Genetic diversity of *Saccharomyces cerevisiae* isolated from different feed sources: applications and implications for biotechnological animal health

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Background
Saccharomyces cerevisiae strains were isolated from various sources and studied due to their significant potential as probiotics for treating different diseases. They act as adjuvants against gastrointestinal tract disorders, including bowel disease and various types of diarrhea in humans and animals. Consequently, identifying different strains of *S. cerevisiae* and confirming their safe consumption by humans and animals became crucial.

Objective
This study aims to isolate, identify, and characterize 36 *S. cerevisiae* isolates from different animal feed sources. The primary goal is to examine the diversity among these isolates.

Materials and methods
Yeast isolation from feed involved using Rose Bengal agar, which was incubated at a temperature of 30°C for 48 h. Isolate identification was performed using an internal transcribed spacer and *S. cerevisiae*-specific primers. Molecular markers, specifically sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeat (ISSR), were used to determine the genetic diversity. Polymorphic information content was estimated, and genotype-specific markers were detected. A phylogenetic relationship was established based on data obtained from the molecular markers.

Results and conclusion
S. cerevisiae-specific primers confirmed that all 36 yeast isolates belonged to the *S. cerevisiae* species. Molecular identification was achieved through amplification of internal transcribed spacer sequences, followed by sequencing and database matching. Phylogenetic analysis further confirmed their classification as *S. cerevisiae*. Genetic diversity among the 36 strains was assessed using SRAP and ISSR markers, which show 23.4 and 30 polymorphism percentages, respectively. The results revealed that 26 SRAP and 18 ISSR genotype-specific markers were identified for the 36 *S. cerevisiae* isolates. The cluster analyses indicated that the strains were separated into 20 distinct clusters based on their genetic similarities. These findings demonstrate the effectiveness of *S. cerevisiae*-specific primers, molecular identification techniques, and genetic marker analyses in characterizing and understanding the genetic diversity and relatedness within a population of *S. cerevisiae* isolates.

Keywords:
inter-simple sequence repeat, molecular markers, *Saccharomyces cerevisiae*, sequence-related amplified polymorphism

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Introduction

Probiotics, according to the Food and Agriculture Organization of the United Nations, are live microorganisms that, when administered in sufficient quantities, provide health benefits to the host [1]. Among the microbial genera commonly used as probiotics are *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Pediococcus*, *Propionibacterium*, *Bifidobacterium*, *Escherichia*, *Bacillus*, *Staphylococcus*, and some yeast genera, particularly *Saccharomyces* [2]. Potentially probiotic yeast strains can be used in the production of multiple fermented foods, which improves their nutritional and sensory properties [3]. Yeasts are unicellular fungi that reproduce through budding, fission, or spore formation, and there are currently recognized 500 yeast species [4]. One of the most widely used yeasts is *Saccharomyces cerevisiae*, extensively used in winemaking and brewing. This single-celled eukaryote is among the most

extensively studied and applied microorganisms in industry, with applications in the production of several industrial compounds, heterologous proteins, alcohol fermentation, baking, and bioethanol processes [5]. However, there is ongoing research aimed at enhancing their use, as many industrial processes fail to utilize optimal yeast strains for their specific applications [4]. Furthermore, increasing demands for productivity, shorter fermentation periods, broader substrate utilization, production of nonconventional aromatic flavors, and evolving consumer preferences have great interest in developing novel strains and optimizing existing ones for industrial use [6]. In this context, accurate and definitive identification of yeast species becomes crucial, particularly when dealing with genetically closely related species. Identifying yeasts through classical standard microbiological methods based on phenotypic characteristics has been challenging, as they often yield inconclusive results at the species level and are time-consuming [7].

Molecular techniques, specifically the ones that require PCR, have been broadly utilized to identify yeast species. Analysis of the internal transcribed spacer (ITS) region, which includes ITS-1, ITS-2, and the 5.8S ribosomal gene, as well as segments of the 18S and 26S (D1/D2 domain) genes, has been widely implemented in the studies of yeast biodiversity in fermented foods. This approach enables rapid differentiation of specific isolates at the species level [8]. The clinical mycology field has adopted the ITS region of the ribosomal region in the nuclear genome as the primary DNA barcode for the identification of fungal species [9]. The ITS region has noncoding regions flanked by the ribosomal small subunit or 18S subunit sequences at the 5' end and the large subunit (LSU) or 28S subunit sequences at the 3' end. It contains two parts, ITS-1 and ITS-2, where the 5.8S subunit separates them. [9]. One of the advantages of the ITS region is that it can be amplified easily through universal primers, which bind to conserved regions located at the ends of the 18S and 28S regions, which subsequently avails PCR amplification and Sanger sequencing from small clinical samples. Moreover, the hit rate of PCR amplification is commonly high, and the length of the ITS region is relatively short, which makes it optimum for Sanger sequencing [10]. Another advantage of the ITS region is the presence of numerous high-quality reference sequences deposited in multiple online databases [11]. Furthermore, the noncoding ITS region exhibits a faster mutation rate than the small subunit and LSU rRNA coding regions, leading to accumulated sequence variations that help in discriminating different taxa [12].

