Plant Protection (Scientific Journal of Agriculture)

49(1), Spring, 2026

10.22055/ppr.2026.50779.1850

Short Communication

Decontamination of fungal cultures and inhibition of bacterial growth using molasses agar

S. Abbasi [1*], E. Seidmohammadi [2]

1. ***Corresponding Author:** Department of Plant Protection, Razi University, Kermanshah, Iran (abbasikhs@yahoo.com)
2. Department of Plant Protection, Faculty of Agriculture, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Received: 6 October 2025 Revised: 1 February 2026 Accepted: 9 February 2026

Abstract

Bacterial contamination remains one of the most persistent challenges in maintaining pure fungal cultures, often leading to data inaccuracy and the loss of valuable isolates. In this study, a natural and antibiotic-free approach was developed to overcome this issue using molasses agar, either alone or in combination with the Cabin-Sequestering (CS) physical isolation method. Sugar beet molasses, rich in phenolic and organic compounds, was incorporated into agar medium at various concentrations (75–150 g/L) and tested against multiple fungal taxa (*Rhizoctonia solani*, *Fusarium redolens*, *Rhizopus stolonifer*, and *Pythium aphanidermatum*) and two bacterial species (*Bacillus subtilis* and *Xanthomonas translucens*). The results revealed a clear dose-dependent antibacterial effect, with complete inhibition of bacterial growth at 125–150 g/L, while most true fungi maintained viable growth. In addition, molasses agar effectively supported the germination and single-spore isolation of *Cercospora beticola* without bacterial interference. When integrated with the CS method, molasses agar further enhanced purification efficiency, enabling the recovery of uncontaminated fungal hyphae and the establishment of stable subcultures free of bacteria. This combined system provides a sustainable, cost-effective, and environmentally safe strategy for fungal culture purification and maintenance, offering a reliable alternative to antibiotic-based techniques for mycological research.

Keywords: Polyphenol, Cabin-Sequestering method, Oomycetes, decontamination, antimicrobial effect

Associate editor: R. Rezaei (Ph.D.)

Citation: Abbasi, S., & Seidmohammadi, E. (2026). Decontamination of fungal cultures and inhibition of bacterial growth using molasses agar. *Plant Protection (Scientific Journal of Agriculture)*, *49*(1), 57-67. https://doi.org/ 10.22055/ppr.2026.50779.1850

گیاه‌پزشکی (مجله علمی کشاورزی)
جلد ۴۹، شماره ۱، بهار ۱۴۰۵

10.22055/ppr.2026.50779.1850

گزارش کوتاه انگلیسی

آلودگی‌زدایی از کشت‌های قارچی و بازداری رشد باکتری‌ها با استفاده از محیط کشت ملاس‌آگار

سعید عباسی*[۱]، الهام صیدمحمدی[۲]

۱- * **نویسنده مسوول**: گروه گیاه‌پزشکی، دانشکده علوم و مهندسی کشاورزی، دانشگاه رازی، کرمانشاه، ایران

۲- گروه گیاه‌پزشکی، دانشکده کشاورزی، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران

تاریخ دریافت: ۱۴۰۴/۰۷/۱۴ تاریخ بازنگری: ۱۴۰۴/۱۱/۱۲ تاریخ پذیرش: ۱۴۰۴/۱۱/۲۰

چکیده

آلودگی باکتریایی یکی از چالش‌های پایدار و مهم در حفظ خلوص کشت‌های قارچی است و می‌تواند موجب کاهش دقت داده‌ها و از دست رفتن جدایه‌های ارزشمند شود. در این پژوهش، روشی طبیعی و بدون استفاده از آنتی‌بیوتیک برای رفع این مشکل توسعه داده شد که مبتنی بر به‌کارگیری محیط ملاس آگار، به‌تنهایی یا در ترکیب با روش فیزیکی Cabin-Sequestering (CS)، بود. ملاس چغندرقند که سرشار از ترکیبات فنولی و آلی است، در غلظت‌های مختلف (۷۵ تا ۱۵۰ گرم در لیتر) به محیط آگار افزوده شد و اثر آن بر چندین گونه‌ی قارچی شامل *Rhizoctonia solani*، *Fusarium redolens*، *Rhizopus stolonifer* و *Pythium aphanidermatum* و دو گونه باکتری شامل *Bacillus subtilis* و *Xanthomonas translucens* ارزیابی شد. نتایج نشان داد که ملاس آگار دارای اثر ضدباکتریایی وابسته به غلظت است؛ به‌طوری‌که در غلظت‌های ۱۲۵ تا ۱۵۰ گرم در لیتر، رشد باکتری‌ها را به‌طور کامل متوقف کرد، حال آن‌که، بیشتر قارچ‌های حقیقی همچنان رشد قابل قبولی داشتند. افزون بر این، ملاس آگار بدون ایجاد تداخل باکتریایی، جوانه‌زنی و تهیهٔ جدایه‌های تک‌اسپور *Cercospora beticola* را نیز به‌خوبی پشتیبانی کرد. ترکیب ملاس آگار با روش CS کارایی خالص‌سازی را بیشتر افزایش داد و امکان به‌دست‌آوردن ریسه‌های عاری از آلودگی و زیرکشت‌های پایدار و بدون باکتری را فراهم ساخت. این سامانهٔ ترکیبی روشی پایدار، کم‌هزینه و سازگار با محیط‌زیست برای خالص‌سازی و نگهداری کشت‌های قارچی فراهم می‌کند و می‌تواند جایگزینی قابل‌اعتماد برای روش‌های مبتنی بر آنتی‌بیوتیک در پژوهش‌های قارچ‌شناسی باشد.

