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Screening of Potential Yeast Starters from *Cola cordifolia* Fruit Pulp

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Abstract The aim of this study is to add value to *Cola cordifolia* fruit from northern Côte d'Ivoire. Owing to its high carbohydrate content, *Cola cordifolia* could be a suitable substrate for research into fermentative flora for food industry. In this way, yeasts were isolated from *Cola cordifolia* pulp and screened for potential starters. Following screening, the best strains were identified using a molecular technique to determine the species involved. The results were used to select five (5) strains showing the best growth compared with the other strains tested. These strains belong to the *Candida parapsilosis*, *Hanseniaspora uvaru*m and *Pichia manshurica* species. These strains could thus be used as potential starters in biotechnological applications. *Cola cordifolia* would be crucial to the development of yeast culture collections useful for controlled fermentations.

Keywords: *characterization, Cola cordifolia, selection, starter, yeast*

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1. Introduction

Côte d'Ivoire is the West Africa's top producer of fruit and vegetables. Every year in Côte d'Ivoire, large quantities of fruit are produced among which some are sold in Côte d'Ivoire, others are exported, and others are for local consumption only without resulting incomes. Therefore, a great part of the production is lost because many fruits are highly perishable [1]. Over the past 20 years, Côte d'Ivoire has lost an average of 15% of its annual production, due to the lack of local processing units [1]. Although some alternatives to direct consumption have already been implemented (jams, fruit concentrates, fruit juices, nectars, etc.), a large quantity of fruit is still left in the fields to rot or to be collected and then disposed of as waste. Among others, we can state *Cola cordifolia*, a wild fruit that generally grows in savannah regions. This fruit is poorly exploited, less known by Ivorians, from both nutritional and industrial point of view since its consumption remains seasonal. The production is abundant, but the product has never been taken into account as a means of adding value. In some countries, the alternative solution implemented has been to process most wild fruits through fermentation. Most often, the resulting product is wine derived from fruit, which is often distilled and has a variable alcohol concentration, or fruit vinegar [2]. During these alcohol fermentation processes, several yeasts are involving whose identity and functional properties are unknowns. Knowledge of the functional properties of these yeasts will make it possible to select starter culture who will be able to optimize the alcoholic production of the *Cola cordifolia* fruit and other fruit notably cocoa fermentation. The aim of this study is to contribute to the valorization of *Cola cordifolia* fruits through the identification and screening of yeast strains involving of alcohol fermentation of this fruits to be used as a starter culture for the improvement and optimization of alcoholic fermentation.

2. Materials and Methods

2.1. Material

The biological material used in this study is the fruit (pulp) of *Cola cordifolia* (Figure 1) from trees at the PELEFERO GON COULIBALY University in Korhogo (Côte d'Ivoire). The fruits have been harvested and sent to the University's laboratory for microbiological analysis.

Figure 1. *Cola cordifolia* fruits

3. Methods

3.1. Isolation and Identification of Yeasts

Cola cordifolia beans (25 g) are diluted in 225 ml of peptone salt solution (0.1% (w/v) bactopeptone and 0.85% (w/v) NaCl). The solution thus prepared constitutes the stock solution, which undergoes successive decimal dilutions (10^{-1} to 10^{-4}) with tryptone salt solution. A volume of 100 µL of each dilution has been streaked onto MYGP agar (3 g/L yeast extract, 3 g/L malt extract, 5 g/L bactopeptone and 10 g/L glucose) containing 100 mg/L chloramphenicol (Sigma). After inoculation, Petri dishes have incubated and yeast strains have morphologically been identified after 3 days of incubation at 30°C, then yeast cells have been observed fresh under a precision optical microscope (Zeiss Microlmaging GmbH 37081, Germany) at objective ˣ100. Presumptive yeast isolates were stored in cryotubes containing MYGP broth supplemented with 20% glycerol at -20°C for subsequent testing [3].

