” ($n = 5$), *Rhodotorula mucilaginosa* ($n = 2$), *Wickerhamomyces anomalus* ($n = 4$), and *Yamadazyma paraaseri* ($n = 3$) were exclusively found on the surface of fruits, while “*H. phyllophila*” ($n = 2$), *R. diobovata* ($n = 3$), and the new species of *Pseudozyma* sp. ($n = 2$), exclusively on leaves (Supplementary Table S2).

Antagonism of yeasts

Twenty isolates (nineteen yeast species) were selected for antagonism assays against *M. roreri* based on previous research reports suggesting that they may have biocontrol

Fig. 2 Morphology of some of the isolated yeasts at 21 days of growth: (A) Isolate KLG-051; (B) Isolate KLG-117; (C) Isolate KLG-108; (D) Isolate KLG-036; (E) Isolate KLG-014; (F) Isolate KLG-044; (G) Isolate KLG-043; (H) Isolate KLG-099; (I) Isolate KLG-055; (J) Isolate KLG-A03; (K) Isolate KLG-095; (L) Isolate KLG-109



properties (Alkuwari et al. 2022; Choińska et al. 2020; Hernandez-Montiel et al. 2021; Junior et al. 2022; Kheireddine et al. 2018; Khunnamwong et al. 2020; Kulakovskaya et al. 2004; Nandhini et al. 2021; Neto et al. 2021) (Table 3).

***M. roreri* area of growth**

Strains of *M. roreri* from Amazonas, Cusco, and Loreto showed significant differences in growth when confronted with the yeast species isolated in this study (Fig. 3; Supplementary File S2). In all cases, the most effective yeasts were KLG-104 (*Candida sorboxylosa*) and KLG-014 (*W. anomalus*), which completely or almost completely inhibited

the growth of the three *M. roreri* strains. In addition to these two strains, the yeast KLG-112 (*Y. paraaserei*) also inhibited the growth of the three *M. roreri* strains at the same significant level (Fig. 3). Conversely, yeast strain KLG-068 (*Meira plantarum*) showed the least antagonistic ability against the three strains of *M. roreri* (Fig. 3; Supplementary File S2).

The growth of the *M. roreri* strain from Amazonas was also significantly inhibited by the confrontation with the yeast strains KLG-109 (*Saturnispora diversa*), KLG-115 (*Pichia sporocuriosa*), KLG-112 (*Y. paraaserei*), KLG-095 (*Meyerozyma guilliermondii*), and KLG-108 (*Papiliotrema ruineniae*), which did not differ significantly with KLG-104 (*C. sorboxylosa*) and KLG-014 (*W. anomalus*) and the two

Table 2 General data on the classification of the identified isolates from this study by phylum, genus and species

Phylum	Genus	Species	Isolated	Source	Origin	Coordinates		Altitude (m a.s.l)	ITS Accession number	LSU Accession number
						Latitude (S)	Longitude (W)			
<i>Ascomycota</i>	<i>Candida</i>	<i>C. boidinii</i>	KLK-036	Cacao fruit	Nieva, Condor-canqui	04°35'42.9"	083°52'5.9"	202	PQ055828	PQ055900
		" <i>C. jaroonii</i> "	KLK-103	Cacao leaf	Cajaruero, Utcu-bamba	05°43'36.1"	078°21'0.7"	762	PQ055829	PQ055901
		<i>C. metapsilosis</i>	KLK-111	Cacao leaf	Cajaruero, Utcu-bamba	05°44'37.0"	078°21'37.0"	656	PQ055830	PQ055902
		<i>C. sorboxylosa</i>	KLK-104	Cacao fruit	Cajaruero, Utcu-bamba	05°43'36.1"	078°21'0.7"	762	...	PQ055903
		<i>C. sorboxylosa</i>	KLK-118	Cacao fruit	Cajaruero, Utcu-bamba	05°43'5.5"	078°17'1.7"	1195	...	PQ055904
<i>Yamadazyma</i>	<i>Yamadazyma</i>	<i>Y. paraasari</i>	KLK-112	Cacao fruit	Cajaruero, Utcu-bamba	05°44'37.0"	078°21'37.0"	656	PQ055825	PQ055897
		<i>Y. paraasari</i>	KLK-114	Cacao fruit	Cajaruero, Utcu-bamba	05°44'37.0"	078°21'37.0"	656	PQ055826	PQ055898
		<i>Y. paraasari</i>	KLK-120	Cacao fruit	Cajaruero, Utcu-bamba	05°43'45.4"	078°20'28.1"	740	PQ055827	PQ055899
		<i>Me. guilliermondii</i>	KLK-005	Cacao fruit	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055831	PQ055905
		<i>Me. guilliermondii</i>	KLK-007	Cacao fruit	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055832	PQ055906
<i>Meyeromyces</i>	<i>Meyeromyces</i>	<i>Me. guilliermondii</i>	KLK-008	Cacao fruit	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055833	PQ055907
		<i>Me. guilliermondii</i>	KLK-009	Cacao fruit	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055834	PQ055908
		<i>Me. guilliermondii</i>	KLK-010	Cacao leaf	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055835	PQ055909
		<i>Me. guilliermondii</i>	KLK-011	Cacao leaf	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055836	PQ055910
		<i>Me. guilliermondii</i>	KLK-013	Cacao fruit	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055837	PQ055911
		<i>Me. guilliermondii</i>	KLK-017	Cacao fruit	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055838	PQ055912
		<i>Me. guilliermondii</i>	KLK-018	Cacao leaf	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055839	PQ055913
		<i>Me. guilliermondii</i>	KLK-019	Cacao fruit	Nieva, Condor-canqui	04°35'21.3"	083°51'1.6"	242	PQ055840	PQ055914
		<i>Me. guilliermondii</i>	KLK-033	Cacao fruit	Nieva, Condor-canqui	04°35'42.9"	083°52'5.9"	202	PQ055841	PQ055915
		<i>Me. guilliermondii</i>	KLK-035	Cacao fruit	Nieva, Condor-canqui	04°35'42.9"	083°52'5.9"	202	PQ055842	PQ055916
		<i>Me. guilliermondii</i>	KLK-041	Cacao leaf	Río Santiago, Condorcanqui	04°01'38.8"	083°45'25.6"	219	PQ055843	PQ055917

