Antifungal Metabolites as Food Bio-Preservative

Subjects: Food Science & Technology
Contributor: Awdhesh Mishra

Perishable food spoilage caused by fungi is a major cause of discomfort for food producers. Food sensory abnormalities range from aesthetic degeneration to significant aroma, color, or consistency alterations due to this spoilage. Bio-preservation is the use of natural or controlled bacteria or antimicrobials to enhance the quality and safety of food. It has the ability to harmonize and rationalize the required safety requirements with conventional preservation methods and food production safety and quality demands. Even though synthetic preservatives could fix such issues, there is indeed a significant social need for “clean label” foods. As a result, consumers are now seeking foods that are healthier, less processed, and safer. The implementation of antifungal compounds has gotten a lot of attention in recent decades. As a result, the identification and characterization of such antifungal agents has made promising advances.

anti-fungal bio-preservation food spoilage perishable foods shelf life

1. Mode of Action for Various Metabolites

1.1. Citric Acid and Phenyllactic Acid

Among the many organic acids (which are generally weak acids), citric acid shows as high as 80% antifungal properties. Citric acid can be biosynthesized using fungal fermentation, either liquid surface fermentation or submerged fermentation. The ability of citric acid to inhibit mycelial growth proves its efficacy as an antifungal agent (**Figure 1**) [1]. Because of their solubility, flavor-enhancing qualities, and low toxicity, organic acids are commonly utilized as antibacterial or acidulant preservatives in the food industry. Sorbic acid and its sodium, potassium, and calcium salts are widely used as powerful antifungal and antibacterial agents, extending the shelf life of food goods.

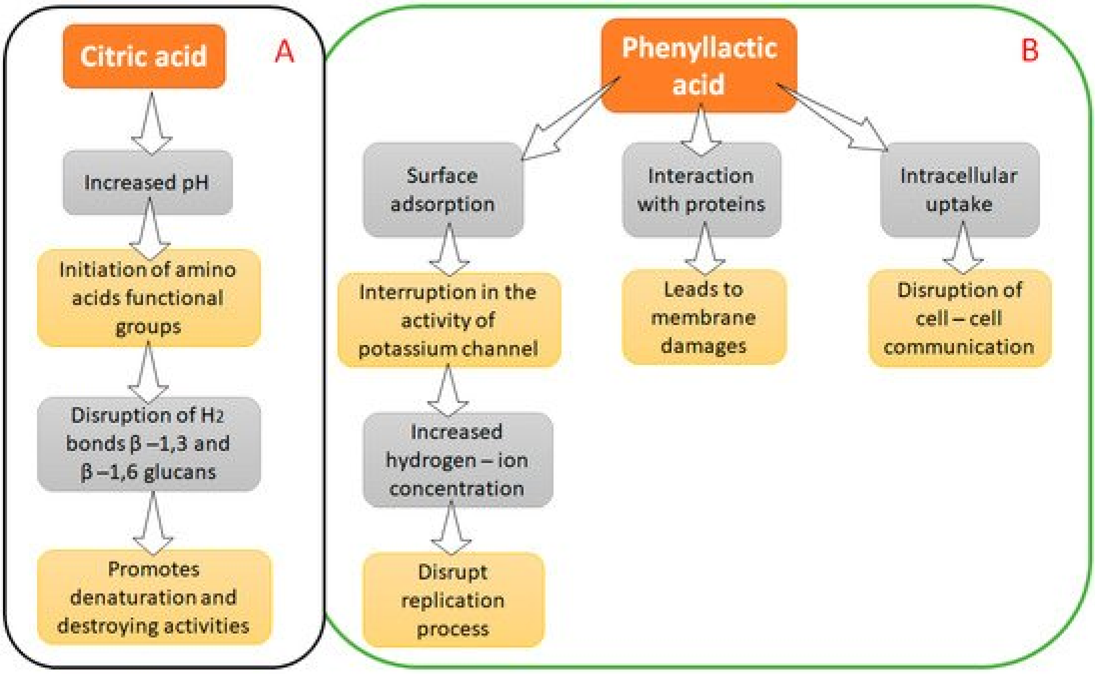

Figure 1. Mechanism of organic acids ((**A**) for Citric acid; (**B**) Phenyllactic acid) as bio-preservatives.