3.2. Yeast Screening

Depending on the test to perform, strains are grown overnight at 25◦C either on MYGP agar or in MYGP broth and then the cultures are used to inoculate either MYGP broth or specific media. For the latter, each strain is harvested by centrifugation (5000 rpm for 10 min), washed once in NaCl 0.9% (w/v) solution, re-suspended to Optical Density (OD) 600 of 1.0 in the same solution [4]. Subsequently, each strain has been spotted (5 µL) in duplicate onto specific media.

3.2.1. Screening of High Fermentative-capacity Yeasts

The fermentative capacity of yeast strains isolated from *Cola cordifolia* pulp has been studied according to the method of [5] with a slight modification. From pre-culture of 24 hours, pure yeasts culture has been suspended in saline tryptone to get an optical density of 0.7 at 600 nm and 100 µL of this suspension have been used to inoculate 10 mL of YPG medium containing a Durham tube into essay tube. Then, the culture has been incubated at 30°C for 6 days, without agitation. Fermentative capacity has also been determined by measuring the gas production in Durham test. Yeasts usually oxidize sugars into ethanol with production of gas [6]. This volume of gas and ethanol produced is related to the fermentative strength of strain [7].

3.2.2. Catalase Activity

The yeast biomass, taken with a sterile 1µL loop, has been added to a drop of 3% (v/v) H2O2 [8]. The development of bubbles has indicated positive activity.

3.2.3. Acetic Acid Production

A loopful (1 µL) of biomass of each strain has been streaked onto Chalk agar (yeast extract 3 g/L, glucose 10 g/L, calcium carbonate 3 g/L, agar 15 g/L) plates and has incubated for 7 d at 25 °C [9]. The presence and extent of a clear halo around the yeast biomass has indicated the rate of acetic acid production.

3.2.4. H_2S Production

To assess hydrogen sulphide production, 10 µL of each strain from the previously prepared YPD liquid has been inoculated on the BIGGY medium and kept for 2–3 days at 24 C, respectively. Visual scale has been used as a function of the increasing level of H_2S produced [10,11,12,13].

3.2.5. Protease Activity

Each strain culture has been spotted onto Petri plates with a medium prepared by mixing the two following solutions: malt extract 3 g/L, yeast extract 3 g/L, peptone 5 g/L, glucose 10 g/L, NaCl 5 g/L, agar 20 g/L (separately sterilized), adjusted to pH 3.5 with 0.1 M HCl; and a skim milk solution (10% w/v) prepared and treated at 100◦C for 10 min. After incubation for 3 days at 25◦C, the presence of a clear halo around the yeast spot indicated protease activity [14].

3.2.6. Growth at Various Temperatures and pH

Yeast strains have been grown in standard liquid medium containing 0.05% yeast extract; 0.3% casein peptone; 1% glucose at pH 5.6. To assess the influence of

temperature on the growth of yeast isolates, 10 ml of standard liquid medium contained in a test tube has been inoculated with 100 µL of yeast pre-culture, OD600 = 0.7. The cultures have then been incubated for 72 h at temperatures ranging from 30 to 50°C. The influence of pH variations on the growth of yeast isolates has been analyzed in the same medium at different pH values (2.5; 4; 5 and 7) and has been incubated at 30°C. Yeast isolate growth has been determined by measuring the turbidity of the culture medium at 600 nm using a spectrophotometer.

3.2.7. Determination of Ethanol by Distillation

An Ethanol assay has been carried out at the end of fermentation. Fermented molasse has been distilled to ethanol, using the powerful Quickfit/FC3/13 column distiller, 85 cm long and 4.45 cm in diameter (Fischer Scientific, Sweden) A temperature of 79°C has been maintained at the top of the column until all the alcohol in the fermented juice evaporated and condensed. The use of an alcoholmeter (Biobase, china) has enabled to determine Ethanol content. Three independent experiments have been performed.

