scientific reports

Check for updates

OPEN

Typing of clinical and reference strains of *Saccharomyces cerevisiae* using pulsed-field gel electrophoresis and MALDI-TOF MS

Justyna Cieślik[1,5], Zofia Bakuła[2,5], Katarzyna Roeske[2], Aleksandra Kuryłek[2], Alicja Okrasińska[3], Jacek Bielecki[2], Marta Wróblewska[4] & Tomasz Jagielski[2]✉

In recent years matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a rapid and reliable tool for microbial identification and diagnosis. However, its use for molecular typing of *S. cerevisiae* has been investigated in a limited number of studies, mainly based on brewing strains. The purpose of the study was to compare the results of the gold standard pulsed-field gel electrophoresis (PFGE) typing with MALDI-TOF MS on a subset of *S. cerevisiae* clinical and reference strains. The study comprised 50 clinical isolates, collected from single patients hospitalized in the Central Clinical Hospital of the Medical University of in Warsaw between 2014 and 2016. Furthermore eight reference strains i.e. three probiotic, four baker and one winery strains, were included. Strain typing was performed using PFGE and MALDI-TOF MS. PFGE split the study sample into six clusters and two unique profiles. Whereas MALDI-TOF MS typing produced five clusters. Overall, the results of PFGE and MALDI-TOF MS were congruent for all (49/50; 97%) but one clinical isolates. In both analyses, three probiotic strains, unlike baker's and winery strains, clustered only with clinical isolates. Although PFGE had a higher resolution capacity than MALDI-TOF MS, both methods allowed for a clear discrimination between clinically relevant (clinical & probiotic) and irrelevant (baker's and winery) strains. This is the first time that MALDI-TOF MS has proven useful in the epidemiological studies of *S. cerevisiae*.

Keywords *Saccharomyces cerevisiae*, Yeasts, Probiotics, Fungemia, PFGE, MALDI-TOF MS

Saccharomyces cerevisiae are among the most well-known and best studied microorganisms in the world. These yeasts have been isolated from a variety of natural sources and have been widely used in food industry since ancient times. They have also been exploited as cell factories for the production of livestock feed, biofuels, and live biotherapeutic products (LBPs), commonly referred to as probiotics[1,2]. Furthermore, *S. cerevisiae* has been a robust experimental model organism for studying fundamental aspects of eukaryotic genetics.

Although *S. cerevisiae* are normally saprophytic colonizers of human alimentary, respiratory, and genitourinary tracts, they have been reported to cause opportunistic infections in patients with underlying immunosuppressive conditions. Over the last decade, about 200 papers reporting on *S. cerevisiae* infections have been published according to the PubMed database, averaging 20 articles per year. Captivatingly, more than a hundred cases of *S. cerevisiae* fungemia have been described in the literature since 2000[3,4]. Still, the role of this yeast as a pathogen is not yet understood. Most often it causes fungemia, including catheter-related fungemia, in adult patients with a documented probiotic intake, suffering from chronic diseases, malignancy, acquired immune deficiency syndrome (AIDS), or after hematologic transplantations. Very rarely, *S. cerevisiae* fungemia has been described in pediatric patients or in patients without prior history of yeast-containing probiotic supplementation[5,6]. The presumed pathomechanism of *S. cerevisiae* infection involves abundant multiplication of the yeast in patients with gut microbiota dysbiosis, for instance after a long-term antibiotic therapy[7–9].

[1]Department of Medical Biology, National Institute of Cardiology, Stefan Cardinal Wyszyński State Research Institute, Warsaw, Poland. [2]Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland. [3]Institute of Evolutionary Biology, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland. [4]Independent Public Clinical Ophthalmology Hospital in Warsaw, Warsaw, Poland. [5]These authors contributed equally: Justyna Cieślik and Zofia Bakuła. ✉email: t.jagielski@uw.edu.pl

Historically, identification of the yeasts has been performed with phenotype-based methods, including microscopy evaluation or auxanography assays. Since the early 2000s, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a robust diagnostic alternative, offering the advantages of rapidity, simplicity, and cost-effectiveness[10–13]. MALDI-TOF MS has proven to be a powerful tool for the identification of the yeasts, with high sensitivity and specificity, both exceeding 90%[14,15]. The method has also been used to differentiate strains within a given species or complex, as it was done for several bacterial[16–20] and fungal[21–23] pathogens, yet not for clinically relevant *Saccharomyces* spp. isolates.