Organic acids producing bacteria comprise the larger class of LAB (lactic acid bacteria), which have been used in the food industry for a long time. Organic acids are extensively synthesized from lactic acid bacterial species such as, *Pediococcus acidilactici* which can be cultivated in labs or even found in traditional Chinese medicines [2]. The whole class of LAB shows a wide range of mechanisms depending upon the species used as an antifungal agent. This broad spectrum includes increased oxidative stress and cell permeability, enzyme inhibition, proton gradient interference, etc. [3][4]. Phenyllactic acids (PLA) (also called 3-Phenyl lactic acid or 2-Hydroxy-3-Phenylpropionic acid) inhibited *Penicillium roqueforti*, *Aspergillus ochraceus*, *Fusarium graminearum*, *Penicillium expansum*, *Aspergillus niger*, *Monilia sitophila*, *Aspergillus flavus*, *Penicillium verrucosum*, *Penicillium citrinum*, and other fungi [5]. In a study, PLA had a minimum inhibitory concentration (MIC) of 6.5–12.0 mg/mL against fungus [6]. The mechanism of the antifungal activity of PLA is poorly understood. Various researchers have suggested that PLA interferes with the proton gradient and inhibits cellular enzymes, often coactively working with other metabolites [7]. PLA's antifungal activities are thought to be inhibiting fungal radial growth and sporulation. PLA also inhibited the development and sporulation of fungal radicals on malt extract agar [8].

1.2. Essential Oils and Phytochemicals

Essential oils are the substances released by plants as a defense mechanism against extraneous factors. They can be easily extracted from various parts of plants such as flowers, stems, roots, leaves, etc. They have also been used as perfumery agents for centuries. Though the number of EOs produced by plants is relatively high, it would be a sophisticated process to characterize every EO, synthesis, and mechanism. Therefore, a few of them have been summarized in **Figure 2** below [9].

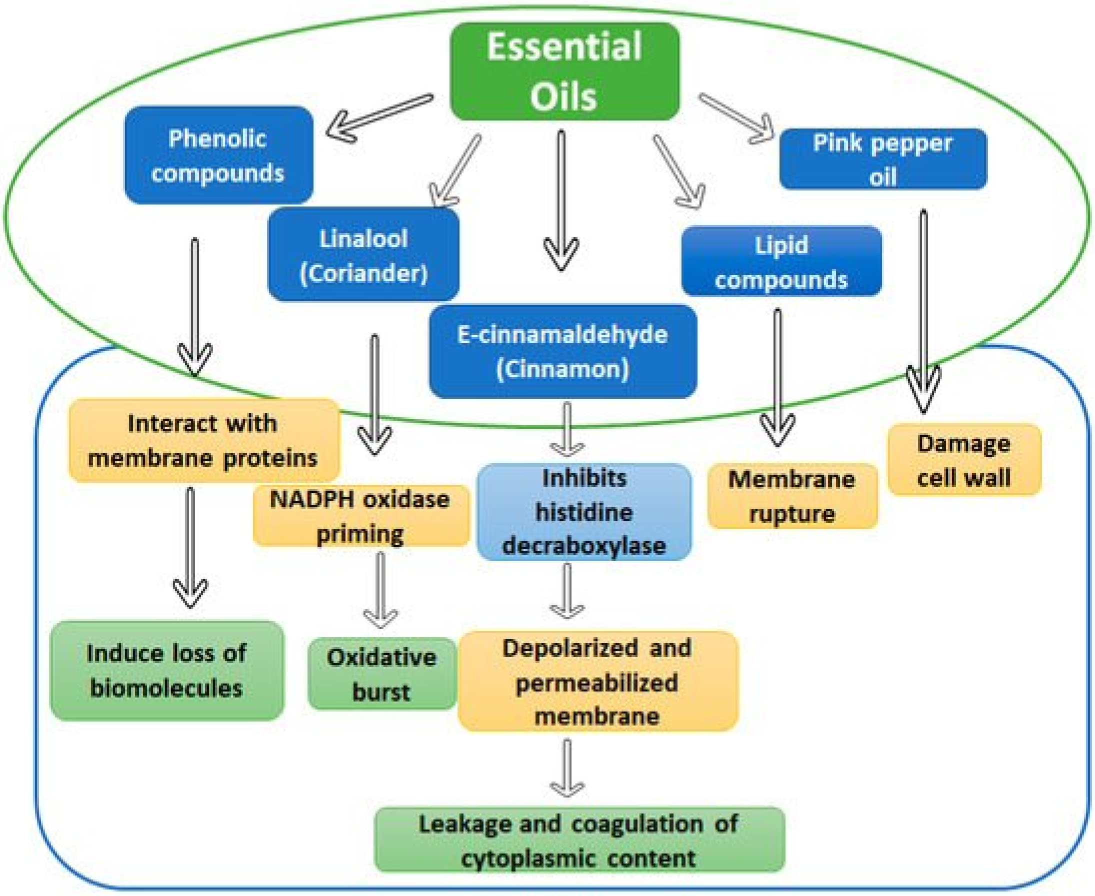

Figure 2. Mechanism of various essential oils as bio-preservatives.