3.2.8. Molecular Characterization of Potential Starters

Yeasts have been grown at 30 °C to the mid-log phase in YPD medium before harvesting. Yeast genomic DNA has been extracted using the classic phenol / chloroform method described by [15]. The D1/D2 region of 26S rDNA has been PCR amplified as described by [16] using the eukaryotic universal primer gc-NL1 (5’ CGCCCGCCGCGCGCGGCGGGCGGGC GGGGGCCATATCAATAGCGGAGGA AAAG 3’) and the reverse primer LS2 (5’ ATTCCCAAACAACTCGACTC 3’). (5’ ATTCCCAAACAACTCGACTC 3’). The D1/D2 PCR program consisted to one cycle at 94°C for 3 min, followed by 30 cycles (95°C for 1 min, 52°C for 1 min, 72°C for 1 min), and a final extension at 72°C for 10 min. PCR-amplified D1/D2 regions of 26S rDNA were sequenced by BIOFIDAL (Lyon, France) using the Pseq D1/D2 primer (5’GGGCCATATCAATAAGC3’). Sequences have then been compared to the NCBI data Genbank using the BLAST program (http: //blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences showing a high percentage of identity (≥ 97 %) have been considered as belonging to the same species.

3.2.9. Statistical Analysis

All measurements have been performed in triplicate. Statistical analyses of the data have been performed through Statistica version 7.1 software. Means have been compared through Tuckey's HSD test with a significance level of only 5% (p < 0 ,050).

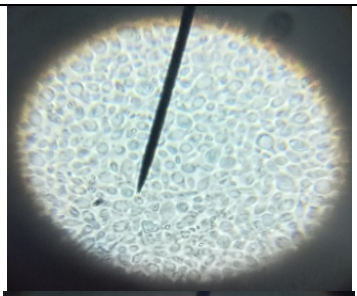
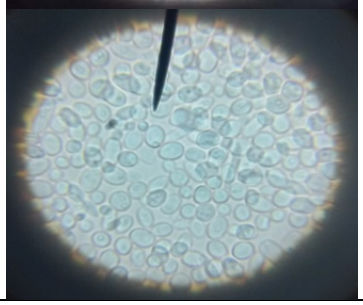
4. Results and discussion

4.1. Results

4.1.1. Morphology of yeast involving in fermentation of *Cola cordifolia* pulp

Fifty-six (56) yeast isolates have been isolated from *Cola cordifolia* pulp. Table 1 shows the morphology, color, and texture of the colonies of the different strains grown on MYGP agar.

Table 1. Colony characteristics of yeast strains grown on MYGP agar

Strains	Colony Morphology, Color, and Texture	Optical microscopy of representative strains
YC1-YC12	Convex, White, Smooth/Glossy	
YC13-YC17	Convex, White, Matt/opaque	
YC18-YC56	Convex, Cream-colored, Smooth/Glossy	

4.1.2. Fermentative Capacity of Yeast Strains

Among the 56 analyzed yeast strains for the fermentative capacity, only Sixteen (16) have shown a high fermentative capacity with gas amount above to 4 cm^3. While five (5) isolates have a gas production between 1 and 4 cm3 and Thirty-Five (35) have gas production between 0 and 1 cm3 (Table 2).

Table 2. CO2 production of yeast isolate from *Cola cordifolia* pulp

Fermentative capacity	Volume of CO2 (cm3)	Number of isolates
High level	[4 – 6 cm^3]	16
Middle level	[1 – 4 cm^3[	05
Low level	[0 – 1 cm^3[	35

4.1.3. Biochemical Properties of High Fermentative Capacity Yeasts Involving of *Cola cordifolia* Pulp Fermentation

All the yeasts isolates involving of *Cola cordifolia* pulp fermentation tested have the catalase and H_2S production capacity. But the level of production is different from one yeast isolate to another. About protease activity, nine (9) have produced with high activity for five (5) isolates (YC 16, YC 21, YC 42, YC 46, YC 49). While only tree (3) yeasts isolates (YC 8, YC 53, YC 56) are able to produce acetic acid (Table 3).

