International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

https://gssrr.org/index.php/JournalOfBasicAndApplied/index

Isolation and Characterization of Yeast Strains from Burdur Sugar Factory

Nermin Sarıgül*

Burdur Mehmet Akif Ersoy University, Science and Literature Faculty, Molecular Biology and Genetics Department, Burdur 15010, Turkey

Email: sarigul@mehmetakif.edu.tr

Abstract

This study aimed to isolate and characterize yeast strains from diverse sampling points within the Burdur Sugar Factory, including pulp, bagasse, transport water, molasses, and filter press soil. Yeast isolation was performed using Rose Bengal Chloramphenicol Agar, and colonies were analyzed for growth at elevated temperatures, heavy metal resistance, killer toxin production, and textile dye removal capabilities. A total of 16 yeast strains were isolated, with the highest colony counts observed in pulp and bagasse samples, while no growth occurred in molasses or filter press soil. Physiological characterization revealed that 11 strains exhibited growth at 37 °C, and all strains demonstrated lead resistance at 5mM concentration, though resistance to other heavy metals was negligible. Killer toxin assays identified that BS12 effectively inhibited five other strains, highlighting its potential for fermentation applications. In the textile dye removal test, all strains grew in dye-enriched media, but only one strain BS8, isolated from bagasse, showed significant color removal. These findings suggest that the unique microenvironment of the sugar factory supports yeast strains with diverse and potentially valuable metabolic properties. Further molecular characterization is recommended to confirm species-level identification and explore the industrial applications of these yeasts in biotechnology, biofuel production, and environmental remediation.

Keywords: Sugar factory; yeast; heavy metal resistance; azo dye removal.

Received: *10/7/2024*
Accepted: *12/7/2024*
Published: *1/17/2025*

---

* Corresponding author.

1. Introduction

Yeasts are unicellular fungi of significant biotechnological and industrial importance due to their diverse metabolic capabilities and widespread applications. In sugar factories, yeasts play a pivotal role in fermentation processes, particularly in the production of ethanol and other bio-products. These environments, characterized by high concentrations of sugar and specific temperature and pH conditions, serve as an ideal niche for the proliferation of both wild and industrially relevant yeast strains. Isolating yeasts from such settings provides an opportunity to discover strains with unique physiological and metabolic traits, which may have potential applications in biotechnology, biofuel production, and food industries [1].The characterization of yeast strains is equally critical, as it enables the identification of species and the evaluation of their functional properties. Modern techniques, including morphological, biochemical, and molecular approaches, are essential for understanding the genetic and metabolic diversity of isolated yeasts. By focusing on the isolation and characterization of yeasts from sugar factory environments, researchers can explore the adaptive mechanisms employed by these microorganisms and harness their capabilities for industrial applications. This study, therefore, contributes to the growing field of microbial biotechnology by identifying and analyzing yeast strains with potential economic and ecological benefits [2,3]. The aim of this study was to verify whether it is possible to isolate natural yeast species from Burdur sugar factory and to understand their microbiological characteristics.

2. Material and methods

Within the scope of the study, yeast was isolated from "Burdur Sugar Factory". The sampling points are as follows, washing and transport water, pulp, bagasse-1, bagasse-2, bagasse-3, molasses and filter press soil.

2.1. Isolation

Serial dilutions were made from the collected molasses, filter press soil, bagasse, pulp and transport water samples, and they were inoculated on Rose Bengal Chloramphenicol Agar medium and incubated at 25 °C for 1 week. On the 3rd, 5th and 7th days, yeast colonies were counted and single colonies with different appearances were planted on horizontal agar. After purity check, isolates were stocked in 20% glycerol [2].

2.2. Growth at 37 °C

Isolates were inoculated on Yeast Extract Saccharose (YES) medium and incubated at 37 °C for 1 week.

2.3. Heavy metal resistance

Heavy metal enriched yeast peptone dextrose agar used for resistance tests (YPDA, containing (g/L): yeast extract 15, peptone 20, dextrose 20, agar 20, enriched with 5 mM heavy metal. Five heavy metal compounds were used for resistance test: copper (II) sulfate ($CuSO_4$), silver nitrate ($AgNO_3$), cobalt nitrate ($Co(NO_3)_2x6H_2O$), zinc sulfate $ZnSO_4$ and lead nitrate ($Pb(NO_3)_2$) [4].