Table 2 (continued)

Phylum	Genus	Species	Isolated	Source	Origin	Coordinates		Altitude (m a.s.l)	ITS Accession number	LSU Accession number
						Latitude (S)	Longitude (W)			
<i>Debaryomyces</i>	<i>Me. guilliermondii</i>		KLG-047	Cacao leaf	Río Santiago, Condorcanqui	03°56'4.8"	083°44'41.8"	186	PQ055844	PQ055918
			KLG-048	Cacao leaf	Río Santiago, Condorcanqui	04°01'38.8"	083°45'25.6"	219	PQ055845	PQ055919
			KLG-049	Cacao fruit	Río Santiago, Condorcanqui	03°56'4.8"	083°44'41.8"	186	PQ055846	PQ055920
			KLG-054	Cacao fruit	Cenepa, Condorcanqui	04°26'30.1"	078°09'13.5"	232	PQ055847	PQ055921
			KLG-056	Cacao fruit	Cenepa, Condorcanqui	04°26'26.1"	078°09'15.0"	238	PQ055848	PQ055922
			KLG-057	Cacao fruit	Cenepa, Condorcanqui	04°26'26.1"	078°09'15.0"	238	PQ055849	PQ055923
			KLG-064	Cacao fruit	Río Santiago, Condorcanqui	03°56'4.8"	083°44'41.8"	186	PQ055850	PQ055924
			KLG-065	Cacao fruit	Cenepa, Condorcanqui	04°26'30.1"	078°09'13.5"	232	PQ055851	PQ055925
			KLG-086	Cacao fruit	Aramango, Bagua	05°24'39.6"	078°27'31.1"	380	PQ055852	PQ055926
			KLG-093	Cacao fruit	Imaza, Bagua	04°58'29.4"	078°13'29.8"	412	PQ055853	PQ055927
			KLG-095	Cacao leaf	Imaza, Bagua	04°58'29.4"	078°13'29.8"	412	PQ055854	PQ055928
			KLG-071	Cacao fruit	Copallín, Bagua	05°40'20.4"	078°24'38.2"	948	PQ055855	PQ055929
			KLG-075	Cacao fruit	Copallín, Bagua	05°40'20.4"	078°24'38.2"	948	PQ055856	PQ055930
			KLG-077	Cacao fruit	Copallín, Bagua	05°40'20.4"	078°24'38.2"	948	PQ055857	PQ055931
<i>Wickerhamomyces</i>	<i>W. anomalus</i>		KLG-092	Cacao fruit	La Peca, Bagua	05°36'42.6"	078°26'50.5"	782	PQ055858	PQ055932
			KLG-094	Cacao fruit	Imaza, Bagua	04°58'29.4"	078°13'29.8"	412	PQ055859	PQ055933
			KLG-004	Cacao fruit	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055860	PQ055934
			KLG-006	Cacao fruit	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055861	PQ055935
			KLG-014	Cacao fruit	Cajaruero, Utcubamba	05°42'16.5"	078°24'22.5"	648	PQ055862	PQ055936
			KLG-015	Cacao fruit	Cajaruero, Utcubamba	05°42'16.5"	078°24'22.5"	648	PQ055863	PQ055937
<i>Exophiala</i>	<i>E. alcalophila</i>		KLG-020	Cacao leaf	Nieva, Condorcanqui	04°35'21.3"	083°51'1.6"	242	PQ055864	PQ055938
			KLG-044	Cacao fruit	Río Santiago, Condorcanqui	04°01'38.8"	083°45'25.6"	219	PQ055865	PQ055939

Table 2 (continued)