Table 3. Biochemical properties of yeasts isolates involving of *Cola cordifolia* pulp fermentation

strains	Acetic Acid Production[a]	Catalase activity[b]	Protease Activity[c]	H_2S Production[d]
YC 3	-	++	+	++
YC 5	-	+	-	+
YC 6	-	+	-	+
YC 8	+	++	-	+
YC 16	-	+++	+++	+
YC 21	-	+++	+++	+
YC 28	-	+	-	++
YC 40	-	+	-	++
YC 41	-	+	-	+
YC 42	-	+++	+++	+
YC 46	-	+++	+++	+
YC 49	-	+++	+++	+
YC 51	-	++	-	++
YC 53	+	+	+	++
YC 54	-	++	++	++
YC 56	+	+	+	++

[a]Halo: -, none; +, low. [b] Development of bubbles: +, low; ++, medium, +++, high. [c]Activity: −, no halo; +, small diameter; ++, medium diameter; +++, large diameter. [d] Biomass color: ++, black brown; +, brown.

4.1.4. Growth Capacity of the High Fermentative Yeasts Isolate Involving of *Cola cordifolia* pulp Fermentation At Different Temperature

Yeast isolates showed varying growth rates at different temperatures. In fact, all yeast isolates show a decrease in growth with increasing temperature**.** At 30°C, yeasts isolate growth ranged from 0.76 to 1.83. At 35°C, growth ranged from 0.40 to 1.54. At 40°C, growth ranged from 0.20 to 1.01. At 45°C, yeast isolate growth ranged from 0.15 to 0.82, and at 50°C from 0 to 0.35. Among the sixteen (16) isolates tested, ten (10) yeasts isolates (YC16, YC21, YC28, YC41, YC42, YC46, YC49, YC51, YC53 and YC56) showed the best growth at 30°C with an average optical density (OD) greater than 1. But the yeast isolate YC16 shows better growth (1.83) than all isolates tested at 30°C. In addition, yeasts isolate YC 28, YC 42, YC 56 and YC 46 maintain this good growth up to 35°C and YC 46 practically maintains this good growth at 40°C (Table 4).

4.1.5. Impact of pH on the Growth of Selected Yeast Isolates

The result of the growth of the 16 best yeasts isolates with a high fermentation power at different pH give two. The first group consists of yeast isolates (YC 3, YC 8, YC 21, YC 28, YC 40, YC 46, YC 53 and YC 56) with the maximum growth at pH 5. At this pH the yeast isolate YC 46 shows the best growth (2.04 ± 0.05). The second group consists of yeasts isolates (YC 54, YC 51, YC 49, YC 42, YC 41, YC 16, YC 6, and YC 5) which shows good growth at pH 4 and 5. At these pH the yeast isolate YC 54 shows the lowest microbial growth (0.66 ± 0.01) (Table 5).

4.1.6. Ethanol Production

Isolates YC16, YC21, YC42, YC46 and YC49 showed the best characteristics (high CO2 production, no acetic acid production, high catalytic activity, high protease activity, low H2S production, and good resistance to temperature and pH variation) for fermentation. These isolates have been selected for ethanol production and identified. The results of the statistical analysis showed a significant difference in ethanol production. Indeed, the production of strains YC16, YC42, YC46 and YC49 differed from that of strain YC28, which had the lowest ethanol production, while strain YC42 had the highest (Figure 2).

4.1.7. Molecular Characterization of the Five Best Yeast Strains

Molecular characterization of the five (5) selected isolates identified YC49, YC16 and YC46 as *Pichia manshurica*, YC21 as *Candida parapsilosis* and YC42 as Hanseniaspora uvarum