2.4. Killer toxin test

Strains were cultivated in pH: 4.8 YPDA with %0,03 methylene blue at 25 ºC. After 48 incubation inhibition zones were measured and killer toxin production determinated [1].

2.5. Textile dye removal

Spots were made on Kirk's Basal medium enriched with Reactive Red 141 ($C_{52}H_{26}Cl_2N_{14}Na_8O_{26}S_8$; MW:1774,19), a double azo class dye, at varying concentrations and incubated at 25 ºC for 3-5-7 days. Thus, the effect of textile dye on color removal and growth was determined [5].

3. Results

Yeast colony count results are indicated in Table 1. While pulp and pulp 3 samples had the highest number of yeasts, no colony development was observed in molasses and filter press soil.

Table 1: Yeast count results (Log cfu/g)

Sampling	Pulp	Bagasse-1	Bagasse-2	Bagasse-3	Water	Molasses	Filter press soil
1	6.2±0.10	1.8±0.15	5.9±0.08	6.8±0.06	4.8±0.07	<1	<1
2	7.2±0.07	1.9±0.1	6.3±0.11	6.9±0.02	5.3±0.03	<1	<1

Table 2: Growth at 37 ºC and heavy metal resistance

Source	Yeast Code	Growth at 37 ºC	Cu^{2+} (5mM)	Ag^{2+} (5mM)	Co^{2+} (0.1mM)	Zn^{2+} (5mM)	Pb^{2+} (5mM)
Bagasse-2	BS1	+	-	-	-	-	+
Bagasse-3	BS2	+	-	-	-	-	+
Bagasse-3	BS3	+	-	-	-	-	+
Bagasse-2	BS4	+	-	-	-	-	+
Bagasse-2	BS5	-	-	-	-	-	+
Bagasse-2	BS6	+	-	-	-	-	+
Bagasse-2	BS7	+	-	-	-	-	+
Bagasse-1	BS8	+	-	-	-	-	+
Pulp	BS9	+	-	-	-	-	+
Pulp	BS10	+	-	-	-	-	+
Pulp	BS11	-	-	-	-	-	+
Water	BS12	-	-	-	-	-	+
Bagasse-3	BS13	-	-	-	-	-	+
Water	BS14	+	-	-	-	-	+
Water	BS15	+	-	-	-	-	+
Water	BS16	-	-	-	-	-	+

The results regarding growth at 37 ºC and heavy metal resistance are given in Table 2. While growth was detected in 11 of 16 strains at 37 ºC, lead resistance was determined in all strains.

In killer toxin test, strains were tested against each other. BS12 is not affected by the toxins of other yeasts and produces toxins that are effective against 5 yeasts (Table 3).

Table 3: Killer toxin test results

Strain Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	1	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
	2	+	+	+	-	+	+	+	+	+	-	-	+	-	-	-	+
	3	+	+	+	+	-	+	+	+	+	-	-	+	-	+	-	-
	4	+	+	+	+	-	+	+	+	+	-	-	+	-	-	-	-
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	7	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
	9	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	11	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
	12	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	+
	13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	14	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-
	15	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+
Strain Number	16	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+

