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Probiotic Potential of Phytase-Producing Yeasts Isolated from Palm Wine

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Abstract

Phytic acid is a major antinutritional factor that limits mineral bioavailability in plant-derived foods. The identification of microorganisms combining phytate-degrading capacity and probiotic properties is therefore of considerable interest for food and nutritional applications. In this study, the probiotic potential of thirteen phytase-producing yeast strains isolated from palm wine was evaluated. All isolates exhibited phytase activity, with hydrolysis halo diameters ranging from 0.18 ± 0.04 to 2.45 ± 0.07 cm. The highest phytase activities were recorded for *Hanseniaspora guilliermondii* RD31 (2.45 ± 0.07 cm), *Hanseniaspora jakobsenii* ADR3E1 (1.88 ± 0.04 cm) and *Saccharomyces cerevisiae* ADR1B1 (1.58 ± 0.04 cm). The strains were further assessed for tolerance to pH, temperature, sodium chloride, bile salts, pepsin and pancreatin, as well as for autoaggregation, coaggregation and cell surface hydrophobicity. All isolates survived simulated gastrointestinal conditions, with survival rates exceeding 87%, while ADR3E1 exhibited the highest resistance to pancreatin (96.15 ± 1.16%). Autoaggregation capacities after 24 h ranged from 78.40 ± 7.35% to 90.95 ± 8.56%, indicating strong adhesion-related potential. Hierarchical clustering analysis identified ADR1B1, ADR3E1 and RD31 as the most promising probiotic candidates, combining superior phytase activity with high tolerance to gastrointestinal stresses and favorable adhesion-related properties. These findings demonstrate that palm wine constitutes a valuable reservoir of multifunctional yeasts and highlight the potential of *S. cerevisiae* ADR1B1, *H. jakobsenii* ADR3E1 and *H. guilliermondii* RD31 for the development of probiotic cultures with phytate-degrading capacity for food and nutritional applications.

Keywords: Palm wine; Probiotic yeasts; Phytase activity; Phytic acid

1. Introduction

Phytic acid (myo-inositol hexakisphosphate, IP6) is the principal storage form of phosphorus in cereals, legumes, oilseeds and many plant-derived foods. Despite its nutritional importance for plants, phytate is considered an antinutritional factor because it strongly chelates essential minerals such as iron, zinc, calcium and magnesium, thereby reducing their bioavailability in humans and monogastric animals [1, 2]. In regions where cereal- and legume-based foods constitute a major part of the diet, high phytate intake has been associated with reduced mineral absorption and an increased risk of micronutrient deficiencies.

Microbial phytases (myo-inositol hexakisphosphate phosphohydrolases) catalyze the hydrolysis of phytate into lower inositol phosphates and inorganic phosphate, thereby improving mineral bioavailability and nutrient digestibility [2,3]. Because of these properties, phytases have received considerable attention in the food and feed industries as eco-friendly biocatalysts capable of enhancing the nutritional value of plant-based products [4]. Yeasts represent an

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important source of phytases and several species have been reported to produce extracellular enzymes capable of degrading phytate under food-processing conditions [5,6].

In parallel, growing consumer demand for functional foods has stimulated interest in probiotic microorganisms. According to the FAO/WHO [7], probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits on the host. Although lactic acid bacteria are the most extensively studied probiotics, yeasts have emerged as promising alternatives due to their natural resistance to antibiotics, tolerance to gastrointestinal stresses and ability to produce bioactive metabolites [8,9]. Beyond the well-known probiotic yeast *Saccharomyces boulardii*, several yeast species isolated from traditional fermented foods have demonstrated probiotic-related properties such as resistance to acidic conditions and bile salts, autoaggregation, coaggregation and adhesion to intestinal surfaces [10-12].

Traditional fermented foods and beverages constitute valuable reservoirs of microbial biodiversity and represent an important source of novel functional microorganisms. Among these products, palm wine is a naturally fermented beverage widely consumed in many African countries and characterized by a rich and diverse yeast community [13]. Previous studies on palm wine yeasts have highlighted their technological potential, particularly their ability to produce aroma compounds and enzymes of industrial interest [14,15]. However, information regarding the occurrence of phytase-producing yeasts exhibiting probiotic properties in palm wine remains scarce.

Most previous studies have investigated either the phytase-producing capacity of yeasts or their probiotic potential separately [5,15]. Consequently, microorganisms combining efficient phytate degradation and probiotic-related traits remain poorly documented, particularly among non-Saccharomyces yeasts. Such multifunctional strains could contribute simultaneously to improving mineral bioavailability and promoting gastrointestinal health, thereby offering new opportunities for the development of functional fermented foods.

Therefore, the aim of the present study was to evaluate the probiotic potential of thirteen phytase-producing yeast strains isolated from palm wine. The strains were assessed for their tolerance to different environmental and gastrointestinal conditions, including pH, temperature, sodium chloride, bile salts and digestive enzymes, as well as for their autoaggregation, coaggregation and cell surface hydrophobicity properties. A hierarchical clustering approach was subsequently used to identify the most promising strains for future food and nutritional applications.

2. Materials and Methods

2.1. Yeast strains and culture conditions

Thirteen yeast strains belonging to different species were used in this study (Table 1). Ten strains were isolated from raffia palm wine (*Raphia hookeri*) and three strains originated from ron palm wine (*Borassus aethiopum*) collected in Côte d'Ivoire. The isolates were previously identified at the species level using molecular methods as described by Amoikon et al. [14]. The strains are maintained in the culture collection of the Laboratory of Food Biotechnology and Microbiology, University Nangui ABROGOUA (Abidjan, Côte d'Ivoire), and stored at -80 °C in YPD broth supplemented with 20% (v/v) glycerol.

The safety of these isolates for potential food applications was previously evaluated through the assessment of hemolytic activity, hydrolytic enzyme production, and biogenic amine formation. The strains included in the present study were reported as safe and suitable for food-related applications [15].

For all experiments, yeast cultures were reactivated on yeast extract-peptone-dextrose agar (YPDA) and incubated at 30°C for 24 h. Cell suspensions were prepared in sterile phosphate-buffered saline (PBS, pH 6.8) and adjusted to approximately 10^7 CFU/mL.

Table 1 List of yeasts strains of this study

Isolation Source	Strains	Yeast species	CLIB numbers	Accession numbers GenBank
Raffia wine (*Raphia hookeri)*	ADR1B1	*Saccharomyces cerevisiae*	3070	MG833305
	ADR3E1	*Hanseniaspora jakobsenii*	3071	MG833303
	ADR3G51	*Geotrichum candidum*	3072	MG833313
	AR235	*Kodamaea ohmeri*	3074	-
	AR2101	*Candida sorboxylosa*	3075	MG833307
	AR2105	*Meyerozyma caribbica*	3076	-
	AR2157	*Yarrowia deformans*	3078	-
	AR2258	*Pichia manshurica*	3079	-
	AR2322	*Pichia kudriavzevii*	3080	-
	AR323	*Debaryomyces hansenii*	3081	-
Ron wine *(Borassus aethiopum)*	RD31	*Hanseniaspora guilliermondii*	3085	-
	RE231	*Yarrowia lipolytica*	3088	MG833309
	RG11	*Candida ethanolica*	3090	-

2.2. Screening of phytase-producing yeasts

Phytase production was assessed according to the method described by Ranjan and Sahay [16]. The phytase screening medium (PSM) contained glucose (1%, w/v), ammonium nitrate (0.3%, w/v), magnesium sulfate (0.05%, w/v), potassium chloride (0.05%, w/v), calcium chloride (0.01%, w/v), agar (1.5%, w/v), and sodium phytate (0.05%, w/v) as the sole phosphorus source. Aliquots (10 µL) of yeast suspensions (10^7 CFU/mL) were spot-inoculated onto PSM agar plates and incubated at 30°C for 72 h. Phytase activity was evidenced by the appearance of a clear halo surrounding the colonies, indicating phytate hydrolysis.

2.3. Evaluation of probiotic properties

2.3.1. Growth under different pH conditions

The ability of yeast strains to grow under acidic and alkaline conditions was evaluated according to Lohith and Anu-Appaiah [12]. YPD broth was adjusted to pH 2.0, 2.5, 3.0, 8.0, 8.5, and 9.0 using sterile HCl or NaOH solutions. YPD broth at pH 6.8 served as the control.

Each medium was inoculated with 1% (v/v) of a yeast suspension (10^7 CFU/mL) and incubated at 37°C. Growth was monitored after 4 h and 24 h by measuring optical density at 600 nm (OD600). Viable cell counts were determined by plating appropriate serial dilutions on YPDA.

2.3.2. Growth at different temperatures

Yeast tolerance to temperature was evaluated by incubating inoculated YPD broth at 30°C, 37°C, and 42°C. Growth was monitored after 4 h and 24 h through OD600 measurements and viable counts on YPDA plates.

2.3.3. NaCl tolerance

Salt tolerance was assessed in YPD broth supplemented with NaCl concentrations of 0%, 2%, 4%, 6%, and 8% (w/v). The inoculated cultures were incubated at 37°C and analyzed after 4 h and 24 h by OD600 measurements and viable cell counts.

