ORIGINAL ARTICLE

Antibacterial activities of indigenous yeasts isolated from pomegranate peels (*Punica granatum L.*)

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ABSTRACT

Pomegranate peels (PGPs) are known to have the potential as antibacterial not only from their nutrient content but also the microflora. The activities might be caused by the existence of indigenous yeast that can be utilized to inhibit the growth of pathogenic bacteria. This study aims to identify antibacterial and antioxidant activity of indigenous yeast isolated from PGP. The research was conducted by experimental methods and followed by descriptive analysis. The study was done by the isolation of indigenous yeast from PGPs, which was identified using the rRNA sequence analysis of internal transcribed spacer (ITS) region with the primers of ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and then compared with Basic Local Alignment Search Tools (BLAST) algorithm toward the GenBank. Antibacterial activities of indigenous yeast were tested with agar plug diffusion and time kill test toward *Escherichia coli* and *Staphylococcus aureus*. The yeast identification obtained two isolates similar to *Hanseniaspora uvarum* CBS 314 and two isolates of *Pichia kudriavzevii* ATCC 6258 which have antibacterial activity against *E. coli* and *S. aureus*. *P. kudriavzevii* PGP D4 have best antimicrobial activities with a strong activity against *E. coli* (±9 mm) and medium activity against *S. aureus* (±3.1 mm).

Key words: Antibacterial, pomegranate peels, yeast

INTRODUCTION

Pomegranate peels (PGPs) are the outer and inedible part of the fruit and also being the byproduct of pomegranate processing with the amount of 60% of the total fruit.[1] PGP has demonstrated an expansive range of antimicrobial activities, especially toward antibiotic-resistant microorganisms.[2] Alkaloids, flavonoids, sterols, triterpenes, tannins, saponins, and phenols have been found in PGP which have shown antibacterial and antioxidant properties.[3]

Besides the chemical compound, antibacterial activity may originate from indigenous microorganisms such as yeasts. Yeasts can be found on PGP because it has a sugar content of 44.35 ± 0.20 mg/g (dry weight) that is useful as a growth medium.[4] Ko *et al*.[5] reported that PGPs contain several chemical compounds including protein, carbohydrates, fats, fiber, and phenols. PGPs contain several compounds needed by yeast to support its growth, such as about 66% carbohydrates (dry basis).[6] Total yeasts population of 7.73×10^8 cfu/g was found in PGP, which is higher than others.[7]

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Many research found various mechanisms in indigenous yeast inhibitory activities toward other microorganisms. Antagonistic activities are shown between yeasts that entangle mycocins that have been known as second metabolites.[8] Meanwhile, the main metabolites such as organic acids, volatile acids, and H_2O_2 create stress conditions for other microorganisms surround the yeast's growth.[9] The formation of secondary metabolites by yeast is caused by the reduced nutritional condition which causes the accumulation of cell excretion, so that more free radicals start to enter cells and cells begin to form antioxidant compounds to capture free radicals produced by cell metabolism.[10,11]

Yeast antimicrobial activity can inhibit the growth of harmful bacteria and molds.[12] The antimicrobial potential can be expanded to microbiological control that influenced quality control and food safety. *Escherichia coli* and *Staphylococcus aureus* as two of the food safety indicator were used to determine the antibacterial activity in occasion to achieve the food quality control and safety systems objectives.[13,14] Wide scope utilization of yeasts in the food industry is promising. Similarly, yeasts have a positive endurance toward a stress environment while shown their antibacterial properties against pathogenic or spoilage bacteria.[15,16] Hence, it is necessary to do research on the antibacterial and antioxidant activity of indigenous yeast isolated from PGPs.

MATERIALS AND METHODS

The raw materials used in this study include the inner skin of a local red pomegranate (6 months old) taken from a local market in Bandung City, while *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were taken from the Department of Biology, Universitas Padjadjaran.

Isolation and identification of indigenous yeast from pomegranate peels

One gram of PGP is diluted using 9 ml physiological NaCl (Merck) solution of 0.85% until dilution to 10^{-3}. 100 µl of each dilution was inserted into the Petri dish and then poured yeast and mold agar/YMA (Merck) and incubated for 48 h at room temperature (25°C–28°C) and then replicated three times. Yeast isolates were sequenced according to internal transcribed spacer (ITS) region with the primers of ITS1 and ITS4, aligned by MEGA X, and analyzed with Basic Local Alignment Search Tools (BLAST) algorithm.