Phylum	Genus	Species	Isolated	Source	Origin	Coordinates		Altitude (m a.s.l.)	ITS Accession number	LSU Accession number
						Latitude (S)	Longitude (W)			
<i>Saturnispora</i>	<i>Pichia</i>	<i>S. diversa</i>	KLK-109	Cacao fruit	Cajaruero, Utcu-bamba	05°44'37.0"	078°21'37.0"	656	PQ055866	PQ055940
		<i>P. sporocuriosa</i>	KLK-115	Cacao fruit	Cajaruero, Utcu-bamba	05°43'5.5"	078°17'1.7"	1195	PQ055867	...
<i>Basidiomycota</i>	<i>Hannaella</i>	<i>H. theobromatis</i>	KLK-034	Cacao leaf	Río Santiago, Condorcanqui	04°01'38.8"	083°45'25.6"	219	OR086918	OR088043
		<i>H. theobromatis</i>	KLK-063	Cacao fruit	Río Santiago, Condorcanqui	03°56'4.8"	083°44'41.8"	186	OR086919	OR088044
		<i>H. theobromatis</i>	KLK-074	Cacao leaf	Copallín, Bagua	05°40'20.4"	078°24'38.2"	948	OR086920	OR088045
		<i>H. theobromatis</i>	KLK-078	Cacao fruit	Copallín, Bagua	05°40'47.9"	078°24'20.2"	894	OR086921	OR088046
		<i>H. theobromatis</i>	KLK-079	Cacao fruit	Copallín, Bagua	05°40'47.9"	078°24'20.2"	894	OR086922	OR088047
		<i>H. theobromatis</i>	KLK-082	Cacao leaf	Aramango, Bagua	05°24'39.6"	078°27'31.1"	380	OR086923	OR088048
		<i>H. theobromatis</i>	KLK-084	Cacao leaf	Aramango, Bagua	05°24'39.6"	078°27'31.1"	380	OR086924	OR088049
		<i>H. theobromatis</i>	KLK-085	Cacao fruit	Aramango, Bagua	05°24'39.6"	078°27'31.1"	380	OR086925	OR088050
		<i>Hannaella</i> sp.	KLK-023	Cacao fruit	Nieva, Condorcanqui	04°35'21.3"	083°51'1.6"	242	PQ055868	PQ055941
		<i>Hannaella</i> sp.	KLK-025	Cacao fruit	Nieva, Condorcanqui	04°35'21.3"	083°51'1.6"	242	PQ055869	PQ055942
		<i>Hannaella</i> sp.	KLK-069	Cacao fruit	Cenepa, Condorcanqui	04°26'26.1"	078°09'15.0"	238	PQ055870	PQ055943
		<i>Hannaella</i> sp.	KLK-096	Cacao leaf	Imaza, Bagua	04°58'29.4"	078°13'29.8"	412	PQ055871	PQ055944
<i>H. phyllophila</i>	<i>H. phyllophila</i>	"H. phyllophila"	KLK-073	Cacao leaf	Copallín, Bagua	05°40'20.4"	078°24'38.2"	948	OR088112	OR088135
		"H. phyllophila"	KLK-091	Cacao leaf	La Peca, Bagua	05°36'42.6"	078°26'50.5"	782	OR088113	OR088136
		<i>H. taiwanensis</i>	KLK-021	Cacao fruit	Nieva, Condorcanqui	04°35'42.9"	083°52'5.9"	202	OR088116	OR088139
		"H. pagnoccae"	KLK-076	Cacao fruit	Copallín, Bagua	05°40'20.4"	078°24'38.2"	948	OR088114	OR088137
		<i>H. sinensis</i>	KLK-121	Cacao leaf	Cajaruero, Utcu-bamba	05°43'45.4"	078°20'28.1"	740	OR088115	OR088138

Table 2 (continued)