2.3.4. Bile salt tolerance

Tolerance to bile salts was evaluated using YPD broth supplemented with ox bile at concentrations of 0%, 0.3%, 0.6%, 0.9%, and 1.2% (w/v), following the method of Lohith and Anu-Appaiah [12]. After incubation at 37°C for 4 h and 24 h, growth was determined by OD600 measurements and viable counts.

2.4. Survival under simulated gastrointestinal conditions

2.4.1. Pepsin tolerance

Yeast survival under simulated gastric conditions was evaluated using a pepsin solution (0.3%, w/v) prepared in 0.5% NaCl and adjusted to pH 2.0. Yeast suspensions (10^7 CFU/mL) were incubated at 37°C for 4 h. Viable counts were determined before and after incubation [12].

The survival rate was calculated as:

$$\text{Survival Rate (\%)} = \frac{\mathbf{Log\ N}}{\mathbf{Log\ N_0}} \times \mathbf{100} \quad (\mathbf{1})$$

Where N0 is the initial viable count and N is the viable count after incubation.

2.4.2. Pancreatin tolerance

Simulated intestinal conditions were assessed using a pancreatin solution (0.1%, w/v) prepared in 0.5% NaCl and adjusted to pH 8.0. Yeast suspensions were incubated at 37°C for 4 h and survival rates were calculated as described above [12].

2.5. Cell adhesion-related properties

2.5.1. Autoaggregation assay

Autoaggregation ability was evaluated according to Collado et al. [17]. Yeast suspensions adjusted to 10^7 CFU/mL in PBS were incubated at 37°C. Absorbance at 600 nm was measured at 0, 4, and 24 h.

Autoaggregation (%) was calculated as:

$$\mathbf{Autoaggregation}\ (\%) = [\mathbf{1} - (\mathbf{A_t}/\mathbf{A_0})] \times \mathbf{100} \quad (\mathbf{2})$$

Where A0 is the initial absorbance and At is the absorbance after incubation.

2.5.2. Coaggregation assay

Coaggregation with pathogenic bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) was evaluated according to Handley et al. [18].

Equal volumes of yeast and bacterial suspensions (10^7 CFU/mL) were mixed and incubated at 37°C. Absorbance measurements were performed after 4 h and 24 h.

Coaggregation (%) was calculated using the equation:

$$\mathbf{Coaggregation}\ (\%) = \frac{\left(\frac{\boldsymbol{A_{pat} + A_{pro}}}{\mathbf{2}}\right) - \boldsymbol{A_{mix}}}{\left(\frac{\boldsymbol{A_{pat} + A_{pro}}}{\mathbf{2}}\right)} \times \mathbf{100} \quad (\mathbf{3})$$

Where Apat is the absorbance of the pathogen suspension, Apro is the absorbance of the yeast suspension, and Amix is the absorbance of the mixed suspension.

2.5.3. Cell surface hydrophobicity (MATH)

Cell surface hydrophobicity was assessed using the microbial adhesion to hydrocarbons (MATH) method described by Bellon-Fontaine et al. [19]. Yeast suspensions were mixed separately with xylene, chloroform, and diethyl ether. After incubation at 37°C for 1 h, the absorbance of the aqueous phase was measured at 600 nm.

Hydrophobicity (%) was calculated as:

$$\text{Hydrophobicity (\%)} = [\mathbf{1} - \mathbf{A}^{*}/\mathbf{A_0}] \times \mathbf{100} \qquad (\mathbf{4})$$

Where A0 is the initial absorbance and At is the absorbance after phase separation.

2.6. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Statistical analyses were conducted using R software version 4.1.1 [20]. Data obtained under each experimental condition were analysed separately using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a significance level of $p < 0.05$.

To identify yeast strains exhibiting the most promising probiotic characteristics and phytate-degrading ability, a multivariate analysis based on heatmap clustering was performed using the pheatmap package in R. This analysis enabled the visualization of similarities and differences among strains and facilitated the selection of the most promising probiotic candidates.

3. Results

3.1. Phytase production by palm wine yeasts

All thirteen yeast strains isolated from palm wine exhibited phytase activity on phytate screening medium, as evidenced by the formation of clear hydrolysis halos around the colonies (Figure 1). However, significant differences ($p < 0.05$) were observed among the isolates with respect to their phytate-degrading capacity. The diameter of the hydrolysis zones ranged from 0.18 ± 0.04 to 2.45 ± 0.07 cm. Strain RD31 exhibited the highest phytase activity, producing the largest hydrolysis halo (2.45 ± 0.07 cm), whereas strain RE231 showed the lowest activity (0.18 ± 0.04 cm). High phytase activities were also observed for strains ADR3E1 (1.88 ± 0.04 cm) and ADR1B1 (1.58 ± 0.04 cm) (Table 1). These results demonstrate that palm wine yeasts constitute a valuable source of phytase-producing microorganisms and highlight substantial strain-dependent variability in phytate degradation ability.

Table 2 Hydrolysis zones of sodium phytate

Strains	Hydrolysis diameter (cm)
ADR1B1	1.58 ± 0.04^{b}
ADR3E1	1.88 ± 0.04^{b}
ADR3G51	0.25 ± 0.07^{f}
AR235	0.43 ± 0.11^{def}
AR2101	1.00 ± 0.07^{c}
AR2105	0.93 ± 0.18^{c}
AR2157	1.08 ± 0.04^{c}
AR2258	0.75 ± 0.21^{cd}
AR2322	0.95 ± 0.07^{c}
AR323	0.30 ± 0.14^{ef}
RD31	2.45 ± 0.07^{a}
RE231	0.18 ± 0.04^{f}

RG11	0.68 ± 0.04^{cde}

Values are expressed as mean ± standard deviation. Different superscript letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

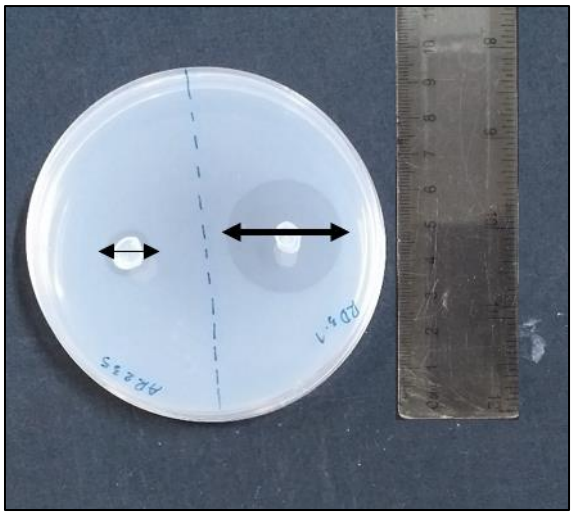

Figure 1 Phytase activity

3.2. Growth of phytase-producing yeasts under different pH conditions

The thirteen phytase-producing yeast strains exhibited different growth responses under acidic and alkaline conditions. Overall, several strains maintained substantial viability across the entire pH range tested (pH 2-9), indicating a remarkable tolerance to environmental stress.

Based on their growth profiles, the strains could be classified into three groups. The first group, including AR2105, AR2322, AR323, RE231, and RG11, showed a preference for acidic conditions. The second group, consisting of ADR3G51, AR235, AR2101, AR2157, and AR2258, exhibited better growth under alkaline conditions. In contrast, ADR1B1, ADR3E1, and RD31 demonstrated strong growth under both acidic and alkaline environments, suggesting superior physiological adaptability.

After 4 hours of incubation, ADR3E1 displayed the highest viable counts at pH 2 (1.6×10^6 CFU/mL) and pH 2.5 (5.1×10^6 CFU/mL), whereas ADR1B1 showed the best growth at pH 3 (7.0×10^6 CFU/mL). After 24 hours, ADR3E1 remained the most acid-tolerant strain at pH 2, reaching 4.1×10^7 CFU/mL. At pH 2.5 and pH 3, RD31 and ADR1B1 exhibited the highest cell densities, respectively.

Under alkaline conditions, ADR3G51 exhibited the highest viable counts after 4 h of incubation at pH 8, 8.5 and 9.0. However, after 24 h, ADR1B1, AR2157 and AR2101 recorded the highest cell densities at pH 8, 8.5 and 9.0, respectively. Significant differences ($p < 0.05$) were observed among strains at all pH values tested.

The ability of ADR1B1, ADR3E1 and RD31 to maintain high viability under strongly acidic conditions is particularly relevant for probiotic applications, as these conditions mimic the gastric environment.