کلید واژه‌ها: پلی‌فنول، روش Cabin-Sequestering، اومیست‌ها، آلودگی‌زدایی، اثر ضدمیکروبی

دبیر تخصصی: دکتر رسول رضائی

Introduction

Fungal cultures play a crucial role in microbiology, biotechnology, agriculture, and pharmaceuticals, and maintaining their purity is essential for obtaining accurate and reproducible results. However, bacterial contamination remains a common challenge in the isolation and maintenance of fungal species, often resulting in compromised culture quality and the loss of valuable isolates.

Traditional approaches to bacterial contamination primarily rely on antibiotic-supplemented media; however, antibiotics often fail to eradicate contaminants completely, may promote antibiotic-resistant strains, negatively affect fungal growth, and raise environmental concerns. Several non-antibiotic agar-based techniques have therefore been developed. Brown (1924) introduced an inversion method sampling from the agar undersurface, while Machacek (1934) used coverslip sealing on molten agar, a technique requiring precise timing and unsuitable for thermolabile fungi. Other methods, including agar segmentation (Sleeth, 1945), sporulation-based detection (Schmitthenner & Hilty, 1962), and physical isolation using rings (Raper, 1937; Harrower, 1989), also present limitations such as labor intensity, specificity, or ineffectiveness for slow-growing fungi.

Shi et al. (2019) proposed the Cabin-Sequestering (CS) method, which uses a small cabin hole covered by a sterile coverslip to confine bacteria while allowing fungal hyphae to grow uncontaminated spatially; however, this method alone may be insufficient for complete decontamination.

Despite these advances, challenges remain in achieving complete bacterial removal, highlighting the need to optimize culture media that inhibit bacteria while supporting fungal growth. Molasses, a natural by-product rich in polyphenolic compounds with antimicrobial properties (Obata et al., 1963; Filipčev et al., 2010), has been identified as a promising bacteriostatic substrate (Chen et al., 2017). Recent studies have characterized sugar-beet-derived polyphenols and confirmed their antibacterial activity (Arjeh et al., 2022; Sharma et al., 2024). In addition, high sugar concentrations can reduce water activity and exert osmotic stress on microorganisms (Chirife et al., 1983; Molan, 1992), potentially inhibiting bacteria while allowing fungal growth, although responses may vary among fungal groups, including some oomycetes.

This study aims to investigate the integration of molasses agar with the CS method as an antibiotic-free approach for bacterial decontamination in fungal cultures.

Materials and Methods

Fungal and Bacterial Isolates

Fungal species representing diverse taxa were used, including *Pythium aphanidermatum* (Oomycota, Pseudofungi, causal agent of cucumber damping-off), *Rhizopus stolonifer* (Mucoromycota, causing soft rot of strawberry), *Fusarium redolens* (Ascomycota, causing chickpea yellows), and *Rhizoctonia solani* (Basidiomycota, causing root and hypocotyl rot in common bean). Bacterial isolates included *Xanthomonas translucens* (Gram-negative, causal agent of bacterial leaf streak in wheat) and *Bacillus subtilis* (Gram-positive, isolated from soil). All isolates were obtained from the Plant Protection Department, Razi University, Kermanshah, Iran.

Preparation of Molasses Agar Media

Sugar beet molasses (82° Brix) was obtained from Bisotun Sugar Factory (Iran) and diluted in distilled water to final concentrations of 75, 100, 125, and 150 g/L. Media were autoclaved at 121°C for 20 min and poured into Petri dishes. Potato Dextrose Agar (PDA), prepared according to the manufacturer's instructions, was used as the control medium.