Table 4. Impact of the temperature on the growth of selected yeast isolates

Selected yeast strains	Optical density at 600 nm per degree Celsius, OD = 0.7				
	30 °C	35 °C	40 °C	45 °C	50 °C
YC 3	0.95 ± 0.01[a]	0.95 ± 0.01[a]	0.52 ± 0.02[c]	0.21 ± 0.01[d]	0.13 ± 0.05[e]
YC 5	0.96 ±0.03[e]	0.96 ± 0.04[e]	0.50 ± 0.05[b]	0.30 ± 0.00[c]	0.14 ± 0.03[d]
YC 6	0.87 ± 0.02[e]	0.75 ± 0.02[e]	0.49 ± 0.07[b]	0.31 ± 0.00[c]	0.09 ± 0.08[d]
YC 8	0.94 ± 0.04[d]	0.93 ± 0.04[d]	0.44 ± 0.16[c]	0.19 ± 0.04[e]	0.18 ± 0.04[e]
YC 16	**1.83 ± 0.23[b]**	0.93 ± 0.05[c]	0.33 ± 0.04[d]	0.25 ± 0.03[de]	0.20 ± 0.00[e]
YC 21	**1.42±0.14[b]**	0.88 ± 0.02[d]	0.71 ± 0.09[de]	0.63 ± 0.02[e]	0.31 ± 0.03[c]
YC 28	**1.05 ± 0.06[a]**	**1.04 ± 0.05[b]**	0.74 ± 0.02[c]	0.27 ± 0.01[d]	0.08 ± 0.01[e]
YC 40	0.97 ± 0.04[b]	0.82 ± 0.00[c]	0.49 ± 0.07[d]	0.28 ± 0.01[e]	0.24 ± 0.02[e]
YC 41	**1.40 ± 0.03[c]**	0.86 ± 0.07[d]	0.81 ± 0.14[d]	0.24 ± 0.01[e]	0.23 ± 0.00[e]
YC 42	**1.54 ± 0.12[b]**	**1.54 ± 0,11[e]**	0.93 ± 0.02[e]	0.28 ± 0.07[c]	0.20 ± 0.00[d]
YC 46	**1.76 ± 0.15[c]**	**1.00 ± 0.04[e]**	**1.01 ± 0.01[e]**	0.82 ± 0.00[e]	0.16 ± 0.01[d]
YC 49	**1.63 ± 0.06[b]**	0.85 ± 0.00[c]	0.57 ± 0.02[e]	0.53 ± 0,06[e]	0.35 ± 0.03[d]

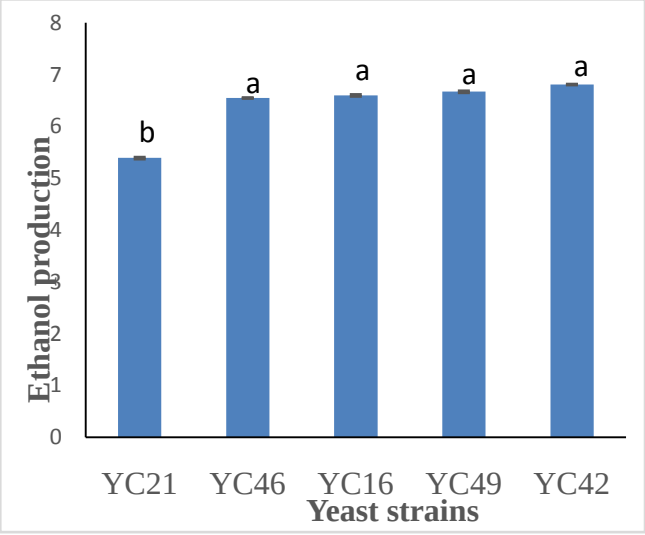
YC 51	**1.44 ± 0.03^{c}**	0.64 ± 0.02^{e}	0.57 ± 0.00^{e}	0.54 ± 0.09^{e}	0.07 ± 0.04^{d}
YC 53	**1.02 ± 0.01^{d}**	0.97 ± 0.04^{d}	0.20 ± 0.00^{e}	0.20 ± 0.05^{e}	0.04 ± 0.01^{c}
YC 54	0.76 ± 0.00^{a}	0.40 ± 0.01^{b}	0.66 ± 0.00^{c}	0.28 ± 0.02^{d}	0.10 ± 0.07^{e}
YC 56	**1.01 ± 0.01^{d}**	**1,00 ± 0.00^{d}**	0.66 ± 0.07^{c}	0.26 ± 0.01^{e}	0.15 ± 0.07^{e}

a, b, c, d, and e: in the averages assigned, different letters are significantly distinct (Tuckey's HSD test, $p < 0.050$)