The aim of the study was to compare the results of the gold standard pulsed-field gel electrophoresis (PFGE) typing with those of MALDI-TOF MS performed on a sample of *S. cerevisiae* clinical and reference strains. Implementation of MALDI-TOF MS in the routine *S. cerevisiae* epidemiological surveillance protocols will substantially improve the workflow in clinical microbiology laboratories by decreasing the time and technical expertise required to perform each typing.

Results

PFGE produced eight distinct patterns with 9 to 16 bands, supported by UPGMA clustering and a cophenetic correlation coefficient (r) equal to 0.99 (Fig. 1). Among these were six clusters (A–F) comprising 2–46 strains and two unique pulsotypes. The largest cluster C accommodated 43 clinical and 3 probiotic (MYA-447, Enterol and Floralis) strains. Clusters D, E, F contained two clinical isolates each. Clusters A and B included baker's yeast strains only (two strains in each cluster). Strains harbouring unigue pulsotypes were a clinical strain (G6721) and a winery (Lalvin 71B) strain.

The MALDI-TOF MS typing resolved five different clusters (nos. 1–5) (Fig. 1). The most abundant cluster (no. 1) included 44 clinical isolates, and 3 probiotic strains (MYA-447, Enterol and Floralis). Clusters 2–4 comprised of two clinical strains each. In cluster 5 there were five strains in total, i.e. four baker's strains (BY4741-1, BY4741-2, W303, S288c) and one winery strain (Lalvin 71B).

The results of PFGE and MALDI-TOF MS profiling were congruent, i.e. isolates shared MALDI-TOF profile and clustered together by PFGE, for all but six strains (1 clinical and 5 food-related; 52/58; 89.6%). One clinical isolate (G6721) which grouped with other clinical isolates upon MALDI-TOF MS fell outside any PFGE cluster. In addition, MALDI-TOF cluster no. 5 was subdivided by PFGE into 2 clusters of two brewer's strains each, and a singleton (winery strain). With both approaches, three probiotic strains, unlike baker's and winery strains, clustered only with clinical isolates (Fig. 1).

Clinical isolates from the largest clusters, that is cluster C (PFGE) and cluster 1 (MALDI-TOF MS) were recovered from different clinical materials (i.e. blood, vascular catheters, lower respiratory tract materials, wounds). Likewise, clinical isolates from minor PFGE and MALDI-TOF MS clusters could not be associated with any specific isolation source.

Discussion

For many years *S. cerevisiae* yeasts used in probiotics were designated as a separate species, namely *Saccharomyces boulardii*. This was subsequently challenged with molecular studies which showed unequivocally that *S. boulardii* should be denominated as *S. cerevisiae*[24].

S. cerevisiae is characterized by a significant genotypic and phenotypic intra-species variability, and the properties of probiotic strains are considered to be strain-specific. For instance, strains of *S. cerevisiae* vary in survival rates in the gastrointestinal tract, adhesion to epithelial cells, and production of bioactive compounds[25,26]. It is thus important to differentiate isolates at the strain level.

In this study, *S. cerevisiae* clinical isolates were identical with probiotic strains under implemented typing schemes. While *S. cerevisiae* is generally regarded as safe organism, there have been occasional reports of infections, particularly in immunocompromised individuals, resulting from the overgrowth of probiotic strains. Between 1991 and 2019, a total of 58 cases of *S. cerevisiae* fungaemia related to probiotic use were reported in the literature[27]. The bulk (76%) of those patients had a central venous catheter and were treated with broad-spectrum antimicrobial drugs (88%)[27]. As the use of probiotics continues to increase, it is crucial to monitor the safety of these strains, particularly in vulnerable populations.