Growth Inhibition Examination

For each fungal isolate, a 6 mm-diameter plug from an actively growing culture was transferred onto molasses agar and PDA, and incubated at 25°C. Plates were monitored until the fungal colony on PDA reached approximately 5 mm from the plate edge; at this standardized time point, colony diameters were recorded for all molasses treatments. Each treatment included three replicates, and the experiment was repeated twice. Data were analyzed using one-way ANOVA, and mean comparisons were performed with Duncan's Multiple Range Test at $\alpha = 0.01$ using SAS software (version 9.3).

Functional Evaluation of Molasses Agar

To assess antibacterial performance, naturally contaminated fungal isolates were subcultured onto molasses agar (150 g/L) and incubated at 25°C. Bacterial presence was monitored visually and fungal growth was compared with PDA controls. In addition, the suitability of molasses agar for fungal isolation was evaluated using *Cercospora beticola*. Conidia were collected from infected leaves, transferred individually onto molasses agar (150 g/L), and incubated at 25°C for 5–7 days. Resulting colonies were subcultured onto V8 agar, and bacterial contamination was assessed visually and microscopically.

Application of Cabin-Sequestering (CS) Method with Molasses Agar

To improve bacterial elimination, molasses agar (125 and 150 g/L) was combined with the Cabin-Sequestering (CS) method as described by Shi et al. (2019). After solidification, 10 mm diameter cabins were created in the agar and inoculated with 6 mm plugs from contaminated fungal cultures. Cabins were covered with sterile coverslips, and plates were incubated at 25°C for 7–10 days. Hyphae growing beyond the coverslip were then transferred onto PDA plates.

Results and Discussion

Growth Inhibition Examination

Fungal growth exhibited a clear concentration-dependent response to increasing molasses content, with ANOVA revealing highly significant differences among concentrations for all tested species ($p < 0.01$), and Duncan's Multiple Range Test at $\alpha = 0.01$ confirming distinct inhibitory effects (Figures 1, 2). The complete inhibition of bacterial growth at 125 and 150 g/L is consistent with the known antimicrobial activity of sugar beet molasses, which contains phenolic compounds, organic acids, and Maillard-derived products that can suppress a broad spectrum of bacteria (Taguri et al., 2004; Chen et al., 2017). These effects, combined with reduced water activity at high sugar concentrations, create conditions highly restrictive for bacterial survival, particularly for Gram-negative species that are susceptible to phenolic-induced membrane damage (Chen et al., 2017).

Among fungi, *R. stolonifer* showed moderate sensitivity. Growth at 75 and 100 g/L did not differ significantly from PDA, while reductions of 9.4% and 18.7% were observed at 125 and 150 g/L, respectively, with significant inhibition only at the highest concentration. *F. redolens* exhibited stronger inhibition, with growth reductions of 29%, 48%, and 62% at 100, 125, and 150 g/L, respectively, indicating progressive suppression. *R. solani* was highly sensitive, showing 37%, 52%, 67%, and 73% reductions at 75, 100, 125, and 150 g/L, respectively. In contrast, the oomycete *P. aphanidermatum* was extremely sensitive, with complete inhibition at ≥100 g/L and only limited growth at 75 g/L.

These results highlight the selective tolerance of true fungi to molasses, likely due to their chitin-reinforced cell walls, melanins, robust osmoregulatory systems, and hyphal growth form, which together provide mechanical stability and resistance to

osmotic and chemical stresses (Jennings & Burke, 1990; Davis et al., 2000; Hohmann, 2002; Ene et al., 2015; Hohmann, 2015; Gow et al., 2017). Overall, molasses agar provides a natural, antibiotic-free medium that selectively suppresses bacterial growth while supporting measurable growth of most filamentous fungi, although some oomycetes may be fully inhibited at higher concentrations.

Functional Evaluation of Molasses Agar

Culturing bacterially contaminated fungal isolates on molasses agar (150 g/L) resulted in complete bacterial elimination in most samples (Figure 3b). In a few cases, resistant bacterial strains were initially suppressed but reappeared in subsequent subcultures, indicating that while molasses agar is highly effective, some persistent contaminants may require additional treatment or physical confinement. Fungal colonies maintained normal morphology and growth, confirming that molasses agar selectively inhibits bacterial proliferation without impairing fungal viability. These findings align with the antibacterial effects of polyphenols and osmotic stress in molasses, which restrict bacterial growth while allowing fungal growth to continue (Hohmann, 2002; Chen et al., 2017; Gow et al., 2017).