Table 5. Impact of pH on the growth of selected yeast isolates

	Optical density at 600 nm per degree Celsius, OD = 0.7			
Selected yeast strains	pH 2.5	pH 4	pH 5	pH 7
YC 3	0.58 ± 0.03^{d}	0.92 ± 0.01^{c}	1.52 ± 0.21^{b}	0.86 ± 0.01^{cd}
YC 5	0.64 ± 0.00^{c}	0.96 ± 0.02^{d}	1.00 ± 0.00^{d}	0.81 ± 0.00^{b}
YC 6	0.642 ± 0.00^{c}	0.934 ± 0.01^{d}	0.948 ± 0.02^{d}	0.732 ± 0.04^{b}
YC 8	0.58 ± 0,07^{b}	0.81 ± 0.04^{cd}	0.93 ± 0.04^{c}	0.72 ± 0.00^{d}
YC 16	1.76 ± 0.16^{d}	1.85 ± 0.18^{d}	1.95 ± 0.04^{d}	0.74 ± 0.01^{c}
YC 21	0.57 ± 0.04^{d}	0.93 ± 0.01^{b}	1.04 ± 0.02^{a}	0.70 ± 0.01^{c}
YC 28	0.48 ± 0.06^{d}	0.96 ± 0.04^{b}	1.74 ± 0.18^{a}	1.43 ± 0.00^{c}
YC 40	0.62 ± 0.00^{c}	0.81 ± 0.05^{d}	1.04 ± 0.01^{b}	0.84 ± 0.01^{d}
YC 41	1.31 ± 0.02^{b}	1.78 ± 0.18^{d}	1.86 ± 0.24^{d}	0.88 ± 0.02^{c}
YC 42	1.27 ± 0.08^{b}	1.66 ± 0.08^{d}	1.77 ± 0.07^{d}	0.89 ± 0.00^{c}
YC 46	1.73 ± 0.22^{d}	1.88 ± 0.02^{cd}	2.04 ± 0.05^{c}	0.82 ± 0.02^{b}
YC 49	1.62 ± 0.03^{d}	1.66 ± 0.12^{d}	1.87 ± 0.15^{d}	0.93 ± 0.01^{c}
YC 51	0.89 ± 0.00^{d}	1.82 ± 0.28^{c}	1.93 ± 0.01^{c}	0.88 ± 0.03^{d}
YC 53	0.28 ± 0.02^{d}	0.86 ± 0.05^{b}	1.33 ± 0.03^{a}	0.71 ± 0.06^{c}
YC 54	0.26 ± 0.04^{c}	0.62 ± 0.05^{d}	0.66 ± 0.01^{d}	0.41 ± 0.01^{b}
YC 56	0.61 ± 0.07^{d}	0.78 ± 0.04^{d}	1.75 ± 0.23^{c}	0.68 ± 0.02^{d}

a, b, c, d and e: in averages assigned, different letters are significantly different (Tuckey's HSD test, $p < 0.050$)



Nb: Histograms assigned to different letters have statistically different values (Tuckey's HSD test, $p < 0.050$).

Figure 2. Ethanol production by selected yeast strains

5. Discussion

The first part of this study consisted in isolating yeasts from Cola cordifolia pulp. Thus, isolation on MYGP medium yielded 56 isolates: YC1 to YC56. The study of phenotypic characteristics has enabled us to distinguish four colonies that have the same criteria: small, Convex, White, Smooth/Glossy colonies with regular outlines, large Convex, White, Matt/opaque colonies with irregular outlines, small Convex, White, Matt/opaque colonies with irregular contours and Convex, Cream-colored, Smooth/Glossy colonies with regular contours. These results are identical to those of [17] who states that the characteristics of pure yeast strains lie in size, color, surface appearance, contour appearance, relief, consistency and transparency. After purification, a study of the microscopic characteristics of the pure isolates showed that the cells of the different isolates are: elongated or oval in shape; large, medium or small in size. These observations testify the definition proposed by [18]

who distinguishes yeasts, which belong to the fungi group, by their unicellular character.