Table 3 Effect of pH on the growth of phytase-producing palm wine yeasts after 4 h and 24 h of incubation

Viable cell count (CFU/mL)								
Strains	**Time**	**pH 2**	**pH 2.5**	**pH 3**	**Control (pH 6.8)**	**pH 8**	**pH 8.5**	**pH 9**
ADR1B1	4h	$1.0\pm0.6\times10^{6\ ab}$	$2.7\pm0.0\times10^{6\ abc}$	$7.0\pm0.1\times10^{6\ a}$	$8.8\pm0.4\times10^{6\ a}$	$2.9\pm0.2\times10^{6\ cd}$	$2.4\pm0.2\times10^{6\ c}$	$1.1\pm0.0\times10^{6\ ef}$
	24h	$3.6\pm0.2\times10^{7\ a}$	$4.3\pm0.5\times10^{7\ a}$	$4.6\pm0.3\times10^{7\ a}$	$4.6\pm0.0\times10^{7\ a}$	$3.2\pm0.4\times10^{7\ a}$	$2.5\pm0.1\times10^{7\ bc}$	$1.8\pm0.0\times10^{7\ a}$
ADR3E1	4h	$1.6\pm0.0\times10^{6\ a}$	$5.1\pm2.3\times10^{6\ a}$	$5.4\pm0.3\times10^{6\ b}$	$6.5\pm1.0\times10^{6\ b}$	$3.9\pm0.8\times10^{6\ c}$	$2.7\pm0.0\times10^{6\ c}$	$2.2\pm0.4\times10^{6\ bc}$
	24h	$4.1\pm1.0\times10^{7\ a}$	$4.5\pm1.0\times10^{7\ a}$	$4.4\pm0.3\times10^{7\ ab}$	$4.9\pm0.8\times10^{7\ a}$	$2.7\pm0.1\times10^{7\ ab}$	$6.9\pm0.1\times10^{6\ c}$	$2.1\pm1.0\times10^{7\ a}$
ADR3G51	4h	$5.0\pm0.4\times10^{5\ ab}$	$1.6\pm0.0\times10^{6\ bc}$	$6.3\pm0.2\times10^{5\ de}$	$3.1\pm0.5\times10^{6\ cd}$	$1.4\pm0.0\times10^{7\ a}$	$1.7\pm0.1\times10^{7\ a}$	$1.9\pm0.0\times10^{7\ a}$
	24h	$7.7\pm1.4\times10^{5\ b}$	$4.7\pm1.7\times10^{6\ b}$	$1.2\pm0.2\times 10^{7\ e}$	$1.7\pm0.0\times10^{7\ ef}$	$2.5\pm0.0\times10^{7\ b}$	$3.0\pm0.0\times10^{7\ a}$	$2.5\pm0.0\times10^{7\ a}$
AR235	4h	$4.8\pm0.4\times10^{5\ ab}$	$9.2\pm2.4\times10^{5\ bc}$	$1.3\pm0.2\times10^{6\ de}$	$2.4\pm0.2\times10^{6\ cdef}$	$9.0\pm1.2\times10^{5\ cd}$	$1.7\pm0.0\times10^{6\ c}$	$2.6\pm0.0\times10^{6\ b}$
	24h	$1.9\pm0.3\times10^{6\ b}$	$7.1\pm1.9\times10^{6\ b}$	$1.4\pm0.1\times10^{7\ de}$	$1.5\pm0.1\times10^{7\ ef}$	$2.5\pm0.0\times10^{7\ b}$	$2.8\pm0.0\times10^{7\ ab}$	$2.6\pm0.0\times10^{7\ a}$
AR2101	4h	$2.2\pm0.8\times10^{5\ b}$	$6.4\pm1.6\times10^{5\ c}$	$1.3\pm0.2\times10^{6\ de}$	$2.0\pm0.4\times10^{6\ def}$	$2.3\pm0.3\times10^{5\ cd}$	$1.4\pm0.1\times10^{6\ c}$	$2.7\pm0.2\times10^{5\ g}$
	24h	$1.2\pm1.4\times10^{6\ b}$	$5.3\pm3.0\times10^{6\ b}$	$7.5\pm0.1\times10^{7\ e}$	$1.3\pm0.0\times10^{7\ f}$	$3.2\pm0.0\times10^{7\ a}$	$2.8\pm0.0\times10^{7\ ab}$	$2.6\pm0.0\times10^{7\ a}$
AR2105	4h	$3.7\pm2.5\times10^{5\ ab}$	$3.4\pm1.6\times10^{5\ c}$	$1.3\pm0.2\times10^{6\ de}$	$8.3\pm2.2\times10^{5\ ef}$	$2.2\pm0.8\times10^{5\ d}$	$1.1\pm0.1\times10^{6\ c}$	$2.0\pm0.0\times10^{6\ bcd}$
	24h	$7.8\pm1.8\times10^{6\ b}$	$1.4\pm0.0\times10^{7\ b}$	$2.2\pm0.8\times10^{7\ cde}$	$2.5\pm0.5\times10^{7\ cde}$	$4.6\pm0.5\times10^{6\ cd}$	$6.0\pm1.5\times10^{6\ d}$	$1.9\pm0.1\times10^{6\ c}$
AR2157	4h	$7.1\pm0.6\times10^{5\ ab}$	$2.1\pm0.1\times10^{6\ bc}$	$2.0\pm0.1\times10^{6\ cd}$	$4.1\pm0.1\times10^{6\ c}$	$2.2\pm0.8\times10^{5\ d}$	$1.9\pm0.7\times10^{6\ c}$	$2.5\pm0.1\times10^{6\ b}$
	24h	$7.5\pm0.5\times10^{6\ b}$	$7.5\pm2.9\times10^{6\ b}$	$1.3\pm0.1\times10^{7\ e}$	$3.4\pm0.0\times10^{7\ bc}$	$3.1\pm0.3\times10^{7\ a}$	$3.0\pm0.3\times10^{7\ a}$	$1.7\pm0.1\times10^{7\ ab}$
AR2258	4h	$4.6\pm2.6\times10^{5\ ab}$	$9.0\pm1.6\times10^{5\ bc}$	$9.0\pm0.4\times10^{5\ de}$	$1.5\pm0.4\times10^{6\ def}$	$2.5\pm0.4\times10^{5\ c}$	$3.2\pm1.4\times10^{5\ c}$	$1.2\pm0.4\times10^{6\ def}$
	24h	$5.2\pm2.5\times10^{6\ b}$	$1.2\pm0.3\times10^{7\ b}$	$1.7\pm0.5\times10^{7\ cde}$	$2.1\pm0.1\times10^{7\ def}$	$7.8\pm0.4\times10^{6\ c}$	$2.5\pm0.0\times10^{7\ bc}$	$1.9\pm0.0\times10^{7\ a}$
AR2322	4h	$1.0\pm0.8\times10^{6\ ab}$	$2.5\pm0.2\times10^{6\ bc}$	$2.9\pm0.9\times10^{6\ c}$	$2.5\pm0.1\times10^{6\ cde}$	$1.8\pm0.0\times10^{6\ b}$	$1.1\pm0.0\times10^{6\ c}$	$5.5\pm1.4\times10^{5\ fg}$
	24h	$2.9\pm0.2\times10^{7\ a}$	$3.5\pm0.0\times10^{7\ a}$	$2.9\pm0.0\times10^{7\ bcd}$	$3.1\pm0.2\times10^{7\ bcd}$	$2.5\pm0.0\times10^{7\ a}$	$5.7\pm0.1\times10^{6\ d}$	$5.6\pm4.0\times10^{6\ bc}$
AR323	4h	$3.6\pm3.2\times10^{5\ ab}$	$2.9\pm1.0\times10^{5\ c}$	$1.5\pm0.5\times10^{6\ de}$	$2.1\pm0.9\times10^{6\ cdef}$	$2.7\pm0.2\times10^{5\ d}$	$6.6\pm0.2\times10^{5\ c}$	$6.3\pm0.2\times10^{5\ fg}$
	24h	$1.1\pm1.4\times10^{6\ b}$	$2.1\pm0.2\times10^{6\ b}$	$1.3\pm0.8\times10^{7\ e}$	$1.7\pm0.0\times10^{7\ ef}$	$1.7\pm0.1\times10^{6\ e}$	$1.3\pm1.0\times10^{6\ e}$	$1.5\pm0.7\times10^{6\ c}$
RD31	4h	$1.1\pm0.0\times10^{6\ ab}$	$3.4\pm0.4\times10^{6\ ab}$	$4.9\pm0.5\times10^{6\ b}$	$7.3\pm0.8\times10^{6\ ab}$	$7.7\pm3.0\times10^{6\ b}$	$6.7\pm2.4\times10^{6\ b}$	$1.5\pm0.4\times10^{6\ cde}$
	24h	$3.8\pm0.7\times10^{7\ a}$	$4.8\pm0.5\times10^{7\ a}$	$4.4\pm0.3\times10^{7\ ab}$	$4.1\pm0.2\times10^{7\ ab}$	$2.8\pm0.0\times10^{7\ ab}$	$2.3\pm0.1\times10^{7\ c}$	$1.9\pm0.2\times10^{7\ a}$
RE231	4h	$2.8\pm1.9\times10^{5\ b}$	$5.5\pm2.6\times10^{5\ c}$	$5.5\pm0.2\times10^{5\ e}$	$2.1\pm0.5\times10^{6\ cdef}$	$4.6\pm0.1\times10^{5\ d}$	$1.4\pm0.2\times10^{5\ c}$	$2.1\pm0.6\times10^{5\ g}$

	24h	$4.2\pm1.4\times10^{6\ b}$	$1.4\pm0.4\times10^{7\ b}$	$2.1\pm0.0\times10^{7\ cde}$	$2.1\pm0.2\times10^{7\ def}$	$2.3\pm0.2\times10^{6\ e}$	$8.4\pm2.4\times10^{5\ e}$	$4.3\pm2.2\times10^{5\ c}$
RG11	4h	$3.4\pm1.2\times10^{5\ ab}$	$1.1\pm0.0\times10^{6\ bc}$	$1.6\pm0.2\times10^{6\ cde}$	$3.4\pm1.2\times10^{5\ f}$	$1.4\pm0.0\times10^{6\ cd}$	$1.7\pm0.0\times10^{6\ c}$	$2.7\pm0.2\times10^{5\ g}$
	24h	$5.5\pm2.2\times10^{5\ b}$	$5.9\pm0.0\times10^{6\ b}$	$2.9\pm0.0\times10^{7\ bc}$	$3.0\pm0.0\times10^{7\ bcd}$	$4.2\pm0.0\times10^{6\ de}$	$2.5\pm0.2\times10^{6\ de}$	$4.1\pm1.8\times10^{5\ c}$