Molasses agar also successfully supported germination and colony formation of single *Cercospora beticola* spores isolated from infected sugar beet leaves. No bacterial contamination was observed during incubation, and single-spore colonies developed normally after transfer to V8 agar, displaying typical *C. beticola* morphology and sporulation (Figure 4). Overall, these results confirm that molasses agar functions as an effective, antibiotic-free medium for bacterial suppression while maintaining fungal growth and viability.

Application of Cabin-Sequestering (CS) Method with Molasses Agar

Combining the Cabin-Sequestering (CS) method with molasses agar at 125 and 150 g/L demonstrated high efficiency in eliminating bacterial contamination from fungal cultures. In this system, the agar cabin served as a physical barrier, while antibacterial compounds in molasses synergistically inhibited bacterial growth. After 5–7 days of incubation, fungal hyphae extended beyond the coverslip edge with no bacterial growth observed on the surrounding agar, indicating effective separation and complete bacterial suppression.

Subcultures derived from uncontaminated hyphal tips and transferred to PDA exhibited stable, bacteria-free growth through successive transfers, confirming complete elimination of contaminants, with similar results across multiple fungal species. In contrast, application of the CS method on PDA without molasses occasionally allowed bacterial regrowth around emerging hyphae (Figure 5f), highlighting the critical role of molasses agar in enhancing CS efficiency. These findings demonstrate that molasses agar not only has strong bacteriostatic properties but also significantly improves the performance of the CS method by reducing the likelihood of bacterial escape and regrowth (Figure 5). This integrated chemical and physical approach overcomes the limitations of each method alone and supports its use as a natural, antibiotic-free strategy for fungal culture purification.