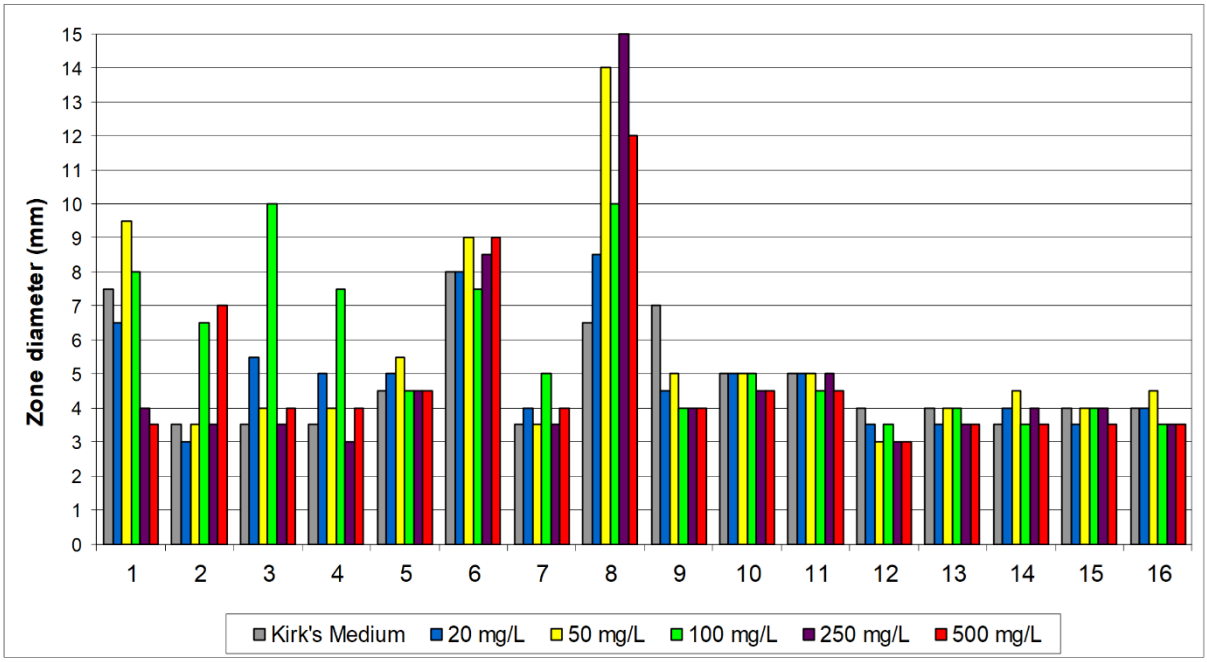

Figure 1: textile dye removal

In the textile dye removal test, all yeasts grew in the dye-containing medium. However, effective color removal has not been determined in most of them. The most effective color removal was observed in BS8 isolated from bagasse sample number 1.

4. Discussion

Yeasts are isolated from many sources such as soil, water and foods. However, when it comes to yeast isolation, the first sources that attract attention are fruits and foods rich in sugar. Samples taken from different points of the sugar factory were preferred because they may contain yeast with different characteristics.

Heavy metal resistance attracts attention because soil and water are polluted with these metals every day. The yeasts we were isolated in our study are not considered resistant. 5 mM is a very low value for resistant microorganisms. In the study conducted with ES10.4, the MIC value of 13 mM for copper and 17 mM for lead was determined [4].

The ability to produce killer toxins is important as it provides an advantage to the producer yeast during fermentation. The production of killer toxin by the industrial strain in mixed cultures during fermentation increases the yield by preventing the development of other strains. In the study, the ability of Sachharomyces cerevisiae strains to produce killer toxin was determined and it was revealed that killer toxin producing yeasts also have better biotechnological properties [1].

Textile dyes are one of the main factors causing water pollution. Removing dyes and cleaning the environment using advanced biological techniques attracts attention because it is economical and effective [5, 6]. *Candida oleophila, Candida tropicalis, Candida zeylanoides, Debaryomyces polymorphus, Galactomyces geotrichum,*

Issatchenkia occidentalis, Saccharomyces cerevisiae and *Trichosporon beigelii* have been studied in the removal of textile dyes and has been determined to be effective [7, 8, 9, 10, 11, 12, 13, 14].

The sugar factory has a different character from general yeast isolation environments such as fruits, and the enzymes of the yeasts isolated from there are special. 61 cold-adapted yeast isolated from Antarctica were tested for dye removal and successful results were obtained [15].

5. Conclusion

However, morphological, physiological and biochemical tests have commonly been used for phenotypic characterization of yeast species. These methods are complex and time consuming and can lead to incorrect classification at species level. Hence, in this research work, molecular identification is needed for classification at species level.

References

[1] G. A. M. Soares and H. H. Sato. "Killer toxin of Saccaharomyces cerevisae Y500-4L active against fleischmann and itaiquara commercial brands of yeast." *Revista de Microbiologia*, vol. 30, pp. 253-257 ISSN 0001-3714, 1999.