Phylum	Genus	Species	Isolated	Source	Origin	Coordinates		Altitude (m a.s.l)	ITS Accession number	LSU Accession number
						Latitude (S)	Longitude (W)			
<i>Rhodotorula</i>	<i>R. diobovata</i>	<i>R. diobovata</i>	KLK-101	Cacao leaf	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055872	PQ055945
			KLK-117	Cacao leaf	Cajaruero, Utcu-bamba	05°43'5.5"	078°17'1.7"	1195	PQ055873	PQ055946
			KLK-122	Cacao leaf	Cajaruero, Utcu-bamba	05°43'45.4"	078°20'28.1"	740	PQ055874	PQ055947
			KLK-055	Cacao fruit	Cenepa, Condor-canqui	04°26'30.1"	078°09'13.5"	232	PQ055875	PQ055948
			KLK-A07	Cacao fruit	Cenepa, Condor-canqui	04°26'26.1"	078°09'15.0"	238	PQ055876	PQ055949
			KLK-119	Cacao fruit	Cajaruero, Utcu-bamba	05°43'45.4"	078°20'28.1"	740	PQ055877	PQ055950
			KLK-043	Cacao fruit	Río Santiago, Condorcanqui	04°01'38.8"	083°45'25.6"	219	PQ055878	PQ055951
			KLK-026	Cacao leaf	Nieva, Condor-canqui	04°35'21.3"	083°51'1.6"	242	PQ055880	PQ055953
			KLK-058	Cacao leaf	Nieva, Condor-canqui	04°35'21.3"	083°51'1.6"	242	PQ055881	PQ055954
			KLK-051	Cacao leaf	Cenepa, Condor-canqui	04°26'30.1"	078°09'13.5"	232	PQ055879	PQ055952
<i>Vishniacozyma</i>	<i>V. alagoana</i>	<i>V. alagoana</i>	KLK-001	Cacao leaf	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055882	PQ055955
			KLK-016	Cacao fruit	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055883	PQ055956
			KLK-080	Cacao leaf	Copallín, Bagua	05°40'47.9"	078°24'20.2"	894	PQ055884	PQ055957
			KLK-081	Cacao leaf	Copallín, Bagua	05°40'47.9"	078°24'20.2"	894	PQ055885	PQ055958
			KLK-089	Cacao leaf	La Peca, Bagua	05°36'42.6"	078°26'50.5"	782	PQ055886	PQ055959
			KLK-113	Cacao leaf	Cajaruero, Utcu-bamba	05°44'37.0"	078°21'37.0"	656	PQ055887	PQ055960
			KLK-088	Cacao fruit	La Peca, Bagua	05°36'42.6"	078°26'50.5"	782	PQ055888	PQ055961
			KLK-110	Cacao leaf	Cajaruero, Utcu-bamba	05°44'37.0"	078°21'37.0"	656	...	PQ055962
			KLK-099	Cacao leaf	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055889	PQ055963
			KLK-102	Cacao leaf	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055890	PQ055964
<i>Moesziomyces</i>	<i>Mo. antarcticus</i>	<i>Mo. antarcticus</i>	KLK-101	Cacao leaf	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055872	PQ055945
			KLK-117	Cacao leaf	Cajaruero, Utcu-bamba	05°43'5.5"	078°17'1.7"	1195	PQ055873	PQ055946
			KLK-122	Cacao leaf	Cajaruero, Utcu-bamba	05°43'45.4"	078°20'28.1"	740	PQ055874	PQ055947
			KLK-055	Cacao fruit	Cenepa, Condor-canqui	04°26'30.1"	078°09'13.5"	232	PQ055875	PQ055948
			KLK-A07	Cacao fruit	Cenepa, Condor-canqui	04°26'26.1"	078°09'15.0"	238	PQ055876	PQ055949
			KLK-119	Cacao fruit	Cajaruero, Utcu-bamba	05°43'45.4"	078°20'28.1"	740	PQ055877	PQ055950
			KLK-043	Cacao fruit	Río Santiago, Condorcanqui	04°01'38.8"	083°45'25.6"	219	PQ055878	PQ055951
			KLK-026	Cacao leaf	Nieva, Condor-canqui	04°35'21.3"	083°51'1.6"	242	PQ055880	PQ055953
			KLK-058	Cacao leaf	Nieva, Condor-canqui	04°35'21.3"	083°51'1.6"	242	PQ055881	PQ055954
			KLK-051	Cacao leaf	Cenepa, Condor-canqui	04°26'30.1"	078°09'13.5"	232	PQ055879	PQ055952
<i>Pseudozyma</i>	<i>P. hubeiensis</i>	<i>P. hubeiensis</i>	KLK-001	Cacao leaf	Copallín, Bagua	05°40'14.7"	078°27'20.8"	480	PQ055882	PQ055955
			KLK-016	Cacao fruit	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055883	PQ055956
			KLK-080	Cacao leaf	Copallín, Bagua	05°40'47.9"	078°24'20.2"	894	PQ055884	PQ055957
			KLK-081	Cacao leaf	Copallín, Bagua	05°40'47.9"	078°24'20.2"	894	PQ055885	PQ055958
			KLK-089	Cacao leaf	La Peca, Bagua	05°36'42.6"	078°26'50.5"	782	PQ055886	PQ055959
			KLK-113	Cacao leaf	Cajaruero, Utcu-bamba	05°44'37.0"	078°21'37.0"	656	PQ055887	PQ055960
			KLK-088	Cacao fruit	La Peca, Bagua	05°36'42.6"	078°26'50.5"	782	PQ055888	PQ055961
			KLK-110	Cacao leaf	Cajaruero, Utcu-bamba	05°44'37.0"	078°21'37.0"	656	...	PQ055962
			KLK-099	Cacao leaf	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055889	PQ055963
			KLK-102	Cacao leaf	Cajaruero, Utcu-bamba	05°42'16.5"	078°24'22.5"	648	PQ055890	PQ055964

Table 2 (continued)

Phylum	Genus	Species	Isolated	Source	Origin	Coordinates		Altitude (m a.s.l)	ITS Accession number	LSU Accession number
						Latitude (S)	Longitude (W)			
<i>Papilliotrema</i>	<i>Pa. miconiae</i>	<i>Pa. miconiae</i>	KLG-A03	Cacao fruit	Cajaruero, Utcu-bamba	05°43'36.1"	078°21'0.7"	762	PQ055891	PQ055965
						05°44'37.0"	078°21'37.0"	656	PQ055892	PQ055966
						04°35'21.3"	083°51'1.6"	242	...	PQ055967
						04°35'21.3"	083°51'1.6"	242	...	PQ055968
<i>Cutaneotrichosporon</i>	<i>Cu. debeurmannianum</i>	<i>Cu. debeurmannianum</i>	KLG-031	Cacao fruit	Nieva, Condorcanqui	04°01'38.8"	083°45'25.6"	219	...	PQ055969
						04°26'30.1"	078°09'13.5"	232	...	PQ055970
						04°26'30.1"	078°09'13.5"	232	...	PQ055971
						04°26'26.1"	078°09'15.0"	238	...	PQ055972
<i>Meira</i>	<i>Mei. plantarum</i>	<i>Mei. plantarum</i>	KLG-061	Cacao fruit	Imaza, Bagua	04°58'29.4"	078°13'29.8"	412	...	PQ055973
						04°35'21.3"	083°51'1.6"	242	...	PQ055974
						05°40'14.7"	078°27'20.8"	480	PQ055893	PQ055975
						03°56'4.8"	083°44'41.8"	186	PQ055894	PQ055976
<i>Sympodiomyces</i>	<i>Sy. paphiopedili</i>	<i>Sy. paphiopedili</i>	KLG-072	Cacao fruit	Copallín, Bagua	05°40'20.4"	078°24'38.2"	948	PQ055895	PQ055977
						05°36'42.6"	078°26'50.5"	782	PQ055896	PQ055978

Isolate, Yeast isolation code; **Source**, Host or source of yeast isolation; **Coordinates**, Latitude and longitude described in degrees (°), minutes (') and seconds ("); **Altitude**, Altitude of the plots expressed in meters above sea level (m a.s.l.). "Species in quotes", not a valid name according to Index Fungorum (<https://www.indexfungorum.org/>) and Mycobank (<https://www.mycobank.org/>)