Values are expressed as mean ± standard deviation. Different superscript letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

3.3. Growth of phytase-producing yeasts at different temperatures

The ability of the thirteen phytase-producing yeast strains to grow at different temperatures was evaluated at 30, 37, and 42°C. All strains remained viable throughout the temperature range tested, although their growth performance varied significantly ($p < 0.05$) depending on both strain and incubation temperature. Overall, yeast growth was higher at 30 and 37°C than at 42°C. After 4 h of incubation, strain RD31 exhibited the highest viable count at 30°C (9.0×10^6 CFU/mL), whereas strain AR323 showed the best growth at 37°C (1.2×10^7 CFU/mL). At 42°C, ADR3E1 displayed the highest cell density (1.1×10^6 CFU/mL), indicating a greater ability to withstand elevated temperatures than most of the other isolates. After 24 h of incubation, ADR1B1 showed the highest viable counts at both 30°C (4.4×10^7 CFU/mL) and 37°C (4.8×10^7 CFU/mL), while ADR3E1 maintained the best growth at 42°C (1.1×10^7 CFU/mL). In contrast, several strains, including AR235, AR2101, AR2105, AR2258, RE231, and RG11, exhibited markedly reduced growth at 42°C, suggesting lower thermotolerance.

Notably, the candidate probiotic strains ADR1B1, ADR3E1, and RD31 maintained relatively high viable counts at 37°C, which corresponds to human body temperature. Their ability to grow under these conditions further supports their potential suitability for gastrointestinal survival and probiotic applications.

Table 4 Effect of temperature on the growth of phytase-producing palm wine yeasts after 4 h and 24 h of incubation

Viable cell count (CFU/mL)						
Temp	**30°C**		**37°C**		**42°C**	
Strains	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**
ADR1B1	$8.9\pm1.9\times10^{6a}$	$4.4\pm0.2\times10^{7a}$	$3.0\pm0.8\times10^{6b}$	$4.8\pm0.2\times10^{7a}$	$2.0\pm0.8\times10^{5b}$	$1.0\pm0.8\times10^{7a}$
ADR3E1	$7.8\pm0.6\times10^{6ab}$	$4.4\pm0.2\times10^{7a}$	$2.4\pm2.9\times10^{6b}$	$3.8\pm0.5\times10^{7ab}$	$1.1\pm0.7\times10^{6a}$	$1.1\pm0.2\times10^{7a}$
ADR3G51	$5.9\pm2.4\times10^{5c}$	$1.3\pm0.2\times10^{7d}$	$6.3\pm0.6\times10^{5b}$	$7.7\pm1.8\times10^{5f}$	$1.8\pm0.2\times10^{5b}$	$7.1\pm1.8\times10^{5c}$
AR235	$1.4\pm1.3\times10^{6c}$	$1.6\pm0.2\times10^{7cd}$	$2.0\pm0.8\times10^{6b}$	$1.6\pm0.1\times10^{7e}$	$1.7\pm0.4\times10^{5b}$	$3.4\pm1.2\times10^{5c}$
AR2101	$5.7\pm4.6\times10^{5c}$	$1.0\pm0.3\times10^{7d}$	$1.3\pm0.6\times10^{6b}$	$1.7\pm0.6\times10^{7de}$	$1.8\pm0.2\times10^{5b}$	$2.7\pm0.2\times10^{5c}$
AR2105	$2.1\pm1.2\times10^{6c}$	$2.7\pm0.3\times10^{7ab}$	$8.3\pm2.2\times10^{5b}$	$2.4\pm0.3\times10^{7cde}$	$2.0\pm0.4\times10^{5b}$	$2.1\pm0.6\times10^{5c}$
AR2157	$8.4\pm0.9\times10^{6a}$	$3.1\pm0.0\times10^{7abc}$	$6.1\pm1.1\times10^{6ab}$	$4.1\pm0.2\times10^{7ab}$	$2.9\pm0.6\times10^{5ab}$	$2.9\pm0.6\times10^{5c}$
AR2258	$2.9\pm0.5\times10^{6c}$	$3.5\pm0.2\times10^{7ab}$	$1.1\pm0.2\times10^{6b}$	$2.0\pm0.2\times10^{7de}$	$3.5\pm1.0\times10^{5ab}$	$3.5\pm1.0\times10^{5c}$
AR2322	$4.2\pm1.1\times10^{6bc}$	$3.2\pm0.0\times10^{7ab}$	$2.1\pm0.5\times10^{6b}$	$3.1\pm0.3\times10^{7bcd}$	$1.5\pm0.2\times10^{5b}$	$4.2\pm0.3\times10^{6bc}$
AR323	$3.9\pm0.6\times10^{6bc}$	$2.0\pm0.2\times10^{7bcd}$	$1.2\pm0.4\times10^{7a}$	$2.9\pm0.5\times10^{7bcde}$	$1.8\pm0.2\times10^{5b}$	$4.4\pm1.2\times10^{6bc}$
RD31	$9.0\pm1.4\times10^{6a}$	$4.0\pm0.3\times10^{7a}$	$3.1\pm1.2\times10^{6b}$	$3.7\pm0.5\times10^{7abc}$	$1.8\pm0.6\times10^{5b}$	$7.0\pm0.2\times10^{6ab}$
RE231	$8.6\pm0.7\times10^{6a}$	$2.2\pm0.3\times10^{7bcd}$	$5.4\pm2.7\times10^{6b}$	$1.7\pm0.3\times10^{7de}$	$3.1\pm0.8\times10^{5ab}$	$3.5\pm1.4\times10^{5c}$
RG11	$3.1\pm1.0\times10^{6c}$	$3.1\pm0.0\times10^{7abc}$	$5.6\pm2.0\times10^{5b}$	$2.8\pm0.2\times10^{7bcde}$	$2.0\pm0.4\times10^{5b}$	$2.0\pm0.4\times10^{5c}$

Values are expressed as mean ± standard deviation. Different superscript letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

3.4. Tolerance of Phytase-Producing Yeasts to Sodium Chloride

The tolerance of the yeast strains to osmotic stress was assessed in the presence of increasing NaCl concentrations ranging from 0 to 8% (w/v). In general, yeast growth progressively decreased with increasing salt concentration. Significant differences ($p < 0.05$) were observed among strains at all NaCl concentrations tested. At low salt concentrations (2-4% NaCl), most strains remained viable and continued to grow, although growth rates differed considerably. Among the isolates, RD31 exhibited the highest tolerance to osmotic stress. After 4 h of incubation, this strain showed the highest viable counts at both 2% (1.3×10^6 CFU/mL) and 4% NaCl (8.4×10^5 CFU/mL). After 24 h, RD31 also recorded one of the highest cell densities at 2% NaCl (4.6×10^7 CFU/mL), confirming its strong adaptation to saline environments. At higher NaCl concentrations (6 and 8%), the growth of most strains was severely inhibited, with viable counts remaining close to the initial inoculum level. Nevertheless, RD31 was able to maintain substantial growth even at 6% NaCl, reaching 1.7×10^7 CFU/mL after 24 h of incubation, whereas several strains showed little or no growth under the same conditions.

The observed tolerance to moderate salt concentrations is an advantageous characteristic for probiotic microorganisms, as it reflects their ability to withstand stressful environmental conditions encountered during food processing, storage, and gastrointestinal transit.

3.5. Tolerance to Bile Salts

The ability of the phytase-producing yeast strains to tolerate bile salts was investigated at concentrations ranging from 0.3 to 1.2% (w/v). All isolates were able to grow in the presence of bile salts, although significant strain-dependent differences were observed ($p < 0.05$) (Figures 2 and 3). After 4 h of incubation, ADR3E1 exhibited the highest growth at all bile salt concentrations tested, reaching 1.5×10^7, 1.2×10^7, 8.6×10^6 and 7.1×10^6 CFU/mL at 0.3, 0.6, 0.9 and 1.2% bile salts, respectively. Although cell density decreased progressively with increasing bile salt concentration, ADR3E1 maintained substantially higher viable counts than most of the other isolates.

After 24 h of incubation, ADR3E1 and RD31 emerged as the most bile-tolerant strains. ADR3E1 exhibited the highest viable counts in the control medium and at 0.3% bile salts, reaching 5.4×10^7 and 6.0×10^7 CFU/mL, respectively. RD31 showed the strongest growth at 0.6 and 0.9% bile salts, with viable counts of 5.4×10^7 and 5.3×10^7 CFU/mL, respectively.

ADR1B1 also displayed good tolerance to bile salts, maintaining high viable counts throughout the experiment. Together, ADR1B1, ADR3E1 and RD31 consistently outperformed the remaining isolates, highlighting their strong capacity to withstand one of the major physiological barriers encountered in the gastrointestinal tract.