Antibacterial activity test

Yeast colony is aseptically swabbed on YMA and incubated for 48 h at room temperature. The liquid culture of *S. aureus* and *E. coli* was swabbed evenly on nutrient agar/NA (merck). Yeast agar plate was plugged aseptically using a sterile forceps or needle and placed onto each NA plate. Incubate the NA plates at 30°C for 72 h and the diameter of the clear zones was measured at 24, 48, and 72 h (modification of[17]); all the treatments were replicated three times.

Viability of *Escherichia coli* and *Staphylococcus aureus*

The test bacterial suspension which has been standardized with McFarland 0.5 was diluted using 0.85% physiological NaCl. *S. aureus* or *E. coli* was grown in nutrient broth/NB (Merck) and then added 10 µL of yeast indigenous for 72 h at 30°C. 1 ml of bacterial suspension was platted in eosin methylene blue/EMB (Merck) for *E. coli* and mannitol salt agar/MSA (Merck) for *S. aureus* and then incubated at 37°C for 24 h. For positive (+) control treatment, 200 µL of bacterial culture of 1 × 104 CFU/mL + 2 µL amoxicillin 100 mg/mL were used, while 200 µL bacterial culture of 1 × 104 CFU/mL used as negative (-) control treatment. The population of *E. coli* and *S. aureus* were count every 24 h; all the treatments were replicated three times.

RESULTS AND DISCUSSIONS

Isolation and identification of indigenous yeast from PGP

Based on the results of yeasts isolation from PGP, 4 types of indigenous yeast isolates were obtained. The BLAST identification results obtained two indigenous yeast species from four isolates [Table 1]. There are two isolates of *Hanseniaspora uvarum* and two isolates of *Pichia kudriavzevii*.

Isolates D1 dan D2 have a percentage identity of 99.75% and 99.83%, respectively; meanwhile, isolates D3 and D4 have a percentage of identity 100%. Percentage identity states the similarity of the sample to certain species, where a value of percentage identity more than 80% means significant similarity of a species.[18] Percentage identity of all isolates was more than 99%, which means that all isolates are identical with the listed name of yeast in the table, e g., *Hanseniaspora uvarum* CBS 314 for isolate D1 and D2 and *P. kudriavzevii* ATCC 6258 for isolate D3 and D4.

Antibacterial activity of indigenous yeasts

Based on Figure 1, it is known that the yeast which has the highest antibacterial activity against *E. coli* bacteria

Table 1: Basic local alignment search tools results of pomegranate peels yeast isolates

Isolate name	Description		
	Yeast species	Percent identify	Accession
D1	*H. uvarum* CBS 314	99.75	NR_130660.1
D2	*H. uvarum* CBS 314	99.83	NR_130660.1
D3	*P. kudriavzevii* ATCC 6258	100	NR_131315.1
D4	*P. kudriavzevii* ATCC 6258	100	NR_131315.1

H. uvarum: Hanseniaspora uvarum, P. kudriavzevii: Pichia kudriavzevii, CBS: Centraal Bureau voor Schimmelcultures, ATCC: American Type Culture Collection

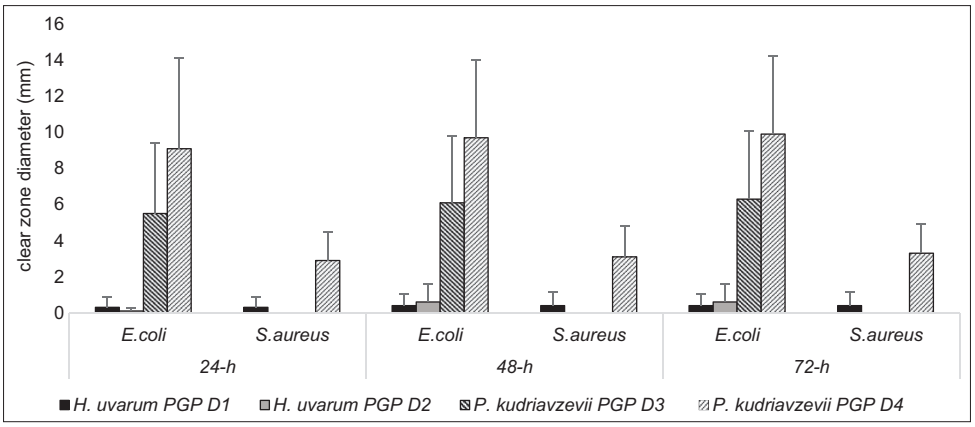

Figure 1: The diameter of clear zone against *Escherichia coli* and *Staphylococcus aureus*

is *P. kudriavzevii* PGP D4. Meanwhile, *Hanseniaspora uvarum* PGP D1 and D2 form a clear zone diameter with a size range below 1 mm. The antibacterial activity of *Hanseniaspora uvarum* PGP D1 and D2 was classified in the weak category (0-3 mm), while the yeast isolates of *P. kudriavzevii* PGP D3 and D4 belong to the strong category (≥6 mm).[19]