Table 3 Yeast species selected for the antagonistic assays in this study

Number	Species	Yeast strain	Source of isolation	Reference that supports selection of species in antagonistic assays
1	<i>Moesziomyces aphidis</i>	KLG-051	Cacao leaf	Srivastava et al. (2021)
2	<i>Rhodotorula mucilaginosa</i>	KLG-055	Cacao fruit	Dávila (2016)
3	" <i>Hannaella phyllophila</i> "	KLG-073	Cacao leaf	Surussawadee et al. (2015)
4	" <i>Hannaella pagnoccae</i> "	KLG-076	Cacao fruit	Junior et al. (2022)
5	<i>Yamadazyma paraaseri</i>	KLG-112	Cacao fruit	González-Gutiérrez et al. (2023)
6	<i>Candida sorboxylosa</i>	KLG-104	Cacao fruit	Wang et al. (2023)
7	" <i>Candida jaroonii</i> "	KLG-103	Cacao leaf	Imanishi et al. (2008)
8	<i>Vishniacozyma alagoana</i>	KLG-016	Cacao fruit	Félix et al. (2020); Vujanovic (2021)
	<i>Vishniacozyma alagoana</i>	KLG-113	Cacao leaf	
9	<i>Pseudozyma</i> sp.	KLG-099	Cacao leaf	Kitamoto (2019)
10	<i>Rhodotorula diobovata</i>	KLG-117	Cacao leaf	Oreamuno-Fonseca and Umaña-Rojas (2023)
11	<i>Wickerhamomyces anomalus</i>	KLG-014	Cacao fruit	Khunnamwong et al. (2020); Wang et al. (2018)
12	" <i>Debaryomyces vindobonensis</i> "	KLG-094	Cacao fruit	Abu-Mejdad (2019)
13	<i>Meira plantarum</i>	KLG-068	Cacao fruit	Li et al. (2022)
14	<i>Exophiala alcalophila</i>	KLG-044	Cacao fruit	Prenafeta-Boldú et al. (2022)
15	<i>Sympodiomyces paphiopedili</i>	KLG-090	Cacao fruit	Kulakovskaya et al. (2004); Wu et al. (2023)
16	<i>Saturnispora diversa</i>	KLG-109	Cacao fruit	Yan et al. (2022)
17	<i>Papiliotrema ruineniae</i>	KLG-108	Cacao leaf	Satianpakiranakorn et al. (2020)
18	<i>Pichia sporocuriosa</i>	KLG-115	Cacao fruit	Elkhairy et al. (2023)
19	<i>Meyerozyma guilliermondii</i>	KLG-095	Cacao leaf	Choińska et al. (2020); Wang et al. (2018)

most outperforming strains (Fig. 3A). Similarly, the growth of the *M. roreri* strain from Cusco was also significantly inhibited by the yeast strains KLG-112 (*Y. paraaseri*), KLG-109 (*S. diversa*), KLG-115 (*P. sporocuriosa*), KLG-103 ("Candida jaroonii"), and KLG-055 (*R. mucilaginosa*), which did not differ with the two most outperforming strains (Fig. 3B). Finally, the growth of *M. roreri* strain from Loreto was also significantly inhibited by the yeast strains, KLG-103 ("C. jaroonii"), KLG-055 (*R. mucilaginosa*), and KLG-112 (*Y. paraaseri*), which did not differ with the two most outperforming strains (Fig. 3C).

M. roreri sporulation

Strains of *M. roreri* from Amazonas, Cusco, and Loreto produced significantly different amounts of spores when confronted with the twenty selected yeast strains (Fig. 4; Supplementary File S2). In all cases, the yeast strains KLG-014 (*W. anomalus*) and KLG-104 (*C. sorboxylosa*) completely or almost completely inhibited the sporulation of all the three strains of *M. roreri*. In contrast, yeast strain KLG-068 (*M. plantarum*) showed the lowest sporulation reduction capacity against the three strains of *M. roreri* (Fig. 4; Supplementary file S2).

The sporulation of the *M. roreri* strain from Amazonas was also significantly reduced by the confrontation of the yeast strains KLG-073 ("Hannaella phyllophila"), KLG-115

(*P. sporocuriosa*), KLG-109 (*S. diversa*), KLG-095 (*M. guilliermondii*), and KLG-108 (*P. ruineniae*), which did not differ with the two most outperforming yeasts strains, KLG-014 (*W. anomalus*) and KLG-104 (*C. sorboxylosa*) (Fig. 4A). Similarly, the sporulation of the *M. roreri* strain from Cusco was also reduced by the yeast strains KLG-115 (*P. sporocuriosa*) and KLG-112 (*Y. paraaseri*), which did not differ with the two most outperforming yeasts (Fig. 4). Finally, the sporulation of the *M. roreri* strain from Loreto was also significantly reduced by yeast strains KLG-103 ("C. jaroonii"), KLG-055 (*R. mucilaginosa*), KLG-073 ("H. phyllophila"), and KLG-112 (*Y. paraaseri*), not differing with the two most outperforming yeasts strains (Fig. 4).

Origin of most outperforming yeasts

An association was identified between the source of isolation of yeasts and their antagonistic activity against *M. roreri*. The three species with the greatest potential to reduce the growth area, KLG-104 (*C. sorboxylosa*), KLG-014 (*W. anomalus*), and KLG-112 (*Y. paraaseri*), were isolated from fruit samples (Table 3). The former two strains were also the two most performing strains in terms of sporulation reduction of *M. roreri*. Therefore, in this study the yeast strains with the highest capacity to reduce *M. roreri* growth and sporulation came from the cacao fruit surface (Table 3).