Table 5 Effect of NaCl concentration on the growth of phytase-producing palm wine yeasts after 4 h and 24 h of incubation

Viable cell count (CFU/mL)										
NaCl Concentrations	**0%**		**2%**		**4%**		**6%**		**8%**	
Strains	4h	24h	4h	24h	4h	24h	4h	24h	4h	24h
ADR1B1	$3.7\pm1.3\times10^{6bc}$	$4.6\pm0.5\times10^{7ab}$	$1.1\pm0.3\times10^{6ab}$	$2.3\pm0.1\times10^{7b}$	$5.9\pm1.6\times10^{5a}$	$1.3\pm0.2\times10^{7d}$	$3.2\pm1.4\times10^{5abcd}$	$9.0\pm0.4\times10^{5cdef}$	$2.0\pm0.8\times10^{5a}$	$2.4\pm0.2\times10^{5a}$
ADR3E1	$5.1\pm1.0\times10^{6bc}$	$4.8\pm0.9\times10^{7a}$	$8.0\pm1.0\times10^{5abc}$	$2.0\pm0.1\times10^{7b}$	$3.9\pm1.6\times10^{5a}$	$3.8\pm0.8\times10^{6e}$	$6.3\pm1.4\times10^{5a}$	$1.1\pm0.2\times10^{6cde}$	$1.8\pm0.2\times10^{5a}$	$1.1\pm0.4\times10^{5a}$
ADR3G51	$6.2\pm2.8\times10^{5c}$	$1.5\pm4.1\times10^{7ef}$	$1.8\pm0.2\times10^{5c}$	$9.4\pm1.4\times10^{5c}$	$3.1\pm0.4\times10^{5a}$	0.0 ± 0.0	$9.8\pm2.0\times10^{4d}$	0.0 ± 0.0	$1.5\pm0.2\times10^{5a}$	0.0 ± 0.0
AR235	$1.8\pm0.6\times10^{6c}$	$1.3\pm0.2\times10^{7f}$	$6.7\pm1.6\times10^{5abc}$	$4.6\pm0.6\times10^{6c}$	$5.3\pm2.4\times10^{5a}$	$2.6\pm0.5\times10^{6e}$	$1.8\pm0.9\times10^{5cd}$	$2.1\pm0.3\times10^{6b}$	$2.0\pm0.8\times10^{5a}$	$5.5\pm1.4\times10^{5a}$
AR2101	$1.7\pm0.8\times10^{6c}$	$1.7\pm0.6\times10^{7def}$	$5.5\pm2.2\times10^{5bc}$	$1.5\pm0.8\times10^{6c}$	$3.4\pm1.2\times10^{5a}$	$1.5\pm1.0\times10^{5e}$	$1.4\pm0.8\times10^{5d}$	$1.4\pm0.8\times10^{5fg}$	$1.4\pm0.8\times10^{5a}$	$1.7\pm0.4\times10^{5a}$
AR2105	$1.1\pm0.6\times10^{6c}$	$2.2\pm0.0\times10^{7cdef}$	$5.5\pm3.4\times10^{5bc}$	$4.6\pm1.4\times10^{6c}$	$3.8\pm1.4\times10^{5a}$	$1.6\pm0.5\times10^{6e}$	$3.4\pm1.2\times10^{5abcd}$	$1.4\pm0.1\times10^{6bc}$	$2.9\pm1.4\times10^{5a}$	$2.1\pm1.0\times10^{5a}$
AR2157	$6.9\pm0.0\times10^{6b}$	$4.5\pm0.4\times10^{7ab}$	$7.6\pm0.8\times10^{5abc}$	$4.2\pm0.6\times10^{7a}$	$4.1\pm1.8\times10^{5a}$	$3.2\pm0.6\times10^{7b}$	$4.1\pm0.6\times10^{5abcd}$	$4.6\pm1.4\times10^{5efg}$	$3.8\pm0.6\times10^{5a}$	$4.1\pm0.6\times10^{5a}$
AR2258	$1.3\pm0.6\times10^{6c}$	$2.0\pm0.1\times10^{7def}$	$7.3\pm0.8\times10^{5abc}$	$4.1\pm0.2\times10^{7a}$	$5.5\pm1.8\times10^{5a}$	$3.1\pm0.0\times10^{7bc}$	$3.5\pm0.2\times10^{5abcd}$	$2.1\pm0.2\times10^{6b}$	$2.0\pm0.4\times10^{5a}$	$3.8\pm0.2\times10^{5a}$
AR2322	$2.1\pm0.5\times10^{6c}$	$3.1\pm0.2\times10^{7bcd}$	$4.8\pm0.8\times10^{5bc}$	$1.7\pm0.2\times10^{7b}$	$2.8\pm0.8\times10^{5a}$	$4.8\pm0.2\times10^{6e}$	$2.8\pm0.8\times10^{5bcd}$	$1.3\pm0.2\times10^{6bcd}$	$2.4\pm0.2\times10^{5a}$	$3.6\pm0.8\times10^{5a}$
AR323	$1.2\pm0.3\times10^{7a}$	$3.1\pm0.2\times10^{7bcde}$	$3.6\pm1.6\times10^{5c}$	$4.7\pm1.4\times10^{6c}$	$3.2\pm1.4\times10^{5a}$	$1.5\pm0.2\times10^{6e}$	$2.2\pm0.8\times10^{5cd}$	$5.7\pm2.6\times10^{5defg}$	$9.8\pm2.0\times10^{4a}$	$2.5\pm0.4\times10^{5a}$
RD31	$3.4\pm0.8\times10^{6bc}$	$3.8\pm0.6\times10^{7abc}$	$1.3\pm0.1\times10^{6a}$	$4.6\pm0.1\times10^{7a}$	$8.4\pm3.0\times10^{5a}$	$2.5\pm0.2\times10^{7c}$	$6.2\pm0.4\times10^{5ab}$	$1.7\pm0.0\times10^{7a}$	$3.5\pm3.0\times10^{5a}$	$9.0\pm1.2\times10^{5a}$
RE231	$7.7\pm0.5\times10^{6ab}$	$2.1\pm0.2\times10^{7def}$	$8.0\pm1.4\times10^{5abc}$	$4.5\pm0.1\times10^{7a}$	$7.6\pm0.8\times10^{5a}$	$4.3\pm0.1\times10^{7a}$	$4.9\pm1.0\times10^{5abc}$	$5.5\pm2.2\times10^{5efg}$	$4.3\pm1.4\times10^{5a}$	$8.7\pm0.3\times10^{5a}$
RG11	$6.2\pm1.2\times10^{5c}$	$2.7\pm0.3\times10^{7cdef}$	$2.0\pm0.8\times10^{5c}$	$3.6\pm1.2\times10^{5c}$	$5.6\pm0.4\times10^{5a}$	$8.7\pm2.4\times10^{5e}$	$1.8\pm0.6\times10^{5cd}$	$5.9\pm2.8\times10^{5defg}$	$2.7\pm1.0\times10^{5a}$	$8.4\pm2.4\times10^{5a}$

Values are expressed as mean ± standard deviation. Different superscript letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

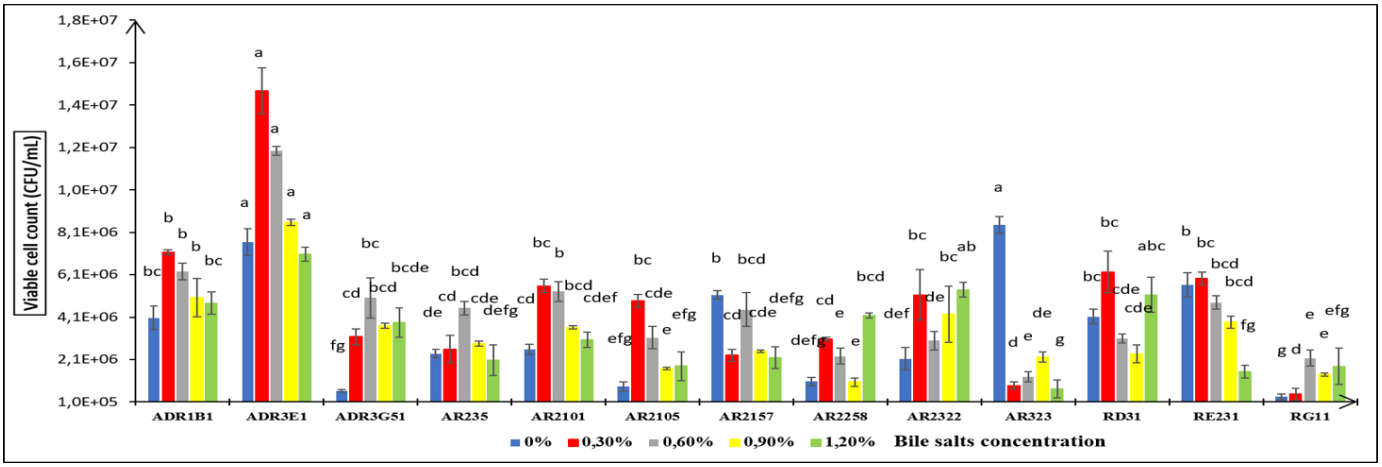

Figure 2 Growth of phytase-producing palm wine yeasts at different bile salt concentrations after 4 h of incubation