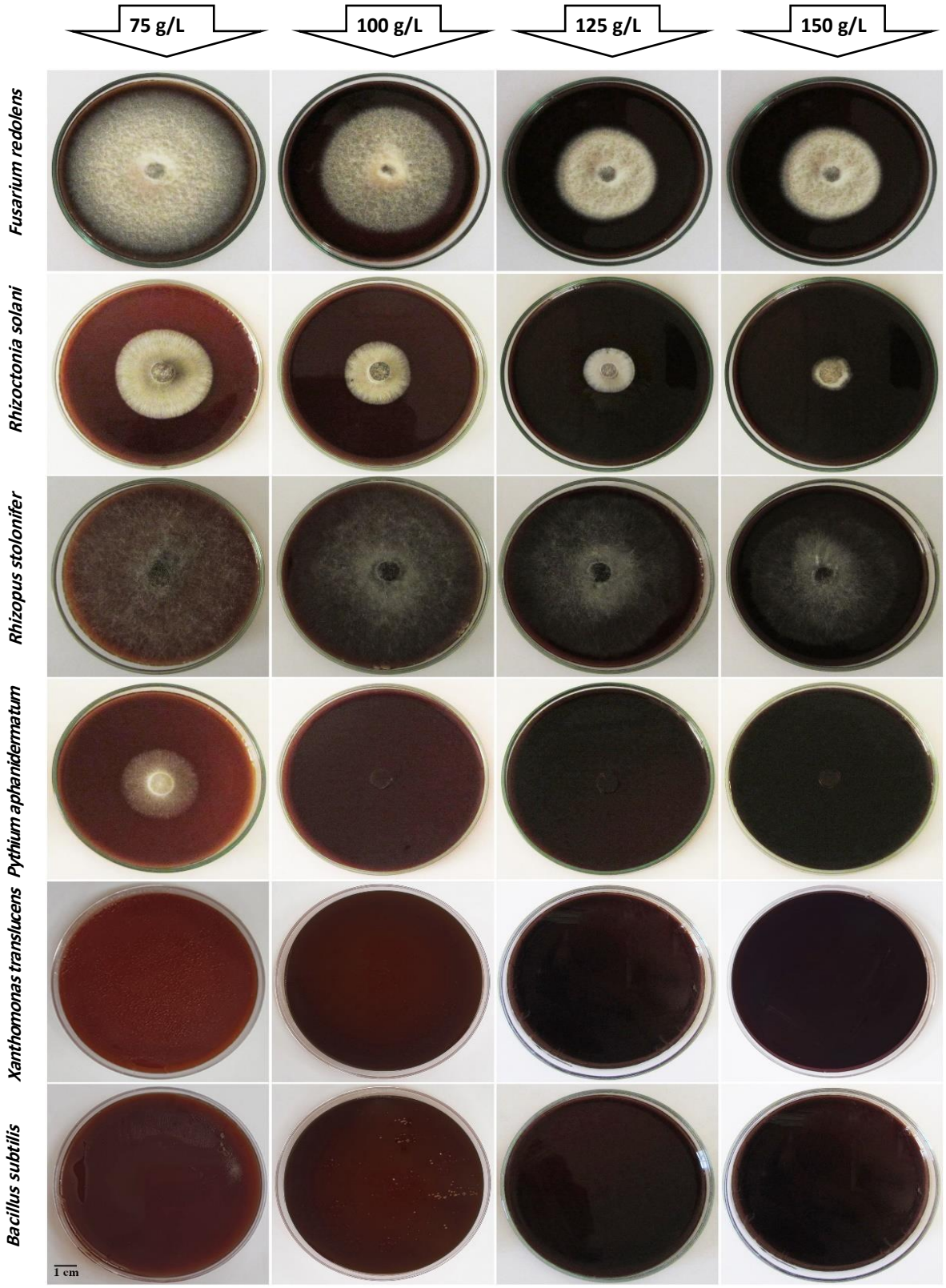


Figure 1. Effect of Different Molasses Concentrations (75, 100, 125, and 150 g/L) on the Growth Patterns of Various Fungal Species and Bacteria (Bar = 1 cm).

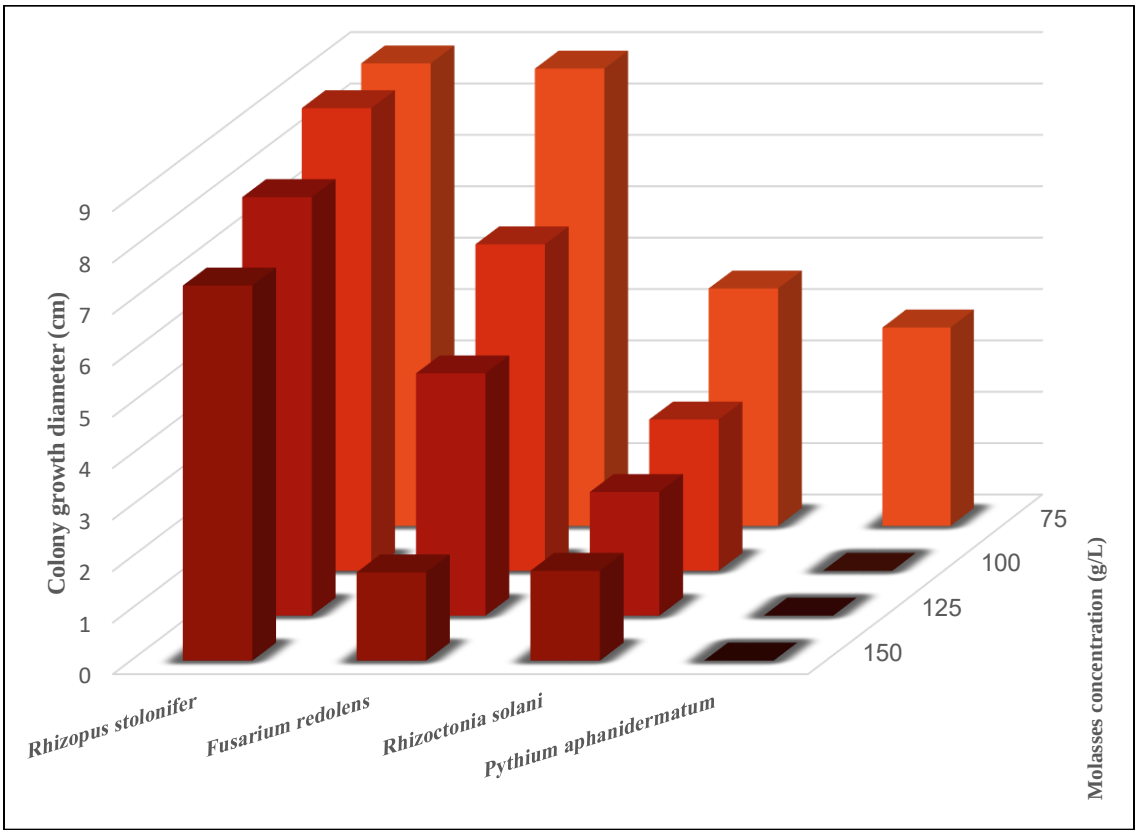


Figure 2. Impact of Molasses Concentration on Colony Growth of Various Fungal Species.