Since the early 1990s PFGE has been the gold standard for molecular typing of *S. cerevisiae*. Although a number of new molecular methods have been developed[28,29], PFGE is still in use, mostly due to its high discriminatory capacity. MALDI-TOF MS has revolutionized clinical diagnostics and today is an integral part of the workflow for identification of bacterial and fungal pathogens at the genus- and species-level. Importantly, MALDI-TOF MS has several advantages over PFGE. It has an automated and user-friendly interface, with less hands-on preparation compared to PFGE, which requires intense and time-consuming sample preparation[30]. Furthermore, while PFGE requires manual interpretation, MALDI-TOF MS utilize well-established databases, allowing for an automated easy comparison to reference data[31]. MALDI-TOF MS is also more sensitive and allows the detection of smaller amounts of DNA or proteins[32]. Overall, MALDI-TOF MS offers an excellent alternative for high-throughput applications and epidemiological surveillance. However, only very few studies have investigated the interspecies variability of fungi (including yeasts) using the MALDI TOF MS[21,23,28,33]. *Saccharomyces* subspeciation with this technology has so far been performed only on brewer's strains[34,35]. It has never been approached for typing of clinically relevant *S. cerevisiae* strains.

In this study, MALDI-TOF MS showed a lower resolution capacity when compared with PFGE, which is in line with previous studies on pathogenic bacteria[36–39]. Furthermore, the clustering results with both methods were consistent (i.e. isolates which shared MALDI-TOF profile were not further subdivided by PFGE) for all but six strains (1 clinical and 5 food-related). These differences might be explained by the fact that MALDI TOF