Terpenes are the most diverse category of chemical compounds identified in plant extracts, with significant antifungal action that can be boosted synergistically by the presence of additional phytochemicals (**Figure 3**). Grifolin, a sesquiterpene chemical derived from the fruiting bodies of the fungus *Albatrellus dispansus*, inhibits the mycelial growth of plant pathogenic fungi such as *Sclerotinina sclerotiorum*, as well as spore germination on *Fusarium graminearum*, *Pyricularia oryzae* and *Gloeosporium fructigenum* [10]. Catechins were shown to rupture the fungal membrane by binding to the ergosterol layer and inhibiting the intracellular and extracellular enzymes [11]. On the other hand, Quercetin proves its antifungal activity by decreasing protein motive forces, thereby increasing membrane permeability [12]. Kaemferol works by blocking the QS pathway, which leads to failure of the cell-to-cell communication which ultimately prevents biofilm formation [13].

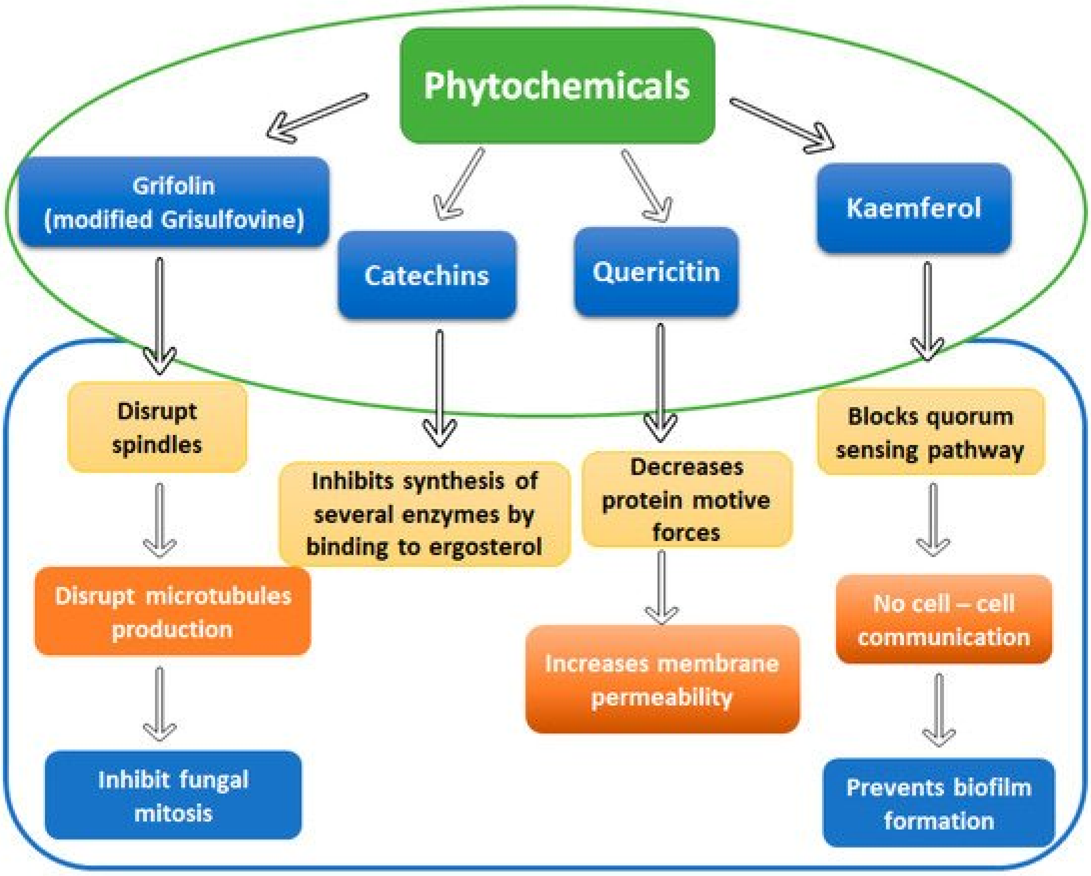

Figure 3. Mechanism of various phytochemicals as bio-preservatives.

1.3. Azoles

Azoles are another class of excellent antifungal agents, which target the fungal cell membrane by acting as competitive inhibitors for CYP51 (a cytochrome P450 enzyme). CYP51 plays a vital role in ergosterol biosynthesis (which is the main component of the fungal cell wall). In addition, the class of azoles includes various sub-components acting as potential antifungal agents that can be categorized based upon their targeting molecules (**Figure 4**) [14].