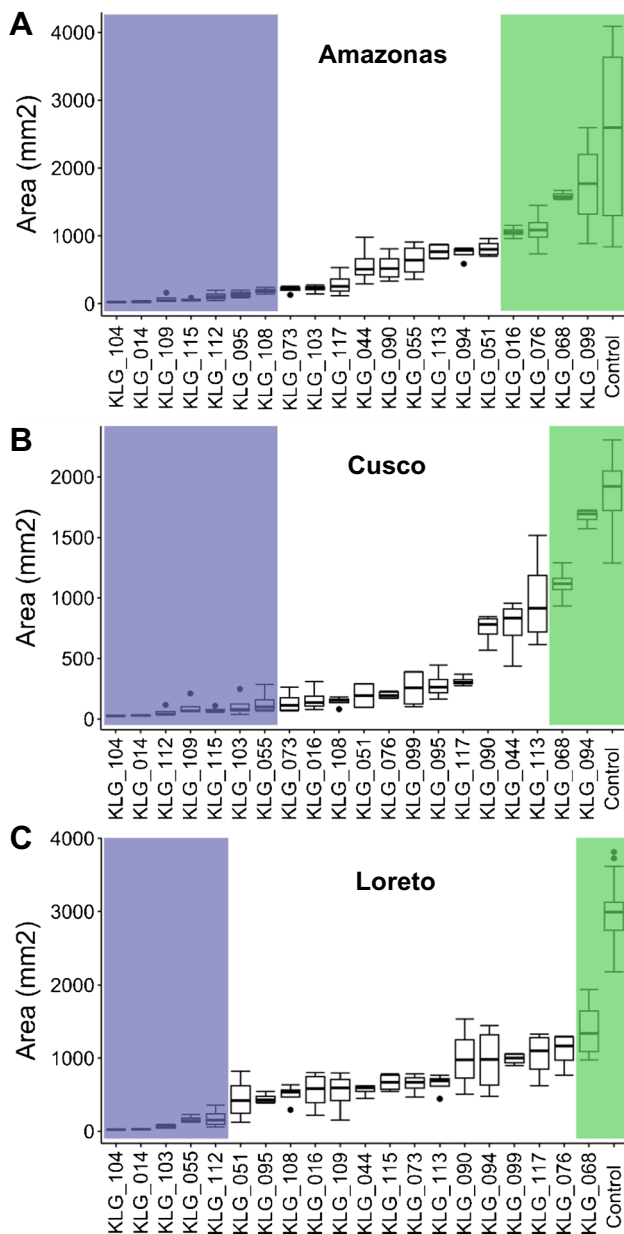


Fig. 3 Area of growth of *Moniliophthora roreri* strains from Amazonas (A), Cusco (B), and Loreto (C) confronted against twenty yeast strains from this study. Yeasts for which boxplot backgrounds are in blue do not differ significantly with KLG-104 (*Candida sorboxylosa*) and KLG-014 (*Wickerhamomyces anomalus*), the two most outperforming yeasts. Yeasts for which boxplot backgrounds are in green do not differ significantly with the control (*M. roreri* without confrontation). Tests applied were Kruskal–Wallis (Chi-square and *p*-values in Supplementary Table S1), and the Post hoc Conover test (Supplementary File S2). Each confrontation had four repetitions (*n* = 4) and a control (total of 20 controls)

Discussion

In this study, we successfully isolated and morphologically and molecularly characterized ninety-six yeast strains