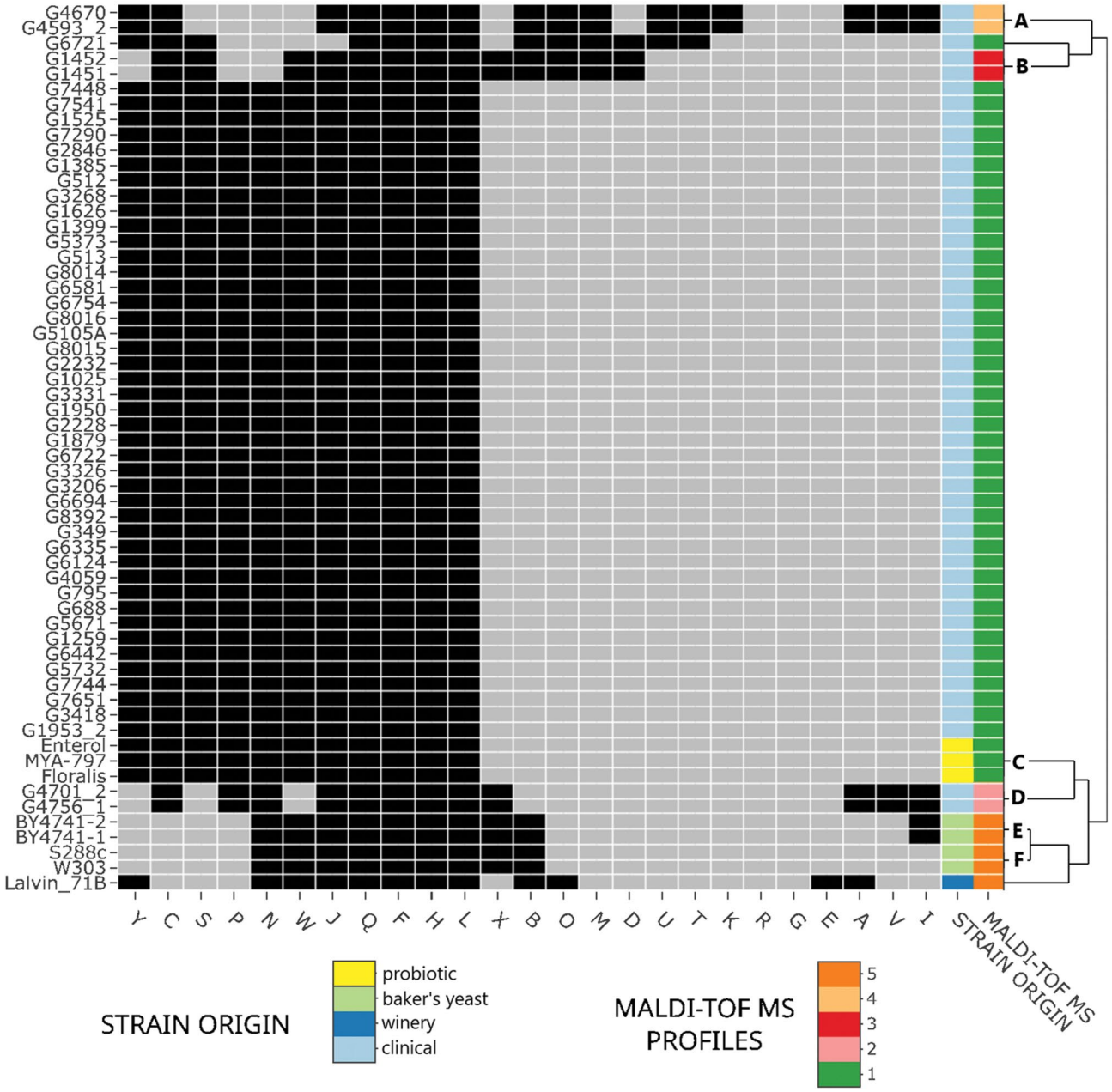

Fig. 1. Results of PFGE and MALDI-TOF typing of 58 *S. cerevisiae* strains under the study. PFGE bands (named A–X) are marked as black (present) or grey (absent) rectangles. Clustering by PFGE is marked on the dendrogram as A–F clusters. MALDI-TOF MS clusters (1–5) are marked with different colours on the right side of the graph.

MS detects only a fragment of bacterial proteome, in a limited mass range, which may not reflect genome-wide differences elucidated by PFGE[40].

Two major limitations of the study have to be mentioned. Firstly, study sample included only 8 strains from non-clinical resources, i.e. the reference strains. Thus, the results could not reflect the overall variability of *S. cerevisiae* strains other than from clinical setting. Secondly, of particular importance would be data concerning probiotics usage in patients, unfortunately unavailable for this study.

In conclusion, to the best of authors' knowledge, there is no study which compares *S. cerevisiae* clinical or nonclinical strains by PFGE and MALDI-TOF MS used in parallel. All but one (49/50; 98%) clinical strains were grouped identically (i.e. shared MALDI-TOF profile and clustered together by PFGE), with both methods, which supports the use of MALDI-TOF MS in the epidemiological studies of *S. cerevisiae*. Furthermore, since most of the clinical isolates were genetically identical with probiotic strains, there is a clear epidemiological link between probiotic strains and *S. cerevisiae* infection.

Reference strains				
No.	**Strain ID (alternate designation)**	**Collection***	**Isolation source**	**Type**
1.	BY4741_1 (ATCC 4040002)	ATCC	Derived from S288C	*Baker's yeast*
2.	BY4741_2**	DMM, UW	Derived from ATCC 4040002	
3.	W303 (ATCC 208352)	ATCC	Natural source	
4.	S288C (ATCC 204508)	ATCC	Natural source	
5.	Lalvin71B***	DMM, UW	winery strain (Lipera, Czech Republic)	*Winery*
6.	MYA-796	ATCC	Ultra Levure (Biocodex, France)	*Probiotic*
7.	Enterol***	DMM, UW	Enterol (Biocodex, France)	
8.	Floralis***	DMM, UW	Floralis entero (Bellis Pharma, Poland)	
Clinical strains				
No.	**Strain ID**	**Collection**	**Isolation source**	**No. of isolates**
1–11	G512, G1626, G1399, G5373, G513, G8014, G6581, G795, G688, G5671, G1259	DM, CCH	Peripheral blood	11
12–23	G7541, G7448, G2232, G3331, G2228, G3326, G3206, G7744, G7651, G4593/2, G3418, G1953/2		Lower respiratory tract material	12
24–29	G1525, G7290, G3268, G6754, G5105A, G8016		Bood taken through a vascular catheter	6
30–34	G1385, G4701/2, G6694, G6124, G4059,		Material from the peritoneal cavity	5
35–38	G1452, G1025, G1879, G6721		Sputum	
39–41	G2846, G4756/1, G5732		Postoperative wound	3
42, 43	G1451, G6722		Oral swab	2
44	G349		Vascular catheter	1
45	G8392		Urine	1
46	G1950		Feces	1
47	G6442		Ulceration - swab	1
48	G4670		Abdominal drain	1
49, 50	G8015, G6335		Blood	2

Table 1. Strains used in this study. *ATTC, American Type Culture Collection; DMM, UW, Department of Medical Microbiology, University of Warsaw; DM, CCH, Department of Microbiology, Central Clinical Hospital of the Medical University in Warsaw; **Clone of ATCC 4040002, deposited in Department of Medical Microbiology, University of Warsaw collection; ***Isolated from commercial product, for the purpose of the study.