However, there are instances where amplification with universal (nonspecific) primers, such as ITS-1 and ITS-4, may be inadequate in the discrimination of yeast species and may require additional sequencing costs. Hence, alternative PCR-based identification techniques remain an interesting topic [13]. DNA polymorphism analysis-based methods have been used to differentiate strains of *Saccharomyces cerevisiae*. Molecular characterization using PCR techniques has proven more suitable for studies of yeast diversity, with molecular markers serving as valuable tools for taxonomic characterization and intraspecific genetic differentiation within microbial communities [14].

In the present investigation, 36 *S. cerevisiae* strains isolated from feed were identified using an ITS and *S. cerevisiae*-specific primers. Genetic diversity was assessed using molecular markers, including sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeat (ISSR). Polymorphic information content (PIC) was estimated, and genotype-specific markers were determined. A phylogenetic relationship was established based on data obtained from molecular markers.

Materials and methods

Sample collection

To isolate yeasts from animal feeds, a total of 36 samples were obtained from local markets in Cairo and Giza governorates. These samples consisted of 13 samples of animal feed additives, 10 samples of dry instant yeast, and 13 samples of fresh compressed yeast.

Determination and isolation of potential yeasts

The agar overlay technique was used for yeast determination and isolation. Rose Bengal agar (Lab M, Neogen Company, Heywood, Greater Manchester, UK) was used as the culture medium. The plates were incubated at 30°C for 48 h. Isolates were stored at −20°C using Rose Bengal broth reinforced with 20% glycerol. This storage medium helps maintain the viability of isolates. The cells were then collected by centrifugation at 12 000 g for 5 min. The harvested yeast cells then undergo genomic DNA extraction. The Gene JET Genomic DNA Purification Kit (Thermo Fisher Scientific, Vilnius, Republic of Lithuania) was used for this purpose. The DNA yield and purity were evaluated through a UV-Vis NanoDrop spectrophotometer (NanoDrop 2000, Thermo Fisher

Scientific, Darmstadt, Germany). Agarose gel electrophoresis equipment from Biorad (USA) was also used to visualize the DNA fragments. For molecular identification, specific primers for *S. cerevisiae* and ITS gene sequencing were used. PCR amplification was carried out using the isolated DNA samples and the specific primers.

Molecular detection of *Saccharomyces cerevisiae* strains

Yeast isolates were characterized by amplifying a specific set of primers designed for *S. cerevisiae* identification. The forward primer sequence used was 5'-AACGGTGAGAGATTTCTGTGC-3', and the reverse primer sequence was 5'-AGCTGGCAGTA TTCCCACAG-3' [15]. The PCR amplification was carried out according to Mostafa *et al.* [15].

Molecular detection through PCR amplification of the internal transcribed spacer region

For the detection and analysis of the ITS region of yeast isolates, PCR amplification was conducted. The amplification was done using the set of primers, ITS forward (5'-CTTGGTCATTTAGAGGAAGTA-3') and ITS reverse (5'-TCCTCCGCTTATTGAT ATGC-3'), based on the method described by White *et al.* [16].

Sequencing analysis of the internal transcribed spacer region

For sequencing analysis, five isolates were randomly selected from the 36 yeast isolates. The sequencing protocol was adapted from Ahmed *et al.* [2]. Subsequently, samples were sequenced using the AB 3500 XL automated DNA sequencers (Applied Biosystems, Foster city, California, USA).

Cluster analysis based on the internal transcribed spacer sequence

Analysis of the sequences was done using the applied software BLAST V2.0 (http://www.ncbi.nlm.nih.gov/BLAST/).

Genetic diversity among the isolated strains

For the assessment of genetic diversity among the isolated strains, SRAP and ISSR molecular markers were used.

Sequence-related amplified polymorphism analysis

A total of 36 yeast isolates were subjected to SRAP analysis using 20 primer combinations. These primer combinations include four forward primers (Me) targeting GC-rich exon regions and five reverse primers (Em) targeting AT-rich intron regions (Table 1). The SRAP-PCR reactions were carried out in a 20 µl volume. Amplification was performed using a Biorad thermocycler (USA) as per the protocol adapted from Li and Quiros [18].

Inter-simple sequence repeat analysis

For the PCRs using nine ISSR primers, with their sequences are shown in Table 2 [17]. ISSR-PCR was done according to Liu *et al.* [14].

Data scoring and statistical analysis

To assess the genetic polymorphism, percentages of polymorphic bands were calculated by summing the polymorphic bands from binary data. ISSR and SRAP bands were scored according to the presence "1" or absence "0" to estimate the similarity between all the analyzed samples. The informative value of these markers in distinguishing yeast isolates was quantified. Pairwise comparisons between the isolates were conducted using the Dice coefficient [19]. The similarity data were used to generate a tree diagram by applying the unweighted pair group method with arithmetic mean clustering method using Systat ver. 7 [20].