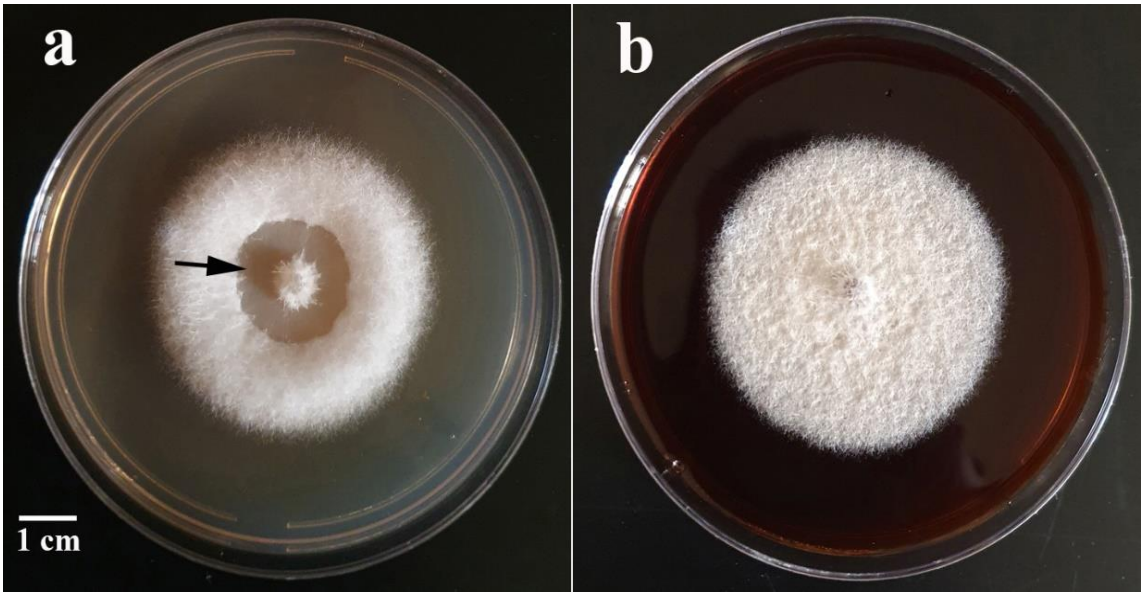


Figure 3. Effect of molasses agar on the elimination of bacterial contamination in fungal cultures. (a) Persistence of bacterial contamination on PDA medium (arrow indicating bacterial contamination). (b) Growth of the same fungal isolate free of bacterial contamination on molasses agar (150 g/L).

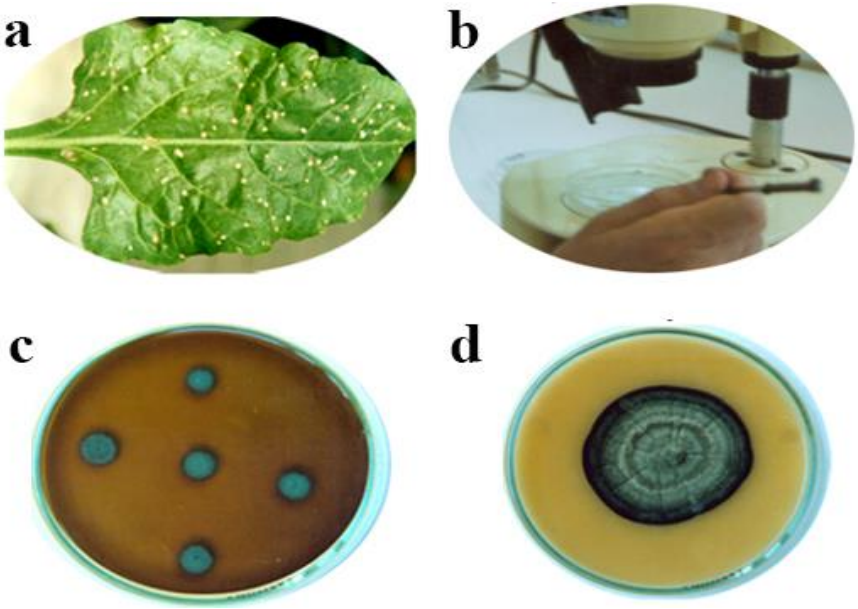


Figure 4. Single-spore isolation of *Cercospora beticola* on molasses agar (150 g/L). (a) Naturally infected sugar beet leaf showing characteristic leaf spot symptoms caused by *C. beticola*. (b) Transfer of individual conidia to molasses agar using a sterile needle under a stereomicroscope. (c) Germination and colony development from single spores on molasses agar, free of bacterial contamination.(d) Single-spore colony subcultured on V8 agar showing normal mycelial growth and typical *C. beticola* morphology.