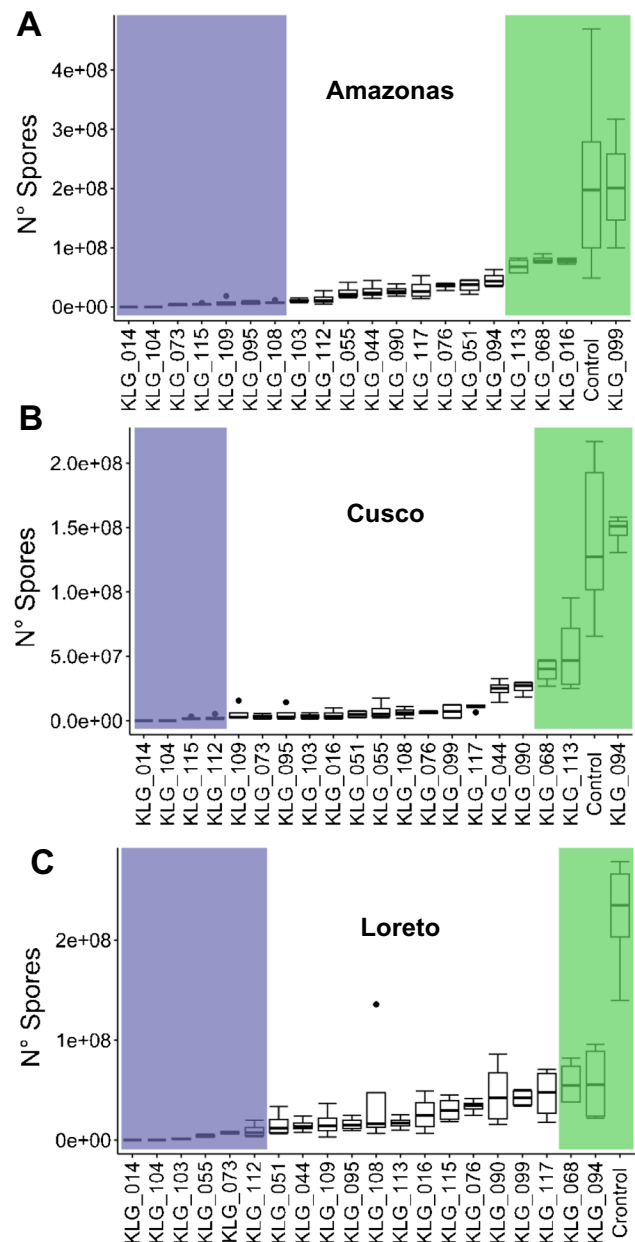


Fig. 4 Number of spores produced by *Moniliophthora roreri* strains from Amazonas (A), Cusco (B), and Loreto (C) confronted against twenty yeast strains from this study. Yeasts for which boxplot backgrounds are in blue do not differ significantly with KLG-104 (*Candida sorboxylosa*) and KLG-014 (*Wickerhamomyces anomalus*), the two most outperforming yeasts in this experiment. Yeasts for which boxplot backgrounds are in green do not differ significantly with the control (*M. roreri* without confrontation). Tests applied were Kruskal–Wallis (Chi-square and *p*-values in Supplementary Table S1), and the Post hoc Conover test (Supplementary File S2). Each confrontation had four repetitions (*n* = 4) and a control (total of 20 controls)

from the phyllosphere of native cacao in different provinces of Amazonas, Peru. In total, we identified thirty-one species across *Basidiomycota* and *Ascomycota*. Within

Basidiomycota, the most species-rich genus was *Hannaella* with six species, followed by *Rhodotorula* and *Moesziomyces* with three and two species, respectively. Similar results were found by Neto et al. (2021), reporting *Hannaella* as the most species-rich genus with four species (twenty-three strains). These genera are common inhabitants of the plant phyllosphere, as reported in previous studies (Landell et al. 2014; Kaewwichian et al. 2015; Surussawadee et al. 2015). Within *Ascomycota*, the most species-rich genus was *Candida* with four species. Species of *Candida* was also found on the surface of plant tissues such as cacao leaves, flowers, and fruits (Neto et al. 2021). Interestingly, we found one strain of *C. metapsilosis*, a species known to cause vaginal candidiasis (Bertini et al. 2013), thus it was not considered in the in vitro tests against *M. roreri* to avoid potential human health issues in the long term.

Regarding frequency of isolation, the most isolates (twenty-four) were identified as *M. guilliermondii* (Table 2; Supplementary Table S2). *Meyerozyma* species are widely distributed in nature and can be isolated from diverse terrestrial and aquatic substrates, and even found in human microflora (Kurtzman and Suzuki 2010; Rosales-López et al. 2018), guts of waxworms (larvae of *Plodia interpunctella*), and on the surface of vegetable plants (Kurtzman and Suzuki 2010; Yurkov et al. 2017). This study provides the first evidence of a widespread distribution of *M. guilliermondii* in the cacao phyllosphere. Its presence was confirmed in the three sampled provinces: Condorcanqui, Bagua, and Utcubamba, with twelve, nine, and three isolates, respectively. Furthermore, it was detected in ten of the eighteen sampled plots. Notably, the majority of isolates were obtained from fruit samples (seventeen isolates), while the remaining ones were recovered from leaves (seven isolates). These findings indicate that *M. guilliermondii* is widely distributed across the cacao agroecosystem.

There is vast evidence that yeasts are naturally present in the phyllosphere of plants and can act as BCAs of pathogens in different crops, including cacao (Bettiol 1997; Bruce et al. 2003; Zhang et al. 2007; Ferraz et al. 2019; Freimoser et al. 2019; Neto et al. 2021; Junior et al. 2022). In this study, out of the thirty-one species found, nineteen (twenty strains) were subjects of the experiments to evaluate their antibiosis activity against the causal agent of frosty pod rot of cacao, *M. roreri*. The results obtained showed that strains KLG-014 (*W. anomalus*) and KLG-104 (*C. sorboxylosa*) have the strongest in vitro antagonistic activity against *M. roreri* as they completely inhibited the growth and sporulation of three strains of the pathogen. Similar results were found by Wang et al. (2018), who reported the biocontrol potential of *W. anomalus* against *B. cinerea*, causal agent of rot in blueberries in the state of Washington, USA. Also, Khunnamwong et al., (2020) found that *W. anomalus* had high potential of antagonism against phytopathogenic fungi such

as *Curvularia lunata*, *Fusarium moniliforme* and *Rhizoctonia solani*. Other studies have also reported the importance of *C. sorboxylosa* as potential biocontrol agent. For example, Taheur et al. (2020) found that this species is able to degrade aflatoxin B1 produced by the plant pathogen *Aspergillus flavus*. Moreover, this species can be effectively preserved and survive up to thirty days on the surface of healthy cacao fruits (Gaslac-Zumaeta et al. 2025). Therefore, the *C. sorboxylosa* KLG-104 strain from this study has strong potential as a BCA.