Results

To identify the yeast species isolated from 20 feed sources, PCR was conducted using specific primers for *S. cerevisiae*. These primers allow amplification of the 1170 bp DNA fragment located between the ITS-1 region and LSU gene of the *S. cerevisiae* strain, which

Table 1 Nucleotide sequence of the primers used in inter-simple sequence repeat analysis [17]

Primer name	Primer sequence (5'-3')	Annealing temperature
ISSR 4	GAGAGAGAGAGAGAGAYC	52.9
ISSR 5	GAGAGAGAGAGAGAGAYG	52.9
ISSR 6	AGAGAGAGAGAGAGAGYT	52.9
ISSR 9	ACACACACACACAGACYG	54
ISSR 12	AGAGAGAGAGAGAGAGYG	51.4
ISSR 14	AGAGAGAGAGAGAAGAGAGT	51.4
ISSR 28	DBDACACACACACACAC	54
ISSR 30	HVHTGTGTGTGTGTG	49.5
ISSR 31	AGAGAGAGAGAGAGAGVC	54

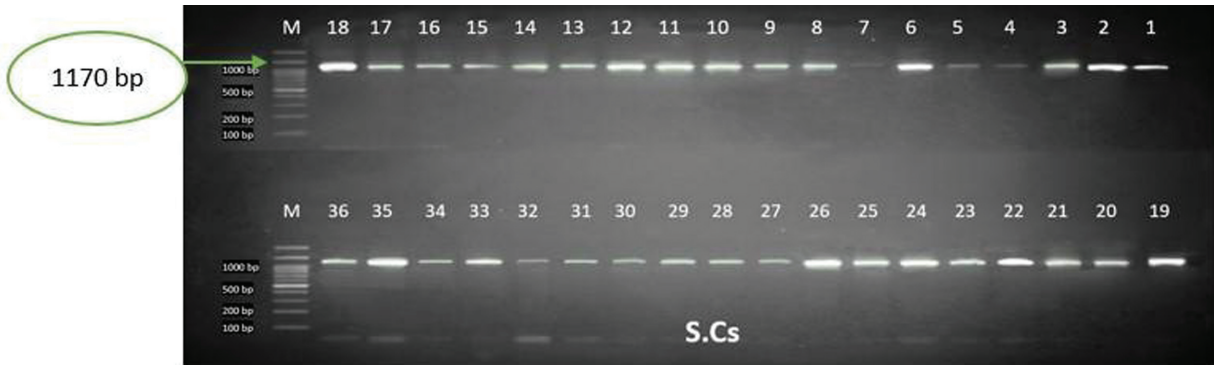
ISSR, inter-simple sequence repeat.

Table 2 Nucleotide sequences of the sequence-related amplified polymorphism primers used for analyzing the yeast species [18]

SRAP forward primer sequence (5'-3')	SRAP reverse primer sequence (5'-3')
Me1 TGAGTCCAAACCGGATA	Em1 GACTGCGTACGAATTAAT
Me2 TGAGTCCAAACCGGAGC	Em2 GACTGCGTACGAATTTGC
Me3 TGAGTCCAAACCGGAAT	Em3 GACTGCGTACGAATTGAC
Me4 TGAGTCCAAACCGGACC	Em4 GACTGCGTACGAATTTGA
	Em5 GACTGCGTACGAATTAAC

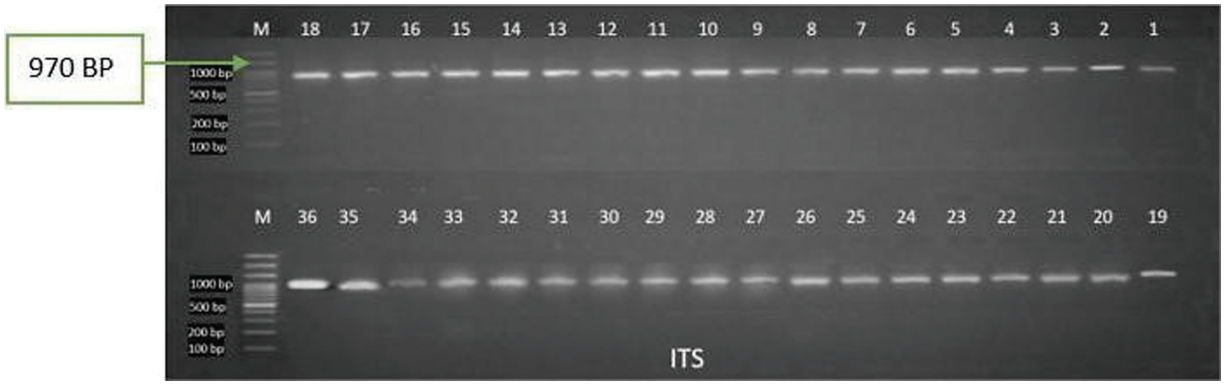
SRAP, sequence-related amplified polymorphism.

Figure 1



PCR of isolated yeasts using SC-specific primer. Lane M, ladder (100BP).