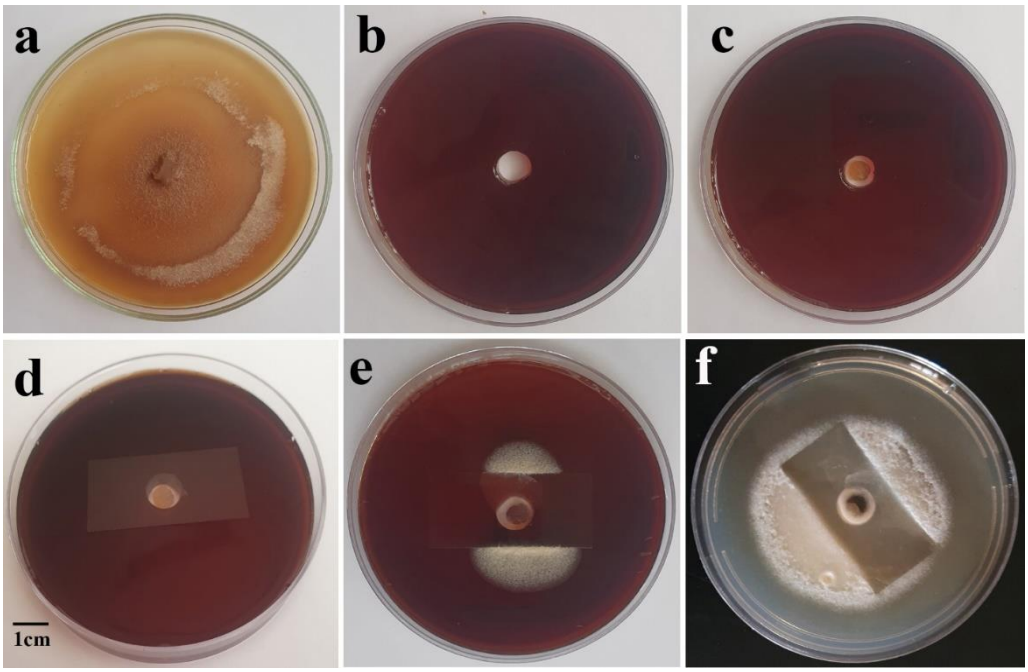


Figure 5. Effect of molasses agar and cabin-sequestering (CS) method on bacterial and fungal growth. (a–f) Step-by-step procedure and outcomes of using molasses agar combined with the CS method to eliminate bacterial contamination. (a) *Fusarium* colony contaminated with a hard-to-remove bacterium on PDA. (b) Creation of a 10 mm diameter hole (cabin) in the surface of a molasses agar plate (125–150 g/L) using a sterile cork borer. (c) Inoculation of a 6 mm diameter plug from a bacterially contaminated *Fusarium* culture into the cabin. (d) Placement of a sterile coverslip over the cabin to seal it without trapping air bubbles. (e) Fungal hyphae growing beyond the coverslip after incubation, showing successful elimination of bacterial contamination. (f) Failure of the CS method combined with the PDA medium to eliminate resilient bacterial contaminants.

Conclusion

The present study demonstrates that molasses agar can serve as a practical, low-cost, and antibiotic-free medium for selective fungal culture. Its strong antibacterial activity, combined with its ability to support fungal growth, makes it well-suited for purification workflows. When combined with the Cabin-Sequestering (CS) method, molasses agar markedly improves the recovery of uncontaminated hyphae, offering a simple, sustainable solution for managing bacterial co-contamination. These findings highlight the potential of molasses agar—alone or in combination with physical separation techniques—to enhance fungal isolation practices across mycological research. Future work should further evaluate species-specific responses, optimize molasses concentrations, and explore scalability for routine laboratory and industrial applications.

Acknowledgments

The authors gratefully acknowledge the graduate students of the Department of Plant Protection, Razi University, Kermanshah, Iran, for supplying bacterial-contaminated fungal cultures utilized in this research.

REFERENCES

Brown, W. (1924). Two mycological methods. I. A simple method of freeing fungal cultures from bacteria. *Annals of Botany*, *38 (2)*, 401-404. https://doi.org/10.1093/oxfordjournals.aob.a089906

Machacek, J. (1934). A simple method of obtaining *Pythium* cultures free from bacteria. *Phytopathology*, *24*, 301–303. https://www.cabidigitallibrary.org/doi/full/10.5555/19341101127

Sleeth, B. (1945). Agar medium and technique for isolating *Pythium* free of bacteria. *Phytopathology*, *35*, 1030–1031. https://www.cabidigitallibrary.org/doi/full/10.5555/ 19461100577

Schmitthenner, A. E., & Hilty, J. W. (1962). A modified dilution technique for obtaining single-spore isolates of fungi from contaminated material. *Phytopathology*, *52*, 582–583. https://www.cabidigitallibrary.org/doi/ full/10.5555/19631100435

Raper, J. R. (1937). A method of freeing fungi from bacterial contamination. *Science*, *85,* 342-342 https://doi.org/10.1126/science.85. 2205.342.a

Harrower, K. M. (1989). A simple technique for purifying mycelial cultures of unicellular contaminants. *The Mycologist*, *3 (2)*, 98.