Materials and methods

Strains

A total of 58 *S. cerevisiae* strains were used in the study (Table 1). Included in this number were 50 single-patient clinical isolates and eight reference strains (Table 1). The isolates were identified to the species level (scores of ≥ 2.0) with MALDI TOF MS Biotyper and the reference database V.3.1.2.0 (Bruker, Germany).

All samples were collected in the Intensive Care Unit, Hematology Clinic, and Surgical Departments of the Central Clinical Hospital of the Medical University in Warsaw, between 2014 and 2016. The clinical strains of *S. cerevisiae* were isolated in culture and identified in the Department of Microbiology, Central Clinical Hospital of the Medical University of Warsaw, Warsaw, Poland.

The requirement for informed consent from the study subjects was waived by the Medical University in Warsaw Bioethics Committee since the study sample was collected during routine clinical practices, and all personal data were anonymized prior the study. All experimental protocols and methods were approved by the Medical University of Warsaw's Bioethics Committee. All methods were carried out in accordance with guidelines and regulations of the Medical University of Warsaw.

Pulsed-field gel electrophoresis

Preparation of agarose plugs

Agarose plugs were prepared following the modified protocol of Hage and Houseley[41]. Briefly, yeast cells were grown in Yeast extract Peptone Dextrose (Biomaxima, Poland) medium at 30°C and 200 rpm for 16 h. Approx. 1×10^8 cells from the overnight culture were centrifuged at 4°C and 2000 rpm for 5 min., washed and suspended in 100 µL of wash buffer (10 mM Tris-Cl pH 7.6, 50 mM EDTA). Then, cell suspension was warmed to 50°C and

mixed with 1 µL of 8500 U/µL lyticase prior to combining with an equal volume of 1.7% (w/v) SeaKem Gold agarose (Lonza, Switzerland) in 0.5x TBE buffer. After vortexing, the mixture was transferred to a CHEF Mapper XA plug mold (Bio-Rad, USA). Solidified blocks were incubated in 500 µL of wash buffer containing 212.5 kU of lyticase at 37°C for 1 h. After incubation, the buffer was removed and 500 µL of PK buffer containing 1 mg/mL proteinase K was added and the samples were incubated overnight at 50°C. The agarose blocks were washed four times for 30 min. in 1 mL of wash buffer and stored at 4°C in 0.5 M EDTA pH 8.0 until use.

Gel loading and electrophoresis

Yeast chromosomes were separated by PFGE using a CHEF Mapper XA unit (Bio-Rad, USA) at 12°C in 0.5x TBE buffer. The quarter of each plug was inserted into 1.0% (w/v) SeaKem Gold agarose gel (Lonza, Switzerland) in 0.5x TBE buffer and electrophoresed under the following conditions: 6 V/cm, pulse 60–120 s and angle 120° for 22 h. The *S. cerevisiae* chromosome marker (Bio-Rad, USA) was used for the determination of DNA size. Six molecular-weight size marker lanes in each 30-well gel enabled normalization within and across gels. The gel was stained with ethidium bromide (5 µg/mL, Sigma Aldrich, USA) for 1 h and rinsed twice with MilliQ water for 30 min. The gels were then visualized in UV transillumination and documented with a BioDoc-It 210 Imaging System (UVP, Germany). Original, unprocessed PFGE gel images are presented in Supplementary Figs. 1A and B. For easier comparison, specific lanes corresponding to particular PFGE profiles were cropped and assembled together in Supplementary Fig. 1B.