Figure 2



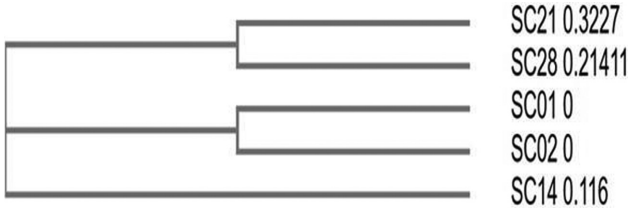
Agarose gel electrophoresis showing amplification of 970 bp. Fragment of the genus *Saccharomyces cerevisiae*. Lane (M): DNA ladder (100 bp), lane (1–36): yeast isolates (samples).

was amplified from 36 yeast isolates (Fig. 1). Furthermore, identification of the yeast isolates was done by detecting the ITS region using primers designed for the conserved ITS DNA region specific to the *Saccharomyces* genus. The data revealed that all 36 isolates had clear bands that correspond to the expected molecular size of the ITS region (970 bp) (Fig. 2). The ITS region of five randomly selected isolates was sequenced to obtain their DNA sequences. These sequences were then compared with the NCBI database using a homology search to confirm their identity. The phylogenetic analysis conducted according to the ITS region sequencing is shown in Fig. 3.

For the evaluation of the genetic diversity among the 36 *S. cerevisiae* isolates, 20 diverse SRAP primers representing four forward and five reverse primer combinations to detect polymorphisms in the open reading frames. Out of a total of 15 435 SRAP markers examined, 3617 markers were found to be polymorphic, resulting in a polymorphism rate of 23.43%.

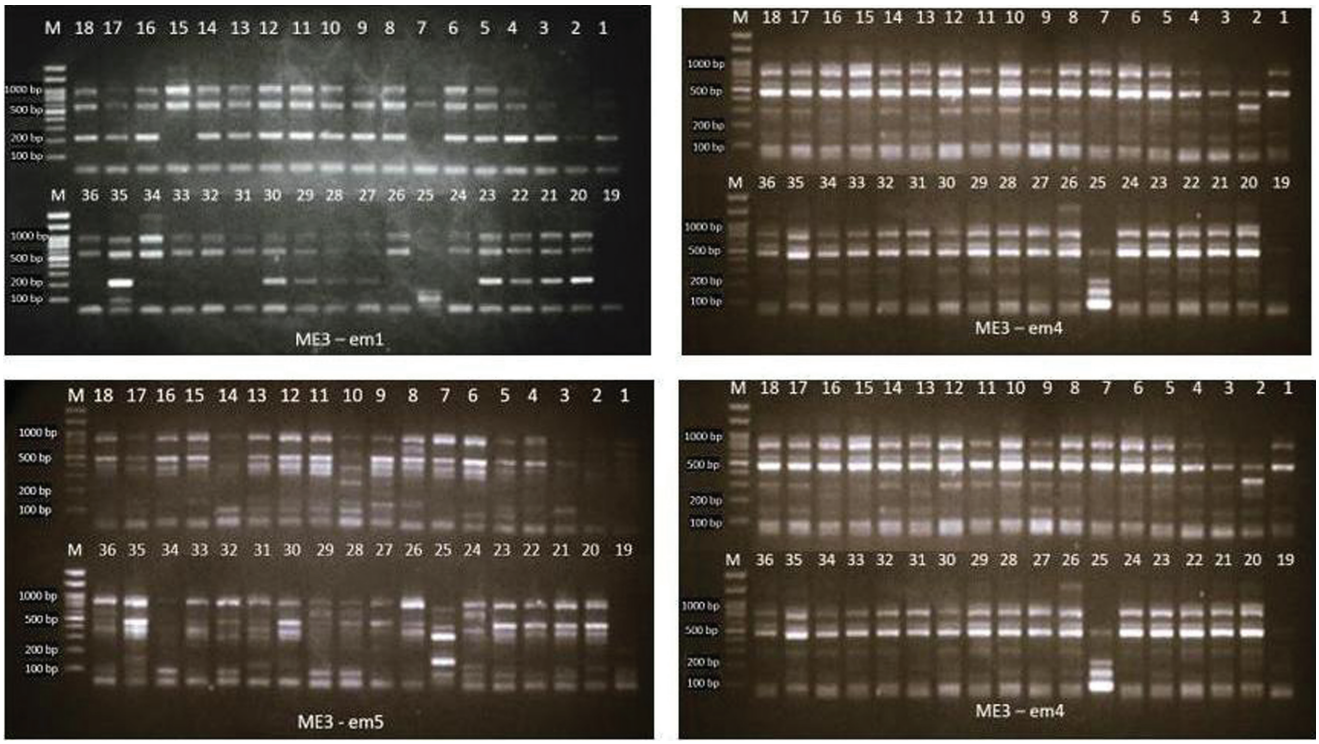
Among the SRAP primer combinations, the Me3 +Em5 combination yielded the highest number of scorable bands (245), while the Me1+Em1 combination produced the lowest number (112) (Fig. 4 and Table 3). The size of the amplified bands ranged from 120 bp (Me5+Em2) to 2000 bp

Figure 3



A neighbor-joining phylogenetic tree based on ITS gene sequencing of five *Saccharomyces cerevisiae* isolates. ITS, internal transcribed spacer.