The antibacterial metabolites produced by yeast can be originated from primary and secondary metabolites. Primary metabolites produced by yeast that could role as an antibacterial compound can be in the form of organic acids and proteins.[20,21] Secondary metabolites produced by yeast can be in the form of flavonoid, phenolic, alkaloid, and polyketide compounds.[22,23] As shown in Figure 1, the diameter of clear zones tends to increase until 72 h. The results showed that the increase of antibacterial activity were shown the logarithmic and stationary phase of yeasts growth occurred which produce both primary and secondary metabolites that gave an antibacterial effect against E. coli.

P. kudriavzevii PGP D4 showed the highest has antibacterial activity that shown by the diameter of the clear zone. The clear zone formed by *P. kudriavzevii* PGP D4 occurred at 24-72 h; this indicates the presence of antibacterial metabolites produced by isolates. The antibacterial activity is derived from primary metabolites and secondary metabolites produced. *P. kudriavzevii* can produce primary metabolites in the form of organic acids, namely, acetic acid, pyruvic acid, and lactic acid.[24] Lactic acid and acetic acid resulted from yeasts metabolism have been proven can control the growth of E. coli.[25,26] *P. kudriavzevii* also can produce ethanol as a secondary metabolite, so that a clear zone in the *E. coli* is formed.[27]

The antibacterial activity of *Hanseniaspora uvarum* PGP D1 and D2 is relatively weak because the diameter of the clear zone formed is <1 mm.[19] *H. uvarum* has the ability to produce killer toxin which can against several pathogenic including *Staphylococcus aureus* and *Escherichia coli*.[28] pH and temperature are factors which give a significant effect on the development of killer toxin.

Based on Figure 1, the yeast which has the highest antibacterial activity against *S. aureus* is *P. kudriavzevii* PGP D4. *Hanseniaspora uvarum* PGP D2 and *P. kudriavzevii* PGP D3 did not show antibacterial activity against *S. aureus* because there were no clear zone diameters showed. The antibacterial activity of *H. uvarum* PGP D1 was classified in the weak category (0–3 mm), while the yeast isolate *P. kudriavzevii* PGP D4 was in the moderate category (3–6 mm).[19]

The formation of primary and secondary metabolites of *P. kudriavzevii* PGP D4 such as organic acids, alcohols, and phenolic compounds, results in clear zone diameter against *S. aureus*. An increase in the diameter of the clear zone occurred at 72 h; it was marked because of the additional activity of the formation of secondary metabolites in the form of phenolic compounds. Phenol compounds were very sensitive to the single-cell wall *S. aureus* because can change the permeability characteristics of bacterial cell membranes and causes leakage of essential constituents of cells so that bacteria dead.[29] Meanwhile, *H. uvarum* produces a small amount killer toxin which influences by environmental factors. The optimum conditions for *H. uvarum* to produce killer toxin and against *S. aureus* are at pH 4 and temperature 25°C with 4% of NaCl.

Based on antibacterial activity, *P. kudriavzevii* PGP D4 has the highest activity against *Escherichia coli* and *Staphylococcus aureus*. So that *P. kudriavzevii* PGP D4 was taken as a candidate isolate that has antibacterial activity. The positive control used was amoxicillin, which has antibacterial activity and the negative control was without amoxicillin nor yeast isolate.

The ability of *Staphylococcus aureus* cells to survive is higher than that of *Escherichia coli* bacteria. This can be seen from