Data analysis

After trimming (range 22–2200 bp), bands from PFGE images were scored for their presence or absence and converted into a binary matrix. Dendrograms were constructed with the PAST programme (Øyvind Hammer, Norway) by unweighted pair-group method with arithmetic averages (UPGMA) using Dice coefficient. The quality of the cluster analysis was verified by calculating the cophenetic correlation value for the dendrogram with the PAST programme (Øyvind Hammer, Norway).

MALDI-TOF MS

Sample preparation and typing

The yeast strains were thawed from the microbank (-70°C) on Sabouraud Dextrose Agar (BioMaxima, Poland) plates, incubated at 30°C for 48 h. After the growth was obtained, a second passage from a single colony was made under the same conditions. A single yeast colony (approx. 10^5 cells) was then suspended in 300 µL of the HPLC-grade water. In the next step, a 900 µL of absolute ethanol was added to each sample. After centrifugation (13 000 rpm, 2 min) at room temperature, excessive ethanol was discarded and the samples were air-dried completely. The cell pellet was then suspended in 50 µL of 70% formic acid and 50 µL acetonitril. The samples were thoroughly mixed, centrifuged (2 min., 13 000 rpm) and 1.0 µL of the clear supernatant was spotted onto the MALDI 96 target stainless steel plate. The typing was carried out on the MALDI Biotyper system using a Microflex LT spectrometer, with Compass v1.4 FlexSeries software (Bruker, Germany). Matrix solution was prepared in accordance with manufacturers' protocol (Bruker, Germany). Briefly, α-Cyano-4-hydroxycinnamic acid (HCCA) was added to standard solvent, containing acetonitrile (50%), water (47.5%), and trifluoroacetic acid (2.5%). Each sample was done in 8 repetitions. Simultaneously, 1 µL of BTS (bacterial test standard) was used for calibration of the device. Each sample was overlaid with 1.0 µL of saturated matrix solution and allowed to dry completely prior to measurement.

Data analysis

The platform was calibrated according to the manufacturer's instructions using the Bruker Daltonics Bacterial Test Standard (Bruker, Germany). The mass spectra were acquired using FlexControl software v3.4 and MBT_AutoX method. All spectra collected were post processed using the Flex Analysis v3.4 software (Bruker, Germany), by the multiple spectrum display for spectra comparison and analysis (Flex Analysis 3.4 User Manual®).

For each isolate, a list of peaks and a numerical value of the mass to charge (m/z) was exported to a spreadsheet. The peaks within the range 3000 to 12,000 m/z were further analyzed for their presence or absence (Supplementary Fig. 2). The repetitions were manually checked to ensure that the shift in the mass of the highest peaks did not exceed 500 ppm.

A MALD-TOF MS cluster was defined as two or more isolates sharing identical peak profiles.

Data availability

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Received: 13 February 2025; Accepted: 7 May 2025
Published online: 16 May 2025

References

1. Parapouli, M., Vasileiadi, A., Afendra, A-S. & Hatziloukas, E. *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiol.* **6**, 1–32.
2. Otto, M., Liu, D. & Siewers, V. *Saccharomyces cerevisiae* as a heterologous host for natural products, 333–367. In *Engineering Natural Product Biosynthesis* (ed Skellam, E.) . (Springer, 2022).
3. Fadhel, M., Patel, S., Liu, E., Levitt, M. & Asif, A. *Saccharomyces cerevisiae* fungemia in a critically ill patient with acute cholangitis and long term probiotic use. *Med. Mycol. Case Rep.* **23**, 23–25 (2019).
4. Munoz, P. et al. *Saccharomyces cerevisiae* fungemia: an emerging infectious disease. *Clin. Infect. Dis.* **40**, 1625–1634 (2005).