Figure 4



SRAP binding patterns of 36 different *Saccharomyces cerevisiae* genotypes. (M) 100 bp DNA ladder. SRAP, sequence-related amplified polymorphism.

(Me5+Em4), with polymorphic bands ranging from 112 (Me1+Em1) to 245 (Me3+Em5), corresponding to polymorphism rates of 15 and 26.17%, respectively.

The differentiation power of the 20 SRAP markers was evaluated by calculating the PIC of their loci (Table 3). The PIC values for these markers ranged from 0.38 (Me4+Em2) to 0.221 (Me2+Em3). Based on SRAP markers, a total of 26 genotype-specific markers were identified, with SC25 exhibiting the highest number of specific markers (14 markers), SC35 (four markers), SC26 (three markers), SC02 and SC16 (two markers each), and SC05, SC11, SC23, SC24, and SC36 (one marker each) (Table 4).

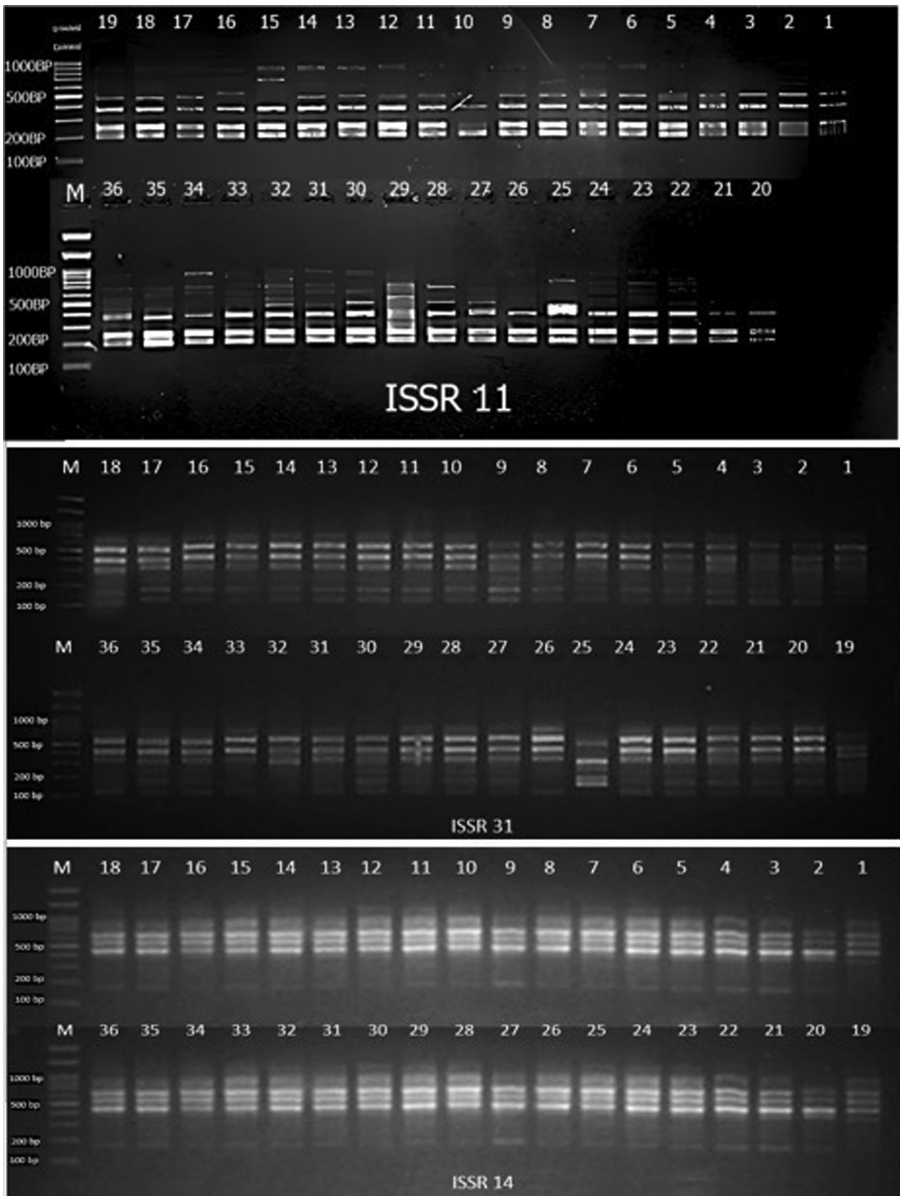
In addition to the SRAP markers, nine ISSR primers were used to detect the genetic variation among the 36 *S. cerevisiae* isolates. The data revealed that the ISSR primers generated 1914 polymorphic markers out of a total of 6408 scorable bands, resulting in a polymorphism rate of 30%. ISSR 9 exhibited the highest number of bands (900), while ISSR 13 had the lowest number (432). Among the ISSR primers, ISSR 12 displayed the highest number of polymorphic bands (308), the highest polymorphism rate (40.74%), and the highest PIC value (0.405), whereas ISSR 13 demonstrated the lowest number of polymorphic bands (82), the lowest polymorphism rate (18.98%), and the lowest PIC value (0.256) (Fig. 5 and Table 5). With regard to the data obtained from ISSR analysis, a total of 18 genotype-specific markers were detected for the 36 *S. cerevisiae* isolates, with SC25 possessing the highest number of specific markers (three markers), followed by SC03, SC07, and SC08 (two markers each), and SC01, SC10, SC16, SC19, SC28, SC29, SC34, SC35, and SC36 (one marker each) (Table 6). The SRAP and ISSR data were combined for similarity and cluster analyses (Fig. 6). The cluster analysis separated the obtained isolates into 20 distinct clusters based on their genetic relatedness.