[2] Z. A. Ibrahim, S. H. Khudheir, A. A. Hussein. "Isolation, screening, and extraction the more efficient local yeast isolates for biosurfactant production." *Earth Environ. Sci.*, vol. 779, pp. 1-8, 2021.

[3] L. He, Y. Chen, H. Zhang, H. Wang, S. Chen, S. Liu, A. Liu, Q. Li, X. Ao, Y. Liu. "Isolation and identification of Lactobacillus and yeast species and their effect on the quality of fermented rice cakes." *Innovative Food Science & Emerging Technologies*, vol. 77, pp. 1-10, 2022. https://doi.org/10.1016/j.ifset.2022.102984.

[4] W. Irawat, Y. Wijaya, S. Christian, E. S. Djojo. "Characterization of heavy metals resistant yeast isolated from activated sludge in Rungkut, Surabaya, Indonesia as biosorbent of mercury, copper, and lead." *AIP Conf. Proc.*, 1744, 020061, 2016. https://doi.org/10.1063/1.4953535

[5] R. Jamee and R. Siddique. "Biodegradation of synthetic dyes of textile effluent by microorganisms: an environmentally and economically sustainable approach." *European Journal of Microbiology & Immunology*, vol *9*(4), pp. 114–118, 2019. https://doi.org/10.1556/1886.2019.00018

[6] M. Tripathi, S. Singh, S. Pathak, J. Kasaudhan, A. Mishra, S. Bala, D. Garg, R. Singh, P. Singh, P. K. Singh, A. K. Shukla, N. Pathak. "Recent Strategies for the Remediation of Textile Dyes from Wastewater: A Systematic Review. *Toxics*, *11*(11), 940. 2023 https://doi.org/10.3390/toxics11110940

[7] R. Khan, P. Bhawana, M. H. Fulekar. "Microbial decolorization and degradation of synthetic dyes: A review." *Rev Environ Sci Bio/Technol*, vol. 12, pp. 75–97, 2013.

[8] R. G. Saratale, G. D. Saratale, J. S. Chang, S. P. Govindwar. "Bacterial decolorization and degradation of azo dyes: A review." *J Taiwan Inst Chem Eng.*, vol. 42(1), pp. 138–57, 2011.

[9] N. Jafaria, M. R. Soudib, R. Kasra-Kermanshahi. "Biodegradation Perspectives of Azo Dyes by Yeasts." *Microbiology*, pp. 484–97.25844460, 2014.

[10] G. Guo, F. Tian, Y. Zhao, M. Tang, W. Liu, C. Liu, S. Xue, W. Kong, Y. Sun, S. Wang. "Aerobic decolorization and detoxification of acid scarlet GR by a newly isolated salt-tolerant yeast strain Galactomyces geotrichum GG." *Int. Biodeterior. Biodegrad.*, vol. 145, 104818, 2019. DOI:10.1016/j.ibiod.2019.104818

[11] A. C. R. Ngo, D. Tischler. "Microbial degradation of azo dyes: Approaches and prospects for a hazard-free conversion by microorganisms." *Int. J. Environ. Res. Public Health.*, vol. 19, pp. 4740, 2022.

[12] J. Jadhav, S.P. Govindwar. "Biotransformation of malachite green by Saccharomyces cerevisiae MTCC 463." *Yeast*, vol. 23, pp. 315–323, 2016.

[13] S. U. Jadhav, S. D. Kalme, S. P. Govindwar. "Biodegradation of methyl red by *Galactomyces geotrichum* MTCC 1360." *Int. Biodet. Biodeg.*, vol. 62, pp. 135–142, 2010.

[14] A. Sadeghi, M.H. Ehrampoush, M.T. Ghaneian, A.A. Najafpoor, H. Fallahzadeh, Z. Bonyadi. "The effect of diazinon on the removal of carmoisine by Saccharomyces cerevisiae." Desalination Water Treat., vol. 137, pp. 273–278, 2019.

[15] F. Ruscasso, L. Cavello, G. Curutchet, G. "Antarctic yeasts: potential use in a biologic treatment of textile azo dyes." *Bioresour. Bioprocess.*, vol. 9 (18), pp. 1-12, 2022. https://doi.org/10.1186/s40643-022-00507-5