Shi, X.-X., Qiu, H.-P., Wang, J.-y., Zhang, Z., Wang, Y.-L. & Sun, G.-C. (2019). A handy method to remove bacterial contamination from fungal cultures. *Plos one, 14 (11),* e0224635. https://doi.org/10.1371/journal. pone.0224635

Obata, Y., Senba, Y. & Koshika, M. (1963). Detection of phenolic compounds by chromatography in beet sugar molasses. *Agricultural and Biological Chemistry*, *27 (5)*, 340-341. https://doi.org/10.1080/00021369.1963.10858110

Filipčev, B., Lević, L., Bodroža-Solarov, M., Mišljenović, N. & Koprivica, G. (2010). Quality characteristics and antioxidant properties of breads supplemented with sugar beet molasses-based

ingredients. *International Journal of Food Properties, 13 (5),* 1035-1053. https://doi.org/10.1080/ 10942910902950526

Chen, M., Zhao, Z., Meng, H., Yu, S. & Technology. (2017). The antibiotic activity and mechanisms of sugar beet (*Beta vulgaris*) molasses polyphenols against selected food-borne pathogens. *Food Science and Biotechnology, 82,* 354-360. https://doi.org/10.1016/j.lwt.2017.04.063

Arjeh, E., Khodaei, S. M., Barzegar, M., Pirsa, S., Karimi Sani, I., Rahati, S., et al. (2022). Phenolic compounds of sugar beet (*Beta vulgaris* L.): Separation method, chemical characterization, and biological properties. *Food Science and Biotechnology, 10 (12),* 4238-4246. https://doi.org/10.1002/fsn3.3017

Sharma, A., Anurag, Kaur, J., Kesharwani, A. & Parihar, V. K. (2024). Antimicrobial potential of polyphenols: An update on alternative for combating antimicrobial resistance. *Medicinal chemistry,* 20 (6), 576-596. https://doi.org/10.2174/ 0115734064277579240328142639

Chirife, J., Herszage, L. n., Joseph, A. & Kohn, E. S. (1983). In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. *Antimicrobial agents and chemotherapy, 23 (5),* 766-773. https://doi.org/10.1128/aac.23.5.766

Molan, P. C. (1992). The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee world, 73 (1),* 5-28. https://doi.org/ 10.1080/0005772X.1992.11099109

Taguri, T., Tanaka, T. & Kouno, I. (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biological and Pharmaceutical Bulletin, 27 (12),* 1965-1969. https://doi.org/10.1248/bpb.27.1965

Hohmann, S. (2002). Osmotic stress signaling and osmoadaptation in yeasts. *Microbiology and Molecular Biology Reviews, 66 (2),* 300-372. https://doi.org/10.1128/mmbr.66.2.300-372.2002

Ene, I. V., Walker, L. A., Schiavone, M., Lee, K. K., Martin-Yken, H., Dague, E., et al. (2015). Cell wall remodeling enzymes modulate fungal cell wall elasticity and osmotic stress resistance. *mBio, 6 (4),* 10.1128/mbio.00986-15. https://doi.org/10.1128/mbio.00986-15

Gow, N. A., Latge, J.-P. & Munro, C. A. (2017). The fungal cell wall: structure, biosynthesis, and function. *Microbiology Spectrum, 5 (3),* 10.1128/microbiolspec.funk-0035-2016.n https://doi.org/10.1128/ microbiolspec. funk-0035-2016

Jennings, D. & Burke, R. (1990). Compatible solutes–the mycological dimension and their role as physiological buffering agents. *New Phytologist, 116 (2),* 277-283. https://doi.org/10.1111/ j.1469-8137.1990.tb04715.x

Davis, D. J., Burlak, C. & Nicholas, P. (2000). Osmotic pressure of fungal compatible osmolytes. *Mycological Research, 104 (7),* 800-804. https://doi.org/10.1017/ S0953756299002087

Hohmann, S. (2015). An integrated view on a eukaryotic osmoregulation system. *Current genetics, 61 (3),* 373-382. https://link.springer.com /article/10.1007/s00294-015-0475-0

Rolfe, C. & Daryaei, H. (2020). Intrinsic and Extrinsic Factors Affecting Microbial Growth in *Food Systems*. In: Demirci A., Feng H., Krishnamurthy K., editors. *Food Safety Engineering*. Cham: Springer International Publishing. p. 3-24.

© 2026 by the authors. Licensee SCU, Ahvaz, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0 license) (http://creativecommons.org/licenses/by-nc/4.0/.