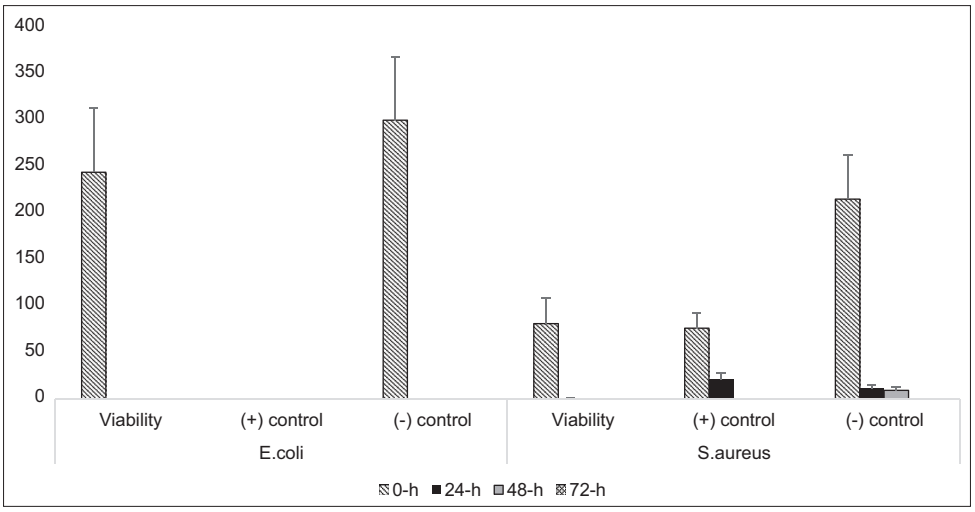

Figure 2: Viability of *Escherichia coli* and *Staphylococcus aureus* against *Pichia kudriavzevii* PGP D4

Figure 2, where the number of *E. coli* bacteria cells decreased drastically at 12 h, while the number of *S. aureus* bacteria cells decreased slowly. *E. Coli* and *S. aureus* are significantly decreased in the range of time 0-24 h. The effectiveness of *P. kudriavzevii* PGP D4 in reducing *E. coli* was 18.58%. Meanwhile, the decreasing effectiveness in reducing *S. aureus* reached 85.18%. This can be caused by several factors including temperature, nutrients, and antibacterial compounds.

The decrease in the number of *E. coli* and *S. aureus* also indicates the formation of antibacterial compounds by *P. kudriavzevii* PGP D4 which can be derived from the metabolites produced. The existence of antioxidant activity in yeast, such as flavonoid, phenolic, isoprenoid, alkaloid, and polyketide compounds, can induce antibacterial activity.[23,30,31] These compounds can cause a decrease in cell surface tension and denature cell proteins so that the number of pathogenic bacteria reduced.[29,32,33]

CONCLUSIONS

Identification of indigenous yeast isolated from PGP resulting in two isolates 99.83%–99.75% identical with *Hanseniaspora uvarum* CBS 314 and two isolates 100% identical with *P. kudriavzevii* ATCC 6258. All isolates showed antibacterial activity against *E. coli* and *S. aureus*, with *P. kudriavzevii* PGP D4 being the highest against *E. coli* (9 mm) and *S. aureus* (3.1 mm).

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Malviya S, Arvind, Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. J Food Sci Technol 2014;51:4132-7.
2. Singh B, Singh JP, Kaur A, Singh N. Antimicrobial potential of pomegranate peel: A review. Int J Food Sci Technol 2019;54:959-65.
3. Makarewicz M, Drożdż I, Tarko T, Duda-Chodak A. The Interactions between Polyphenols and Microorganisms, Especially Gut Microbiota. Antioxidants(Basel) 2021;10:188.
4. Gupta U, Solanki H. Quantification of ash and selected primary metabolites from non-edible parts of several fruits. Int J Pharm Pharm Sci 2015;7:288-90.
5. Ko K, Dadmohammadi Y, Abbaspourrad A. Nutritional and bioactive components of pomegranate waste used in food and cosmetic applications: A review. Foods 2021;10:657.
6. Kaderides K, Kyriakoudi A, Mourtzinos I, Goula AM. Potential of pomegranate peel extract as a natural additive in foods. Trends Food Sci Technol 2021;115:380-90.
7. El Barnossi A, Moussaid F, Iraqi Housseini A. Tangerine, banana and pomegranate peels valorisation for sustainable environment: A review. Biotechnol Rep (Amst) 2021;29:e00574.
8. Hatoum R, Labrie S, Fliss I. Antimicrobial and probiotic properties of yeasts: From fundamental to novel applications. Front Microbiol 2012;3:421.
9. Liszkowska W, Berlowska J. Yeast fermentation at low temperatures: Adaptation to changing environmental conditions and formation of volatile compounds. Molecules 2021;26:1035.
10. Daou R, Joubrane K, Maroun RG, Khabbaz LR, Ismail A, Khoury AE, *et al*. Mycotoxins: Factors influencing production and control strategies. AIMS Agric Food 2021;6:416-47.
11. Santos Sánchez NF, Salas Coronado R, Villanueva Cañongo C, Hernández CarlosB. Antioxidant compounds and their antioxidant

mechanism. Antioxidants 2019;1:1-28. [doi: 10.5772/intechopen.85270].