The combined SRAP and ISSR data were used for similarity and cluster analyses (Fig. 6). The cluster analysis separated the isolated strains into 20 distinct clusters based on their genetic relatedness.

Discussion

S. cerevisiae, a single-celled eukaryote, is widely recognized as the most extensively studied eukaryotic organism. Its significance in fundamental research on eukaryotes comes from its unicellular nature, which allows for the exploration of nearly all biological functions found in eukaryotes. The conservation of

Figure 5



ISSR banding patterns of 36 different *Saccharomyces cerevisiae* genotypes, (M) 100 bp DNA ladder. ISSR, inter-simple sequence repeat.

these functions in *S. cerevisiae* makes it an ideal model organism [21]. Furthermore, natural strains of *S. cerevisiae* exhibit significant genotypic and phenotypic diversity [22].

Yeast species identification often relies on the analysis of ribosomal DNA genes, which includes the regions encoding 5S, 5.8S, 18S, and 26S (D1/D2 domain), as well as the noncoded ITS and IGS regions. The restriction profiles of these regions were used for the identification of yeast species, making ribosomal DNA genes the primary fungal barcode marker due to their high probability of successfully identifying a wide range of fungi [23,24]. In particular, the ITS-1 and ITS-2 regions of the rRNA gene operon were commonly used for the detection and identification of fungal pathogens using different methodologies such as PCR, ITS fragment length polymorphism, restriction fragment length polymorphism, DNA probe hybridization, and DNA sequencing [9,25].

In the current investigation yeast strains isolated from 20 feed sources were identified using molecular techniques. Specifically, PCR was conducted using specific primers for *S. cerevisiae* resulting in a 1170 bp DNA fragment located between the ITS-1 region and the LSU gene. The results showed that the 1170 bp fragment was amplified from 36 yeast isolates, indicating the presence of *S. cerevisiae* in the samples. These outcomes are consistent with previous studies that have identified *S. cerevisiae* as a common yeast species in various environments [17].

Table 3 Total number of scorable bands, polymorphism percentage, polymorphic information content, and a band size of sequence-related amplified polymorphism markers obtained by 20 primers

Primer name	Total bands no.	Polymorphic bands	Polymorphism %	PIC	Band size range
Me1+Em1	756	112	15	0.229	111.26–1034.24
Me1+Em2	432	116	27	0.289	159.10–844.15
Me1+Em3	1044	200	19.15	0.276	82.33–1429.26
Me1+Em4	900	219	24.3	0.344	139.82–1318.27
Me1+Em5	828	237	28.6	0.324	80.70–560.65
Me2+Em1	468	116	24.78	0.29	107.77–355.51
Me2+Em2	603	189	23.86	0.323	140.44–843.60
Me2+Em3	540	106	19.62	0.221	149.61–1063.43
Me2+Em4	792	202	25.5	0.318	127.11–1449.97
Me2+Em5	864	216	25	0.331	108.42–1446.51
Me3+Em1	936	154	16.45	0.244	127.71–2149.47
Me3+Em2	1080	206	23.56	0.286	111.36–1658.66
Me3+Em3	828	206	24.87	0.328	114.92–1057.40
Me3+Em4	612	184	30	0328	183.87–736.34
Me3+Em5	936	245	26.17	0.342	112.43–878.77
Me4+Em1	468	120	25.64	0.275	127.07–1009.21
Me4+Em2	828	242	29.23	0.38	127.70–2013.80
Me4+Em3	972	230	23.66	0.321	122.49–1358.93
Me4+Em4	1080	193	17.87	0.283	107.53–786.46
Me4+Em5	468	124	26.5	0.348	116.17–453.31
Total	15435	3617	23.43	0.304	–

PIC, polymorphic information content.