5. Pillai, U., Devasahayam, J., Kurup, A. & Lacasse, A. Invasive *Saccharomyces cerevisiae* infection: A friend turning foe? *Saudi J. Kidney Dis. Transpl.* **25**, 1266 (2014).
6. Atıcı, S. et al. Catheter-related *Saccharomyces cerevisiae* fungemia following Saccharomyces boulardii probiotic treatment: in a child in intensive care unit and review of the literature. *Med. Mycol. Case Rep.* **15**, 33–35 (2017).
7. Bojsen, R. K., Andersen, K. S. & Regenberg, B. *Saccharomyces cerevisiae* — a model to uncover molecular mechanisms for yeast biofilm biology. *FEMS Immunol. Med. Microbiol.* **65**, 169–182 (2012).
8. Graf, C. & Gavazzi, G. *Saccharomyces cerevisiae* fungemia in an immunocompromised patient not treated with Saccharomyces boulardii Preparation. *J. Infect.* **54**, 310–311 (2007).
9. Jang, K-S. & Kim, Y. H. Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. *J. Microbiol.* **56**, 209–216 (2018).
10. Castano, S. V. & Juanes, F. S. Is MALDI-TOF mass spectrometry a valuable new tool for microorganisms epidemiology? *J. Proteom. Bioinform* **9**. (2016).
11. Dinkelacker, A. G. et al. Typing and species identification of clinical Klebsiella isolates by fourier transform infrared spectroscopy and Matrix-Assisted laser desorption Ionization–Time of flight mass spectrometry. *J. Clin. Microbiol.* **56**, e00843–e00818 (2018).
12. Jung, J. S. et al. Rapid detection of antibiotic resistance based on mass spectrometry and stable isotopes. *Eur. J. Clin. Microbiol. Infect. Dis.* **33**, 949–955 (2014).
13. Hrabák, J., Chudáčková, E. & Walková, R. Matrix-Assisted laser desorption Ionization–Time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. *Clin. Microbiol. Rev.* **26**, 103–114 (2013).
14. Rolland, N. et al. Identification of food spoilage Fungi using MALDI-TOF MS: spectral database development and application to species complex. *J. Fungi*. **10**, 456 (2024).
15. Idelevich, E. A., Nedow, B., Vollmer, M. & Becker, K. Evaluation of a novel benchtop tool for acceleration of sample Preparation for MALDI-TOF mass spectrometry. *J. Clin. Microbiol. E* 00212–00223. (2023).
16. Elabbasy, M. T. et al. MALDI-TOF MS based typing for rapid screening of multiple antibiotic resistance *E. coli* and virulent Non-O157 Shiga Toxin-Producing *E. coli* isolated from the slaughterhouse settings and beef carcasses. *Foods* **10**, 820 (2021).
17. Cordovana, M., Pranada, A. B., Ambretti, S. & Kostrzewa, M. MALDI-TOF bacterial subtyping to detect antibiotic resistance. *Clin. Mass. Spectrom.* **14**, 3–8 (2019).
18. Persad, A. K. et al. Identification and subtyping of Salmonella isolates using Matrix-Assisted laser Desorption–Ionization Time-of-Flight mass spectrometry (MALDI-TOF). *Microorganisms* **10**, 688 (2022).
19. Clark, C. G. et al. Evaluation of MALDI-TOF mass spectroscopy methods for determination of *Escherichia coli* pathotypes. *J. Microbiol. Methods*. **94**, 180–191 (2013).
20. Christner, M. et al. Rapid MALDI-TOF mass spectrometry strain typing during a large outbreak of Shiga-Toxigenic *Escherichia coli*. *PLoS ONE*. **9**, e101924 (2014).
21. Patel, R. A moldy application of MALDI: MALDI-ToF mass spectrometry for fungal identification. *J. Fungi*. **5**, 4 (2019).
22. Angeletti, S. & Ciccozzi, M. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry in clinical microbiology: an updating review. *Infect. Genet. Evol.* **76**, 104063 (2019).
23. Walsh, T. J. & McCarthy, M. W. The expanding use of matrix-assisted laser desorption/ionization-time of flight mass spectroscopy in the diagnosis of patients with mycotic diseases. *Expert Rev. Mol. Diagn.* **19**, 241–248 (2019).
24. Posteraro, B. et al. Molecular tools for differentiating probiotic and clinical strains of *Saccharomyces cerevisiae*. *Int. J. Food Microbiol.* **103**, 295–304 (2005).