On the cacao phyllosphere in Brazil, Neto et al. (2021) found three yeast species with desired traits for a BCA, namely *C. orthopsilosis*, *Hanseniaspora uvarum* and *R. paludigena*. In the present investigation, only *R. paludigena* was retrieved (strain KLG-119). In a similar study on yeasts from the cacao phyllosphere in Brazil, they found a biocontrol capacity of up to 71% in growth reduction of *M. roreri* in confrontation assays of five yeast species: “*H. pagnoccae*”, *H. luteola*, *Hannaella* sp., *Vishniacozyma carnescens* and *R. paludigena* (Junior et al. 2022). Of all these species, only “*H. pagnoccae*” (strain KLG-076) was used in the confrontation assays of the present study. This strain had an antibiosis effect, but it was not the most significant, and even, when tested against the *M. roreri* strain from Amazonas, the growth of the pathogen did not differ with the control. In other words, it did not significantly reduce its growth. On the other hand, we also evaluated “*H. phyllophila*” (strain KLG-073), neither identified nor tested in Junior et al. (2022), finding it significantly reduced sporulation of *M. roreri* strains from Amazonas and Loreto.

In research carried out in Colombia, a technological package based on antagonistic yeasts was formulated for the control of *B. cinerea* in *Rosa* sp. (Dávila 2016). They found that *R. mucilaginosa* and *P. tsukubaensis* showed antagonistic activity against this phytopathogenic fungus through in vitro assays. These results agree with what was found here, as *R. mucilaginosa* KLG-055 also significantly inhibited the growth of *M. roreri* strains from Cusco and Loreto.

In this study, the high in vitro antifungal activity observed in strains *W. anomalus* KLG-014 and *C. sorboxylosa* KLG-104 against *M. roreri* could suggest the production of antagonistic compounds, including killer toxins or mycocins, bioactive VOCs and other secondary metabolites. For example, previous research has shown that the β -glucanase activity of the Panomycocin produced by killer *W. anomalus* strains is effective to protect orange fruits against *Penicillium digitatum* (Platanía et al. 2012). Similarly, *P. membranifaciens* killer toxins (PMKT and PMKT2) inhibit the growth of *B. cinerea* and strains of this species can be used in biocontrol of grey mold on grapevine plantlets (Masih and Paul 2002; Belda et al. 2017). Regarding VOCs, the 3-methyl-1-butanol produced by yeast cells of *Aureobasidium* spp. efficiently inhibit the

mycelial growth of *B. cinerea* (Di Francesco et al. 2020). Also, *P. kudriavzevii*, *P. occidentalis*, and *M. caribbica* produce ethyl esters of medium-chain fatty acids, phenylethyl alcohol, and phenylethyl acetate that are consistently detected during antagonistic interactions against several plant pathogens, including species of *Mucor*, *Penicillium*, *Aspergillus*, *Fusarium*, and *Botrytis*, suggesting that these compounds play an important role in the antagonism of these yeasts (Choińska et al. 2020). Beyond killer toxins and bioactive VOCs, there are other compounds and strategies of yeasts against competitors. Research on *Anthracycystis flocculosa* (syn. *Pseudozyma flocculosa*) has demonstrated its remarkable potential as a BCA via the production of extracellular fatty acids that naturally insert themselves into and degrade the cell membranes of powdery mildews, an important group of phytopathogenic fungi (Avis and Bélanger 2002). Moreover, there are indirect modes of action of biocontrol yeasts, such as the induction of pathogen resistance in the host, as shown in *C. oleophila* (Droby et al. 2007). After inoculation on citrus and pome fruits, *C. oleophila*, enhances ethylene biosynthesis, phenylalanine ammonia lyase activity, and phytoalexin accumulation, and increases chitinase and β -1,3-endoglucanase proteins, which leads to the defense against *Penicillium digitatum* (Droby et al. 2007). These examples reinforce the importance of identifying antagonistic compounds and modes of action of yeasts for commercial application (Olena et al. 2025).

All the diversity of cacao epiphytic yeasts found in this study, and the identification of species and strains with high biocontrol potential against *M. royeri* represents a hallmark opportunity to find an alternative solution to prevent and manage FPR, one of the most important diseases of cacao in Peru and Latin America. The follow-up steps must include exploring the identification and production of killer toxins, metabolites and volatile compounds produced by the yeasts with antagonistic capacity against *M. royeri*, evaluating their antagonistic capacity in the field (Zhang et al. 2020), finding the most efficient ways of preserving, and developing the best protocols and formulations to deliver these epiphytic yeasts on cacao fruits to prevent *M. royeri* infection (Gaslac-Zumaeta et al. 2025). Future research to find potential BCAs also should focus on unaltered Amazonian areas where cacao and its wild relatives grow naturally. These areas harbor a high genetic diversity of the crop and, thus, a high diversity of the natural enemies of their pathogens.

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Author contribution M. Catherine Aime and Jorge R. Díaz-Valderrama contributed to the study conception and design. Material preparation, data collection and laboratory and in silico analyses were performed by Kelvin J. Llanos-Gómez. Some material preparation and data collection were performed by Oswaldo Ananco-Ahuananchi and Jorge Huaman-Pilco. The statistical analyses were performed by Jesús Rascón. Funding acquisition, logistics, resources and supervision of research activities were performed by Segundo M. Oliva-Cruz, Santos Triunfo Leiva-Espinoza, M. Catherine Aime and Jorge R. Díaz-Valderrama. The first draft of the manuscript was written by Kelvin J. Llanos-Gómez and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All sequences generated in this study are available in GenBank with accession numbers cited in Table 2. Crude data about the area of growth and sporulation is available upon request.

Declarations

Ethical approval Not applicable.

Consent to participate This research does not involve human participants and/or animals. An informed consent statement does not apply to this research.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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