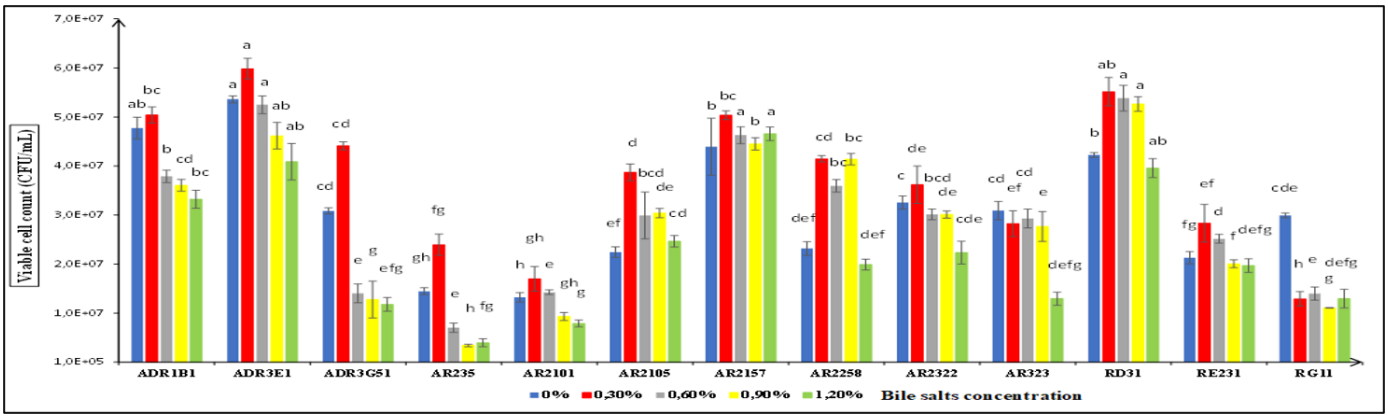

Figure 3 Growth of phytase-producing palm wine yeasts at different bile salt concentrations after 24 h of incubation

3.6. Survival of Phytase-Producing Yeasts under Simulated Gastrointestinal Conditions

3.6.1. Tolerance to Pepsin

The survival of the phytase-producing yeast strains under simulated gastric conditions was evaluated in the presence of pepsin at pH 2.0 (Figure 4). All strains exhibited high resistance to pepsin, with survival rates exceeding 90% after 4 h of incubation. Significant differences ($p < 0.05$) were nevertheless observed among the tested isolates.

The survival rates ranged from 90.07 ± 0.12% to 98.91 ± 0.62%. Among the strains, ADR3E1 displayed the highest survival rate (98.91 ± 0.62%), followed by ADR1B1 and RD31, which also maintained high viability under simulated gastric conditions. In contrast, RE231 exhibited the lowest survival rate (90.07 ± 0.12%), although its viability remained above the threshold generally considered acceptable for probiotic microorganisms.

Overall, the high resistance exhibited by ADR1B1, ADR3E1 and RD31 suggests a strong capacity to withstand gastric stress and reach the intestinal environment in sufficient numbers to exert beneficial effects.

3.6.2. Tolerance to Pancreatin

The ability of the yeast isolates to survive simulated intestinal conditions was assessed in the presence of pancreatin (Figure 5). Similar to the results obtained with pepsin, all strains exhibited high survival rates, ranging from 87.30 ± 1.41% to 96.16 ± 1.41%, indicating a strong resistance to intestinal enzymatic stress. Significant differences ($p < 0.05$) were observed among the isolates.

ADR3E1 again exhibited the highest survival rate (96.16 ± 1.41%), confirming its remarkable tolerance to gastrointestinal conditions. ADR1B1 and RD31 also showed excellent resistance to pancreatin, with survival rates exceeding 93%. In contrast, AR2258 recorded the lowest survival rate, although it remained above 87%.

Taken together, the results obtained with pepsin and pancreatin indicate that ADR1B1, ADR3E1 and RD31 possess a strong ability to survive the successive stresses encountered during gastrointestinal transit, a key requirement for probiotic microorganisms.

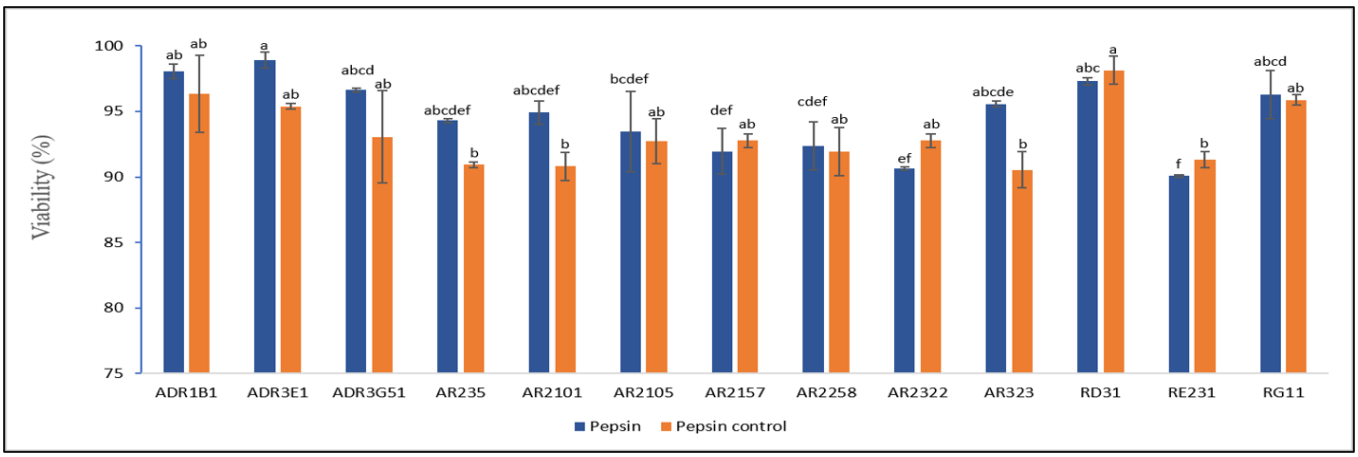

Figure 4 Survival rates of phytase-producing palm wine yeasts in the presence of pepsin after 4 h of incubation

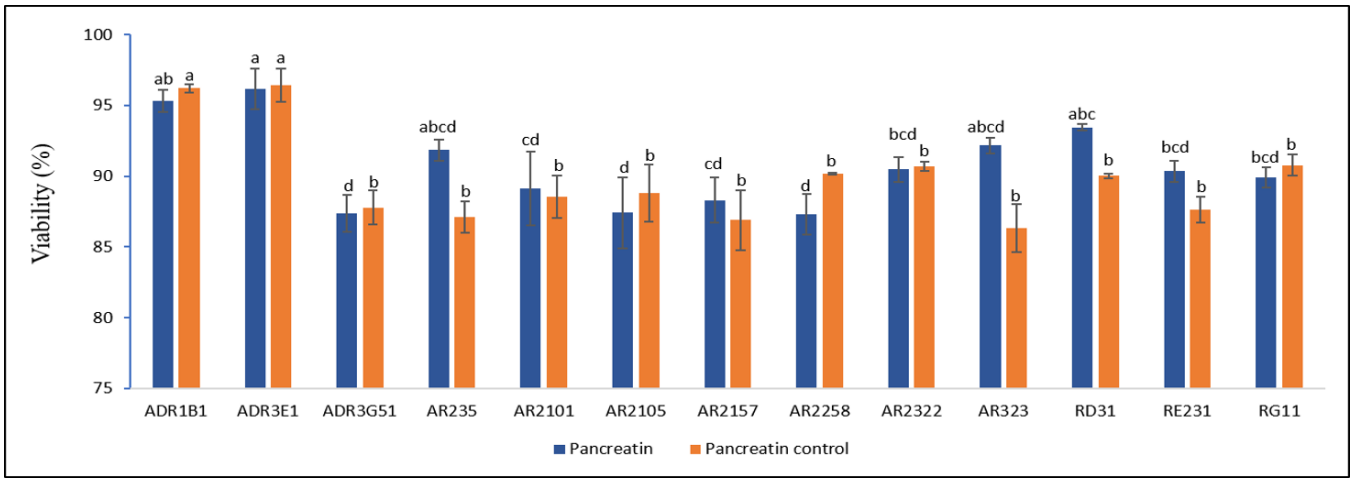

Figure 5 Survival rates of phytase-producing palm wine yeasts in the presence of pancreatin after 4 h of incubation

3.7. Adhesion-Related Properties of Phytase-Producing Yeasts

3.7.1. Autoaggregation Ability

Autoaggregation capacities of the phytase-producing yeast strains are presented in Table 6. After 4 h of incubation, autoaggregation percentages ranged from 31.80 ± 5.37% to 74.60 ± 9.05%, whereas after 24 h they increased substantially, reaching values between 78.40 ± 7.35% and 90.95 ± 8.56%. Most isolates exhibited autoaggregation values above 50% after 4 h, indicating a strong tendency to form cellular aggregates. The highest autoaggregation rate was observed for ADR3G51 (74.60 ± 9.05%), while AR2105 showed the lowest value (31.80 ± 5.37%). After 24 h, all strains displayed high autoaggregation capacities and no significant differences were observed among isolates. ADR1B1 exhibited the highest autoaggregation value (90.95 ± 8.56%), closely followed by AR2322, RE231 and RD31.