Table 4 *Saccharomyces cerevisiae* isolates and their specific sequence-related amplified polymorphism markers

Genotype	SRAP markers	Total marker
SC25	Me1+Em1 (221.75),Me2+Em2 (235.15),Me2+Em3 (503.78), Me2+Em4 (1353.44),Me2+Em5 (406.41),Me3+Em1 (169.03), Me3+Em2 (534.64), Me3+Em3 (319.06).Me3+Em4 (249.18), Me3+Em5 (238.77), Me4+Em1 (966.85), Me4+Em2 (690.27), Me4+Em3(366.90), Me4+Em4 (383.70)	14
SC35	Me2+Em2(824.90), Me3+Em1 (134.55), Me4+Em2 (890.55), ME4+Em3 (486.97)	4
SC36	Me2+Em1 (376.16)	1
SC24	Me1+Em3 (752.64)	1
SC11	Me4+Em3 (180.79)	1
SC05	Me4+Em3 (215.78)	1
SC23	Me4+Em4 (156.04)	1
SC16	Me4+Em4 (180.20),Me1+Em3 (276.15)	2
SC26	Me4+Em3 (1358.92),Me3+Em3 (1258.51),Me2+Em3 (1063.43)	3
SC02	Me4+Em3 (141.12), Me3+Em4 (314.15)	2
Total		26

SRAP, sequence-related amplified polymorphism.

For further identification of yeast isolates, the ITS was targeted using primers specific to the conserved ITS DNA region of the *Saccharomyces* genus. The data showed that all 36 isolates exhibited a distinct band of the expected molecular size (431 bp), indicating their classification as members of the genus *Saccharomyces*. These results are consistent with previous studies that have used the ITS region for molecular identification of yeast species [24,25].

To validate the identification of the isolates as *S. cerevisiae*, DNA sequencing was performed on five randomly selected isolates. The obtained DNA sequences were compared with the NCBI database using a homology search, and the results confirmed that all yeast isolates were *S. cerevisiae*. The sequencing data of the ITS region was used to construct a phylogenetic tree that supported the identification of the isolates as *S. cerevisiae*. These findings are consistent with the species-specific nature of the primers used in the PCR amplification, providing confidence in the accuracy of identification [25].

Conventional yeast identification methods are time-consuming and often fail to differentiate at the species level, resulting in inaccurate identifications that limit

Table 5 Total number of scorable bands, polymorphism percentage, and band size of inter-simple sequence repeat markers obtained by 20 primers

Primer name	Total no. of bands	Polymorphic bands	% of polymorphism	PIC	Band size range
ISSR 5	684	238	34.79	0.389	549.37–946.17
ISSR 6	756	210	27.77	0.305	150.15–850.89
ISSR 9	900	199	22.11	0.306	237.16–1351
ISSR 11	792	230	29.04	0.35	210.60–827.60
ISSR 12	756	308	40.74	0.405	198.49–973.79
ISSR 13	432	82	18.98	0.256	375.46–953.43
ISSR 14	828	277	33.45	0.352	129.50–1182.54
ISSR 31	720	253	35.14	0.359	200.64–1052
ISSR 4	540	127	32.51	0.305	259–1215
Total	6408	1924	30	0.336	

ISSR, inter-simple sequence repeat; PIC, polymorphic information content.

Table 6 Saccharomyces cerevisiae isolates and their specific inter-simple sequence repeat markers

Genotypes	ISSR markers	M.wt	Total marker
SC01	ISSR 6	446.52	1
SC03	ISSR 4	1215.79	2
	ISSR 9	453.58	
SC07	ISSR 13	580.50	2
	ISSR 5	740.226	
SC08	ISSR 13	725.55	2
	ISSR 31	291.35	
SC10	ISSR 13	648.98	1
SC16	ISSR 11	500.89	1
SC25	ISSR 4	1112.10	3
	ISSR 14	311.60	
	ISSR 13	673.33	
SC19	ISSR 14	543.51	1
SC28	ISSR 6	693.31	1
SC29	ISSR 9	756.66	1
SC34	ISSR 5	323.57	1
SC35	ISSR 9	792.25	1
SC36	ISSR 9	1020.14	1
Total			18