12. Petkova M, Stefanova P, Gotcheva V, Angelov A. Isolation and characterization of lactic acid bacteria and yeasts from typical Bulgarian sourdoughs. Microorganisms 2021;9:1346.
13. Lambrechts AA, Human IS, Doughari JH, Lues JF. Bacterial contamination of the hands of food handlers as indicator of hand washing efficacy in some convenient food industries. Pak J Med Sci 2014;30:755-8.
14. Salamandane A, Silva AC, Brito L, Malfeito-Ferreira M. Microbiological assessment of street foods at the point of sale in Maputo (Mozambique). Food Qual Saf 2021;5:fyaa030.
15. Garcia-Gonzalez N, Battista N, Prete R, Corsetti A. Health-promoting role of *Lactiplantibacillus plantarum* isolated from fermented foods. Microorganisms 2021;9:349.
16. Utama GL, Putri F, Indah H, Balia R. Preliminary identification of inhibition activities towards *Eschericia coli* and *Salmonella* spp. by pickle's indigenous halotolerant bacteria. Int J Adv Sci Eng Inf Technol 2015;5:123-5.
17. Roostita LB, Fleet GH, Wendry SP, Apon ZM, Gemilang LU. Determination of yeasts antimicrobial activity in milk and meat products. Adv J Food Sci Technol 2011;3:442-5.
18. Wu HY, Chan KT, But GW, Shaw PC. Assessing the reliability of medicinal *Dendrobium* sequences in GenBank for botanical species identification. Sci Rep 2021;11:3439.
19. Pan X, Chen F, Wu T, Tang H, Zhao Z. The acid, bile tolerance and antimicrobial property of *Lactobacillus acidophilus* NIT. Food Control 2009;20:598–602.
20. Romano P, Ciani M, Fleet GH. Yeasts in the Production of Wine. New York: Springer Nature; 2019.
21. Roostita R, Fleet GH. Growth of yeasts isolated from cheeses on organic acids in the presence of sodium chloride. Food Technol Biotechnol 1999;37:73-9.
22. Rahmat E, Kang Y. Yeast metabolic engineering for the production of pharmaceutically important secondary metabolites. Appl Microbiol Biotechnol 2020;104:4659-74.
23. Siddiqui MS, Thodey K, Trenchard I, Smolke CD. Advancing secondary metabolite biosynthesis in yeast with synthetic biology tools. FEMS Yeast Res 2012;12:144-70.
24. Vilela, A. Use of nonconventional yeasts for modulating wine acidity. Fermentation 2019;5:1-15. [doi: 10.3390/fermentation5010027].
25. Jayabalan R, Malbaša RV, Lončar ES, Vitas JS, Sathishkumar M. A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. Compr Rev Food Sci Food Saf 2014;13:538-50.
26. Raftari M, Jalilian FA, Abdulamir AS, Son R, Sekawi Z, Fatimah AB. Effect of organic acids on *Escherichia coli* O157:H7 and *Staphylococcus aureus* contaminated meat. Open Microbiol J 2009;3:121-7.
27. Utama GL, Kurniawan MO, Natiqoh N, Balia RL. Species identification of stress resistance yeasts isolated from banana waste for ethanol production. IOP Conf Ser Earth Environ Sci 2019;306:1-8. [doi: 10.1088/1755 1315/306/1/012021].
28. Hameed AR, Al-Qaysi SA, Hameed ST. Killer activity of hanseniaspora uvarum isolated from dates vinegar: Partially purification and characterization of killer toxin. Baghdad Sci J 2019;16:141-50.
29. Lopez-Romero JC, González-Ríos H, Borges A, Simões M. Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. Evid Based Complement Alternat Med 2015;2015:795435.
30. Chemler JA, Yan Y, Koffas MA. Biosynthesis of isoprenoids, polyunsaturated fatty acids and flavonoids in *Saccharomyces cerevisiae*. Microb Cell Fact 2006;5:20.
31. Sugita T, Nishikawa A, Ikeda R, Shinoda T. Identification of medically relevant *Trichosporon* species based on sequences of internal transcribed spacer regions and construction of a database for *Trichosporon* identification. J Clin Microbiol 1999;37:1985-93.
32. Koyuncu S, Andersson MG, Löfström C, Skandamis PN, Gounadaki A, Zentek J, *et al*. Organic acids for control of *Salmonella* in different feed materials. BMC Vet Res 2013;9:81.
33. Cendrowski A, Kraśniewska K, Przybył JL, Zielińska A, Kalisz S. Antibacterial and antioxidant activity of extracts from rose fruits (*Rosa rugosa*). Molecules 2020;25:E1365.