The high autoaggregation abilities observed for ADR1B1 and RD31 may contribute to prolonged persistence within the gastrointestinal tract and facilitate interactions with the intestinal mucosa.

3.7.2. Coaggregation with Pathogenic Bacteria

The coaggregation abilities of the yeast isolates with Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 are presented in Table 6. All strains demonstrated the ability to coaggregate with both pathogens, although significant differences ($p < 0.05$) were observed among isolates. For E. coli, coaggregation values ranged from 8.50 ± 0.55% to 33.09 ± 3.21% after 4 h and increased to between 18.14 ± 0.06% and 53.58 ± 3.16% after 24 h. The highest coaggregation rate after 24 h was recorded for AR323, followed by RD31, which exhibited a coaggregation percentage of 42.79 ± 1.71%. In the case of P. aeruginosa, coaggregation values ranged from 8.85 ± 1.01% to 29.64 ± 4.91% after 4 h and from 27.61 ± 3.13% to 67.89 ± 1.73% after 24 h. AR2105 displayed the highest coaggregation ability, whereas RD31 also demonstrated substantial coaggregation capacity, reaching 50.28 ± 1.02% after 24 h.

The ability of probiotic microorganisms to coaggregate with pathogenic bacteria is considered advantageous because it may contribute to pathogen exclusion and colonization resistance. In this regard, RD31 exhibited particularly promising characteristics.

3.7.3. Cell Surface Hydrophobicity

Cell surface hydrophobicity was evaluated using xylene, chloroform and diethyl ether as hydrocarbon solvents (Table 6). Significant differences ($p < 0.05$) were observed among strains for all hydrocarbons tested. Hydrophobicity values varied considerably depending on both the yeast strain and the solvent used. With xylene, adhesion percentages ranged from 11.55 ± 3.19% to 86.41 ± 2.97%, with ADR1B1 exhibiting the highest value. In chloroform, RE231 displayed the strongest adhesion (92.06 ± 1.87%), while RD31 showed the lowest value (37.40 ± 3.29%). For diethyl ether, RE231 again exhibited the highest hydrophobicity (95.50 ± 0.75%), whereas AR323 presented the lowest value (33.83 ± 2.50%).

Among the probiotic candidates, ADR1B1 exhibited particularly high hydrophobicity toward xylene, suggesting a strong capacity for surface interactions. ADR3E1 showed high affinity for chloroform, whereas RD31 displayed moderate hydrophobicity. Despite the observed variability, the overall hydrophobicity profiles support the adhesion potential of these strains and complement the aggregation results.

3.8. Hierarchical Clustering Analysis of Probiotic Properties

To identify the most promising probiotic candidates among the phytase-producing palm wine yeasts, a hierarchical clustering analysis coupled with heatmap visualization was performed using all probiotic-related parameters (Figure 6). The analysis separated the thirteen isolates into two major clusters. Cluster I comprised seven strains (AR323, RE231, ADR3G51, AR235, AR2101, AR2105 and RG11), characterized by relatively low phytase activity, reduced tolerance to acidic conditions and lower resistance to digestive enzymes. Cluster II contained six strains and was further divided into two subclusters.

Subcluster IIa consisted of ADR3E1, ADR1B1 and RD31. These strains were distinguished by high phytase activity, excellent tolerance to acidic pH, strong growth in the presence of bile salts, and high survival rates under simulated gastrointestinal conditions. In contrast, subcluster IIb, composed of AR2258, AR2157 and AR2322, exhibited lower resistance to digestive enzymes despite showing good tolerance to acidic environments and bile salts.

Overall, the clustering analysis clearly identified ADR3E1 (*Hanseniaspora jakobsenii*), ADR1B1 (*Saccharomyces cerevisiae*) and RD31 (*Hanseniaspora guilliermondii*) as the most promising multifunctional strains, combining strong phytase activity with desirable probiotic attributes. These isolates therefore represent the best candidates for future applications in food and feed biotechnology.

Table 6 Adhesion-related properties of phytase-producing palm wine yeasts

Strains	**Autoaggregation**		**Coaggregation**				**Hydrophobicity**		
	Autoaggregation 4 h (%)	**Autoaggregation 24 h (%)**	***E. coli* (%) 4 h**	***E. coli* (%) 24 h**	***P. aeruginosa* (%) 4 h**	***P. aeruginosa* (%) 24 h**	**Xylene (%)**	**Chloroform (%)**	**Diethyl ether (%)**
ADR1B1	71.00 ± 5.80^{ab}	90.95 ± 8.56^{a}	23.65 ± 0.60^{abcd}	43.56 ± 1.38^{ab}	16.68 ± 0.89^{cdef}	28.13 ± 0.27^{cd}	86.41 ± 2.97^{a}	75.77 ± 3.76^{bc}	48.74 ± 2.77^{ef}
ADR3E1	54.50 ± 4.95^{abc}	84.60 ± 9.05^{a}	21.31 ± 0.70^{bcd}	31.47 ± 2.63^{cde}	18.53 ± 3.61^{bcde}	30.57 ± 2.70^{cd}	44.89 ± 6.19^{e}	87.23 ± 2.58^{ab}	43.61 ± 6.97^{fg}
ADR3G51	74.60 ± 9.05^{a}	90.00 ± 4.24^{a}	23.82 ± 4.39^{abc}	27.29 ± 4.30^{def}	26.98 ± 0.07^{ab}	37.40 ± 5.16^{bcd}	51.18 ± 4.71^{de}	57.45 ± 8.04^{d}	91.37 ± 3.33^{ab}
AR235	65.55 ± 9.55^{ab}	89.75 ± 4.60^{a}	21.63 ± 2.78^{bcd}	21.74 ± 2.20^{ef}	16.21 ± 0.87^{cdef}	33.18 ± 2.09^{cd}	83.07 ± 7.37^{ab}	75.78 ± 2.58^{bc}	78.78 ± 3.13^{bc}
AR2101	45.20 ± 1.13^{bc}	85.15 ± 9.40^{a}	8.50 ± 0.55^{e}	29.20 ± 4.16^{de}	11.69 ± 1.28^{def}	41.59 ± 6.63^{bc}	67.55 ± 2.95^{bc}	73.68 ± 4.14^{bc}	72.28 ± 2.17^{cd}
AR2105	31.80 ± 5.37^{c}	86.50 ± 9.19^{a}	33.09 ± 3.21^{a}	35.32 ± 1.96^{bcd}	29.64 ± 4.91^{a}	67.89 ± 1.73^{a}	61.48 ± 3.25^{cd}	67.35 ± 3.61^{cd}	58.04 ± 1.98^{de}
AR2157	70.25 ± 5.30^{ab}	87.70 ± 3.82^{a}	14.36 ± 1.00^{cde}	18.14 ± 0.06^{f}	8.85 ± 1.01^{f}	29.77 ± 1.87^{cd}	15.71 ± 3.46^{f}	38.47 ± 2.65^{e}	36.60 ± 4.08^{fg}
AR2258	70.30 ± 7.92^{ab}	80.55 ± 4.03^{a}	19.04 ± 2.54^{cd}	24.06 ± 0.75^{ef}	10.99 ± 1.36^{def}	36.44 ± 5.17^{cd}	37.15 ± 2.20^{e}	57.77 ± 3.80^{d}	45.48 ± 4.39^{efg}
AR2322	73.20 ± 4.95^{a}	90.40 ± 9.05^{a}	14.22 ± 0.60^{de}	41.51 ± 4.14^{bc}	10.18 ± 0.87^{ef}	27.61 ± 3.13^{d}	11.55 ± 3.19^{f}	84.79 ± 4.38^{ab}	40.56 ± 3.19^{fg}
AR323	71.95 ± 5.73^{a}	84.30 ± 4.53^{a}	30.33 ± 2.63^{ab}	53.58 ± 3.16^{a}	22.57 ± 4.88^{abc}	40.46 ± 4.32^{bcd}	12.77 ± 5.00^{f}	52.31 ± 2.88^{de}	33.83 ± 2.50^{g}
RD31	68.45 ± 5.02^{ab}	89.90 ± 9.05^{a}	17.92 ± 2.36^{cde}	42.79 ± 1.71^{b}	20.00 ± 1.77^{bcd}	50.28 ± 1.02^{b}	41.78 ± 2.71^{e}	37.40 ± 3.29^{e}	45.07 ± 5.23^{efg}
RE231	71.50 ± 4.95^{a}	90.30 ± 8.91^{a}	21.37 ± 2.31^{bcd}	36.44 ± 0.79^{bcd}	21.94 ± 0.23^{abc}	32.79 ± 3.13^{cd}	84.26 ± 1.31^{a}	92.06 ± 1.87^{a}	95.50 ± 0.75^{a}
RG11	65.35 ± 10.39^{ab}	78.40 ± 7.35^{a}	21.86 ± 3.32^{bcd}	36.76 ± 1.04^{bcd}	11.94 ± 2.02^{def}	30.45 ± 0.54^{cd}	67.45 ± 2.55^{bc}	73.68 ± 1.96^{bc}	65.93 ± 1.57^{cd}