25. de Carvalho, B. T. et al. Enhancing probiotic impact: engineering *Saccharomyces boulardii* for optimal acetic acid production and gastric passage tolerance. *Appl. Environ. Microbiol.* **90**, e00325–e00324 (2024).
26. Offei, B., Vandecruys, P., De Graeve, S., Foulquié-Moreno, M. R. & Thevelein, J. M. Unique genetic basis of the distinct antibiotic potency of high acetic acid production in the probiotic yeast *Saccharomyces cerevisiae* Var. Boulardii. *Genome Res.* **29**, 1478–1494 (2019).
27. Roeske, K., Zasuń, A., Cieślik, J., Wróblewska, M. & Jagielski, T. *Saccharomyces cerevisiae* Var. *Boulardii* probiotic yeasts as etiological agents of oportunistic infections in humans. *Postępy Mikrobiol - Adv. Microbiol.* **59**, 291–303 (2020).
28. Hleba, L. et al. Use of MALDI-TOF MS to discriminate between aflatoxin B1-Producing and Non-Producing strains of *Aspergillus flavus*. *Molecules* **27**, 7861 (2022).
29. Spinali, S. et al. Microbial typing by Matrix-Assisted laser desorption Ionization–Time of flight mass spectrometry: do we need guidance for data interpretation?? *J. Clin. Microbiol.* **53**, 760–765 (2015).
30. Bizzini, A. & Greub, G. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. *Clin. Microbiol. Infect.* **16**, 1614–1619 (2010).
31. Singhal, N., Kumar, M., Kanaujia, P. K. & Virdi, J. S. MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. *Front. Microbiol.* **6**. (2015).
32. Calderaro, A. & Chezzi, C. MALDI-TOF MS: A reliable tool in the real life of the clinical microbiology laboratory. *Microorganisms* **12**, 322 (2024).
33. Hof, H., Eigner, U., Maier, T. & Staib, P. Differentiation of *Candida dubliniensis* from *Candida albicans* by means of MALDI-TOF mass spectrometry. *Clin. Lab.* **58**, 927–931 (2012).
34. Gorre, E., Muste, C. & Owens, K. G. Introducing a Cell-Free approach for the identification of brewing yeast (*Saccharomyces cerevisiae*) strains using MALDI-TOF MS. *J. Am. Soc. Mass. Spectrom.* **29**, 2248–2259 (2018).
35. Lauterbach, A., Usbeck, J. C., Behr, J. & Vogel, R. F. MALDI-TOF MS typing enables the classification of brewing yeasts of the genus Saccharomyces to major beer styles. *PLOS ONE*. **12**, e0181694 (2017).
36. Rim, J. H. et al. Insufficient discriminatory power of Matrix-Assisted laser desorption ionization Time-of-Flight mass spectrometry dendrograms to determine the clonality of Multi-Drug-Resistant Acinetobacter baumannii isolates from an intensive care unit. *BioMed. Res. Int.* **2015**, 1–8 (2015).
37. Ibik, Y. E. et al. Evaluating molecular epidemiology of carbapenem non-susceptible Klebsiella pneumoniae isolates with MLST, MALDI-TOF MS, PFGE. *Ann. Clin. Microbiol. Antimicrob.* **22**, 93 (2023).
38. García-Salguero, C. et al. Usefulness of MALDI-TOF and REP-PCR against PFGE for the epidemiological study of Acinetobacter baumannii. *Rev. Esp. Quimioter*. **34**, 207–213 (2021).
39. Jiang, F. et al. Overestimated discriminatory power of MALDI-TOF mass spectrometry for typing of carbapenem-resistant *Klebsiella pneumoniae* clones. *Epidemiol. Infect.* **147**, e324 (2019).
40. Lasch, P. et al. Insufficient discriminatory power of MALDI-TOF mass spectrometry for typing of Enterococcus faecium and Staphylococcus aureus isolates. *J. Microbiol. Methods*. **100**, 58–69 (2014).
41. Hage, A. E. & Houseley, J. Resolution of budding yeast chromosomes using pulsed-field gel electrophoresis, 195–207. In *DNA Electrophoresis* (ed Makovets, S.). (Humana, 2013).

Author contributions

J.C.—investigation, data analysis, writing—original draft; Z.B.—data analysis, writing—original draft; K.R.—investigation; A.Z.—investigation; J.B.—writing—review and editing; A.O.–visualization; M.W.—resources, supervision; T.J.—resources, supervision, writing—review and editing. All authors reviewed the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-01645-4.

Correspondence and requests for materials should be addressed to T.J.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.