The second part of the study consisted in screening yeast isolates to select the best ones for use as starters for ethanol production. In fact, a strain's ability to offer improved technological and functional performance is a key property in its selection as a potential starter [19]. Thus, among the 56 yeasts analyzed for their fermentative capacity, sixteen (16) showed a high fermentative capacity with a CO_2 volume greater than 4 cm^3. These results are similar to those of [20] who showed that among 743 strains, 113 yeast strains were selected for their high fermentative capacity with a CO2 volume greater than 4 cm^3. These yeast strains are likely to produce high quantities of ethanol, since during alcoholic fermentation, the quantity of carbon dioxide (CO_2) would correspond to the quantity of ethanol produced [6].

The majority of strains studied have not produced acetic acid. Acetic acid is one of the compounds that have an impact on the sensory profile of wine, determining its quality. An acetic acid concentration of 0.7–1.1 g/L is not considered pleasant, tasteful; the maximum acceptable limit for volatile acidity in most wines is 1.2 g/L of acetic acid [21,22]. Strains producing little or no acetic acid are the most interesting, tasteful. From this point of view, most of the strains studied are interesting.

Results from the catalase test gave information about the ability of strains to cope with oxidative stress and to perform better during fermentation [23]. All of the strains tested in this study were catalase positive to various extents and in agreement with other authors [24,25]. However, the strains YC16, YC21, YC42, YC46 and YC 49 with the highest catalytic activity are the most interesting for alcoholic fermentation.

The screening of yeast strains that produce zero or low H_2S is particularly important for fermentation since there will be no sulfite formation during fermentation. The difference in H2S production obtained in this study has been confirmed by several authors [14,25,26].

The strains grew well at the different temperatures and pH levels studied. The ability of the strains to grow at high temperatures and low pH levels enables them to adapt to harsh environments. Their resistance to different temperatures corroborates the results of [27] who reported similar optimal temperatures for yeast. In addition, the strains studied had their best growth at pH 5, which differs from the results of [19] indicating that the best yeast growths at the pH level of cocoa pulp is between 3 and 4.

With regard to ethanol production, the alcohol contents of isolates YC 46, YC16, YC 49 belonging to the species *Pichia manshurica* and YC 42 belonging to the species *Hanseniaspora uvarum* have shown no significant difference. However, the ethanol produced by these species is different from that produced by *Candida parapsilosis* species. Production of these strains therefore varies from 5.39 to 6.81%. It should be noted that the production of these strains is higher than that of other strains isolated during cocoa fermentation by [19] whose highest ethanol production is 4.19 %. In addition, the five best strains are non-Saccharomyces strains, which are known to modulate the aroma profile during alcoholic fermentation [28,29].

Isolates YC16, YC21, YC42, YC46 and YC49 showed the best characteristics (high CO2 production, no acetic acid production, high catalytic activity, high protease activity, low H2S production, and good resistance to temperature and pH variation) for fermentation. The growth of these yeast isolates under stress conditions confirms the ability of these yeast strains to be used as starters in many food processes, mainly in the fruit fermentation process.

Conclusion

Fifty-six (56) pure isolates were isolated from Cola cordifolia pulp. Among these fifty-six (56) yeasts isolates, five (5) isolates coded YC16, YC21, YC42, YC46 and YC49 showed the best characteristics, namely high CO_2 production, no acetic acid production, high catalytic and protease activity, low H2S production and good resistance to temperature and pH variation. These strains belong to three species: *Pichia manshurica* (YC49, YC16 and YC46), *Candida parapsilosis* (YC21) and *Hanseniaspora uvarum* (YC42). Thus, these five (5) isolates with interesting technological properties could be used as starters in biotechnological applications.

Competing interests

Authors have declared that no competing interests exist.

Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed and supervised the study. Authors SS and KM managed and performed the experimental and statistical analysis. Authors SL and ML wrote the protocol and wrote the first draft of the manuscript. Authors SS and SY managed the literature searches. All authors read and approved the final manuscript.

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