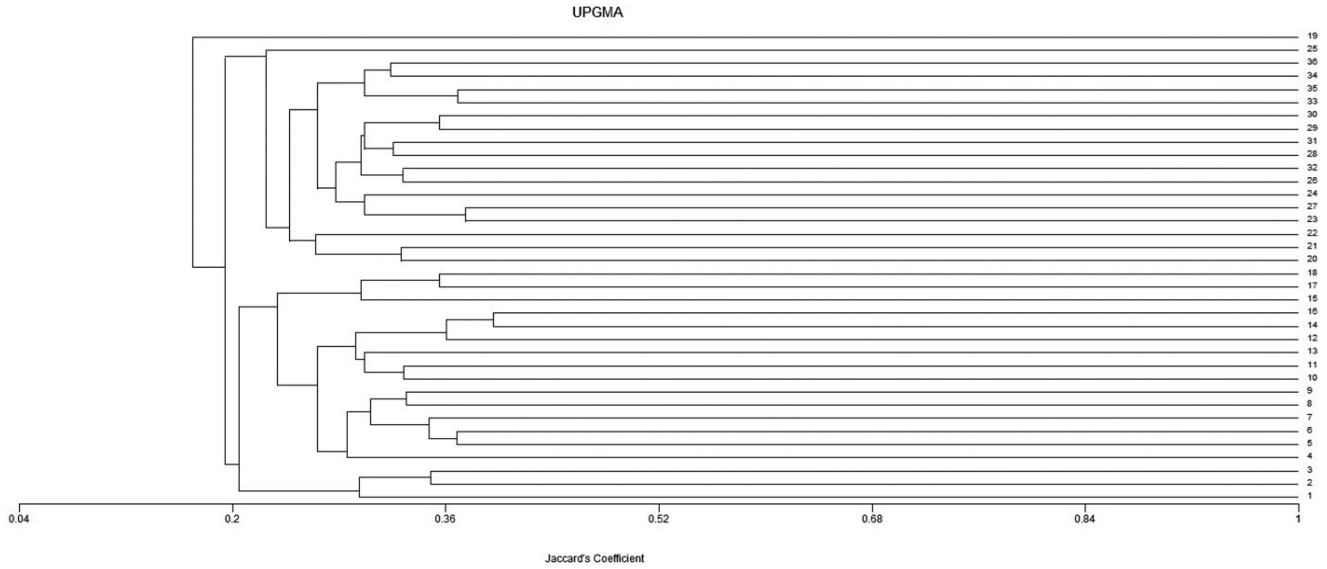
ISSR, inter-simple sequence repeat.

their applicability for characterization purposes. To overcome these limitations, molecular markers are widely used for yeast classification and genetic characterization [17]. Molecular markers have demonstrated their effectiveness in assessing genetic diversity and elucidating genetic relationships within and between species [26]. For the estimation of the genetic diversity among the 36 *S. cerevisiae* isolates, SRAP and ISSR markers were used. SRAP markers, which target polymorphisms in the open reading frames, showed a polymorphism rate of 23.43%. The highest number of scorable bands was obtained with the Me3+Em5 primer combination, while the lowest number was observed with Me1+Em1. This indicates variations in the genetic makeup among the isolates, with different primer combinations revealing different levels of polymorphism. The size of the amplified bands ranged from 120 to 2000 bp, demonstrating the genetic diversity within the *S. cerevisiae* population.

The differentiation power of the 20 SRAP markers was evaluated by calculating the PIC of their loci. The PIC values ranged from 0.38 to 0.221, which indicates the varying degrees of informativeness for distinguishing between the isolates. These markers can provide valuable information for genetic diversity studies and population analyses of *S. cerevisiae* [27–29].

In addition to SRAP markers, ISSR markers were used to detect genetic variation among the isolates. ISSR primers generated a higher polymorphism rate of 30%, with a total of 192 4 polymorphic markers out of 6408 scorable bands. The highest number of bands was observed with ISSR 9, while the lowest number was scored by ISSR 13. Amplicon sizes ranged from 375.46 to 953.43, indicating significant variability in the number of repeats among the strains, resulting in varying levels of polymorphism [25,30]. ISSR 12 exhibited the highest polymorphism rate and PIC value, indicating its effectiveness in detecting genetic diversity. Genotype-specific markers were identified using both SRAP and ISSR markers. A total of 26 genotype-specific markers were recorded using SRAP markers, with SC25 exhibiting the highest number of specific markers (14 markers). For ISSR markers, 18 genotype-specific markers were detected, and again SC25 had the highest number of specific markers (three markers) (Table 6). These genotype-specific markers can serve as valuable tools for strain identification and tracking in future studies [28,31]. The combined SRAP and ISSR data were subjected to similarity and cluster analyses to assess the genetic relatedness among the *S. cerevisiae* isolates. The cluster analysis separated the isolates into 20 distinct clusters based on their genetic profiles. This indicates

Figure 6



Dendrogram based on UPGMA clustering of pooled molecular data obtained from ISSR and SRAP markers among 36 yeast strains. ISSR, inter-simple sequence repeat; SRAP, sequence-related amplified polymorphism; UPGMA, unweighted pair group method with arithmetic mean.

the presence of considerable genetic diversity within the *S. cerevisiae* population, which can be attributed to various factors such as geographical location, feed sources, and environmental conditions. Overall, the molecular identification and characterization of *S. cerevisiae* isolates from feed sources provide valuable insights into their genetic diversity, which can have implications for their application in various biotechnological processes and their impact on feed quality and animal health.

Conclusion

This study successfully identified the yeast species isolated from 20 feed sources as *S. cerevisiae* using a combination of PCR, sequencing, and molecular marker techniques. The results indicated the presence of genetic diversity within the *S. cerevisiae* population, as evidenced by the polymorphic markers obtained through SRAP and ISSR analyses. The genotype-specific markers identified can serve as valuable tools for strain identification and tracking in future studies. Cluster analysis showed distinct genetic clusters, indicating the presence of genetic relatedness among the isolates. These findings contribute to our understanding of the genetic diversity and population structure of *S. cerevisiae* in feed sources.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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