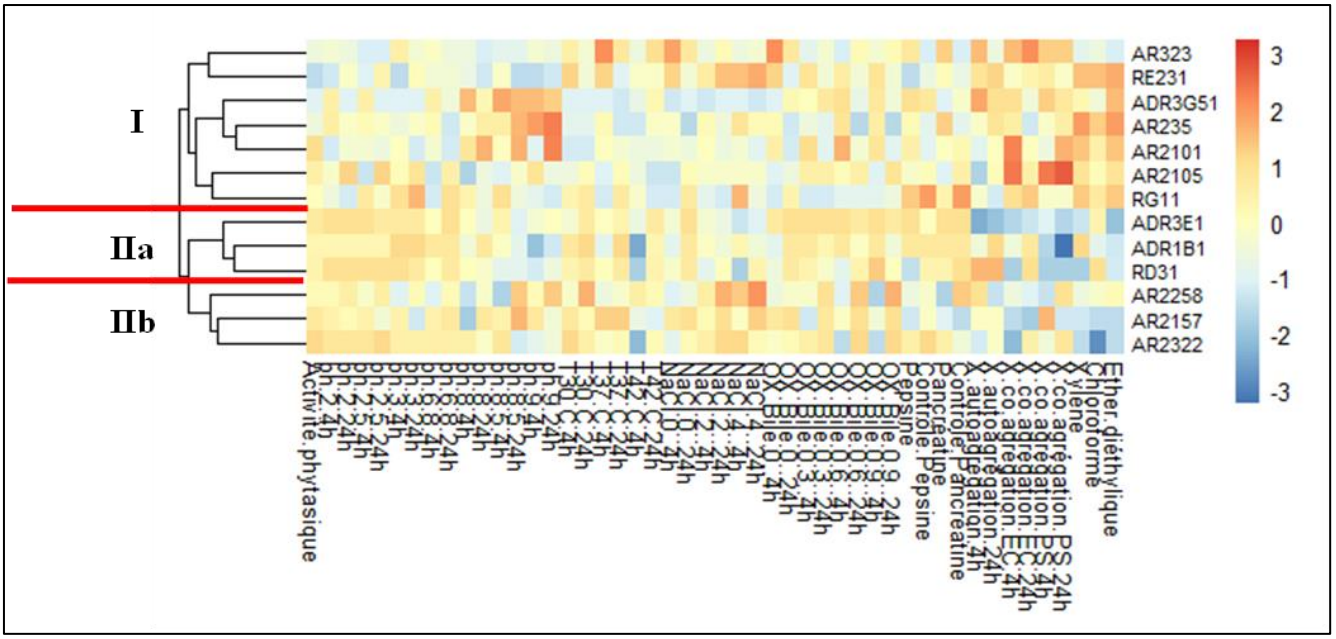

Figure 6 Hierarchical heatmap clustering of phytase-producing palm wine yeasts based on probiotic-related characteristic

4. Discussion

The present study investigated the probiotic potential of thirteen phytase-producing yeasts isolated from palm wine, a traditional fermented beverage known to harbor a rich diversity of indigenous yeasts. Among the tested isolates, *Saccharomyces cerevisiae* ADR1B1, *Hanseniaspora jakobsenii* ADR3E1 and *Hanseniaspora guilliermondii* RD31 consistently exhibited the most desirable characteristics, combining high phytase activity with strong tolerance to gastrointestinal stresses and favorable adhesion-related properties. The convergence of these traits suggests that these strains may constitute promising multifunctional microorganisms for food and nutritional applications.

All thirteen yeast isolates were able to hydrolyze phytate, although significant differences in phytase activity were observed among strains. The highest hydrolysis diameters were recorded for RD31, ADR3E1 and ADR1B1, indicating a superior capacity to degrade phytic acid. Phytate is recognized as one of the main antinutritional factors in plant-derived foods because it chelates minerals and reduces their bioavailability. Consequently, phytase-producing microorganisms are of considerable interest for improving the nutritional quality of cereal and legume-based foods [1,2]. Similar phytate-degrading activities have been reported in yeasts isolated from fermented foods and beverages, including *Saccharomyces, Pichia* and *Hanseniaspora* species [5,6,16]. The fact that all isolates displayed phytase activity suggests that palm wine may represent an ecological niche favoring the development of microorganisms capable of mobilizing organic phosphorus sources. More importantly, the three strains selected as the best probiotic candidates were also the most active phytase producers, highlighting their multifunctional potential.

A fundamental requirement for probiotic microorganisms is their ability to survive the harsh physicochemical conditions encountered during gastrointestinal transit. In the present study, all isolates tolerated a broad range of pH conditions, with ADR1B1, ADR3E1 and RD31 exhibiting the highest viability under acidic conditions. Resistance to low pH is particularly important because microorganisms must survive gastric acidity before reaching the intestine. The observed acid tolerance may be related to physiological adaptations developed in the palm wine environment, where yeasts are naturally exposed to acidic conditions throughout fermentation [14]. Similar acid tolerance has been reported for probiotic yeasts isolated from fermented foods, including *Saccharomyces cerevisiae, Candida tropicalis* and *Pichia kudriavzevii* [9,12,21].

The ability of the selected strains to grow at 37°C and maintain substantial growth at 42°C further supports their probiotic potential. Since body temperature constitutes an important selective pressure for probiotic microorganisms, thermotolerance is generally considered a desirable characteristic. Comparable growth performances at elevated temperatures have been reported for probiotic yeasts such as Saccharomyces cerevisiae var. boulardii and other food-

associated yeasts [11,21]. The capacity of ADR1B1, ADR3E1 and RD31 to remain metabolically active under these conditions may favor their persistence within the gastrointestinal tract.

Bile salts constitute another major physiological barrier for probiotic microorganisms because of their detergent action on cellular membranes. The selected strains maintained high viability at bile concentrations exceeding those normally encountered in the small intestine, demonstrating remarkable bile tolerance. Similar observations have been reported for probiotic yeast species isolated from dairy and fermented food products [10,12]. According to Begley et al. [22], microbial resistance to bile salts is closely associated with membrane integrity and stress-response mechanisms, which contribute to survival under intestinal conditions. The strong bile tolerance exhibited by ADR3E1 and RD31 therefore reinforces their suitability as probiotic candidates.

Likewise, all strains showed high survival rates in the presence of pepsin and pancreatin, with ADR3E1 exhibiting the highest resistance. Survival in the presence of digestive enzymes is considered a critical probiotic attribute because it increases the likelihood that viable cells reach the intestine in sufficient numbers to exert beneficial effects. Similar resistance patterns have been described for *Saccharomyces boulardii* and other probiotic yeasts isolated from fermented foods [8, 23]. The high survival rates observed in the present study suggest that palm wine yeasts possess physiological mechanisms that enable them to withstand enzymatic stress during gastrointestinal transit.

Adhesion-related properties are also important indicators of probiotic functionality because they may contribute to intestinal persistence and competitive exclusion of pathogens. Most isolates exhibited high autoaggregation capacities after 24 h, suggesting a strong ability to form cellular aggregates. Autoaggregation is frequently associated with enhanced colonization potential and improved resistance to environmental stresses [17]. In addition, the ability of the isolates to coaggregate with Escherichia coli and Pseudomonas aeruginosa may facilitate pathogen exclusion through physical interactions and biofilm disruption mechanisms. Similar relationships between aggregation properties and probiotic potential have been reported in both yeasts and lactic acid bacteria [17, 24].

Cell-surface hydrophobicity analyses revealed strain-dependent differences in affinity toward the tested solvents. Hydrophobicity is often considered an indirect indicator of microbial adhesion to epithelial surfaces and has been widely used in the preliminary screening of probiotic microorganisms [19]. Although the most promising strains did not systematically exhibit the highest hydrophobicity values, they combined moderate to high hydrophobicity with excellent performance in the other probiotic tests. This finding suggests that probiotic potential should be evaluated through a combination of complementary physiological traits rather than a single parameter.

Hierarchical clustering analysis integrating all probiotic-related characteristics identified ADR1B1 (Saccharomyces cerevisiae), ADR3E1 (*Hanseniaspora jakobsenii*) and RD31 (*Hanseniaspora guilliermondii*) as the most promising candidates. While the probiotic properties of Saccharomyces species have been extensively documented [9,8], information regarding probiotic potential within the genus *Hanseniaspora* remains scarce. Most studies involving *Hanseniaspora* species have focused on their contribution to aroma formation and fermentation performance [25,26]. Therefore, the present study provides evidence that *H. jakobsenii* and *H. guilliermondii* may represent promising non-conventional probiotic yeasts. Their simultaneous ability to degrade phytate, tolerate gastrointestinal stresses and exhibit favorable adhesion-related properties highlights their potential as multifunctional cultures for the development of functional foods and nutritionally enhanced fermented products.

5. Conclusion

This study demonstrated that all thirteen yeast strains isolated from palm wines exhibited phytase activity, although significant differences were observed among the isolates. Among them, *Saccharomyces cerevisiae* ADR1B1, *Hanseniaspora jakobsenii* ADR3E1, and *Hanseniaspora guilliermondii* RD31 showed the most promising profiles, combining high phytase activity, strong tolerance to simulated gastrointestinal conditions, and favorable adhesion-related properties. Multivariate analysis further confirmed these three strains as the best probiotic candidates. These findings highlight the potential of palm wine yeasts as a valuable source of functional microorganisms and suggest that ADR1B1, ADR3E1, and RD31 could be exploited as multifunctional probiotic cultures for food and nutritional applications. Nevertheless, further in vivo studies are required to confirm their probiotic efficacy and health-promoting effects.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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