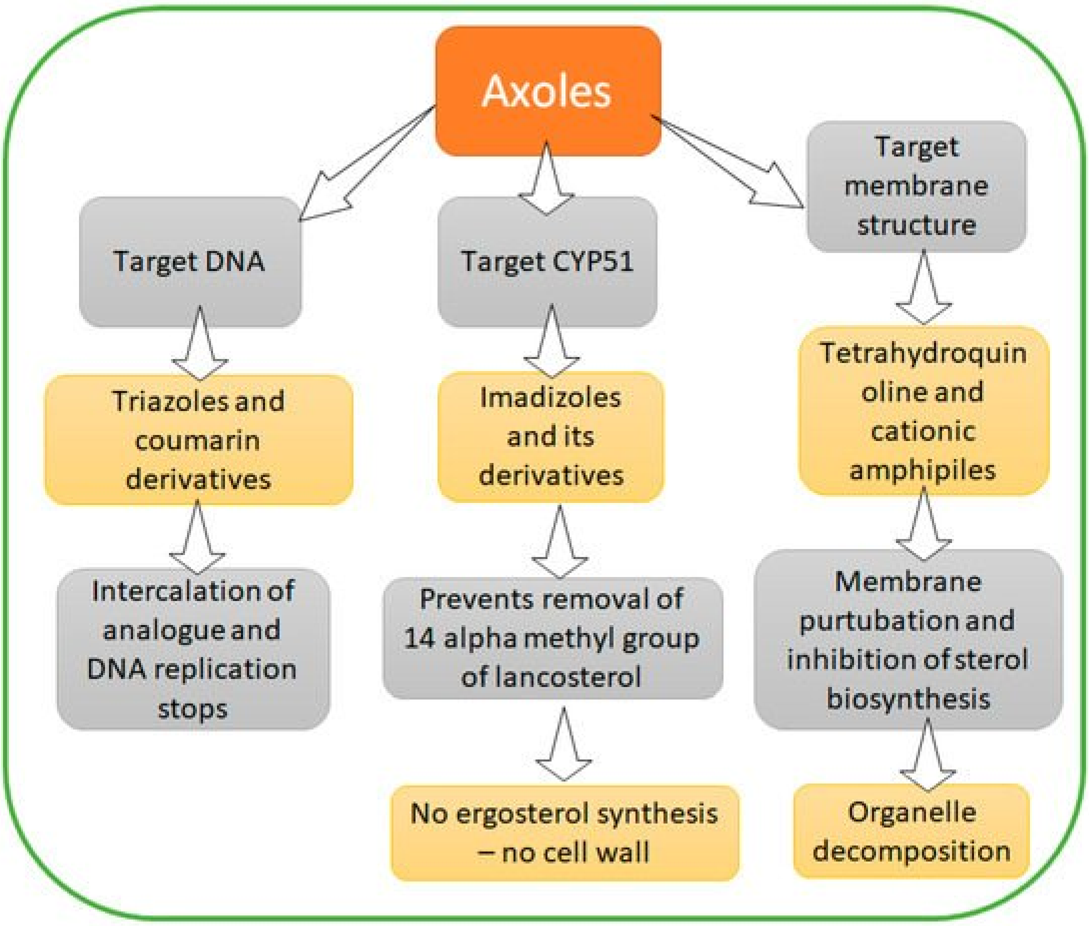

Figure 4. Mechanism of azoles as bio-preservatives.

2. Applications Oriented Studies from Laboratory to Pilot Scale

Conventional suspensions prepared from phytocompounds have antifungal effects. The antifungal range of a nanoemulsion made by ultrasonication using *Cleome viscosa* essential oil and Triton-x-100 was studied. Essential oil nanoemulsion (EONE) was evaluated with foodborne pathogenic *Candida albicans* at a minimum inhibitory and fungicidal dosage. The MIC and MFC values for *C. albicans* isolates ranged from 16.5 to 33 mL/mL, with a considerable reduction in biofilm. Fourier transformed infrared spectroscopy corroborated the shift in compositional fingerprinting, and spectroscopic analysis revealed a drop in chitin levels in cell walls. In *C. albicans* cells, EONE and its biologically active compounds cause massive damage [15].

Several techniques have proven that the primary components of EOs have antioxidant, antibacterial, and antifungal effects. Tea tree oil, lemon oil, cinnamon oil, clove oil, and thyme oil, among other EOs from local plants, have positively influenced antibacterial and antioxidant activity, along with expanded cereal shelf lives and enhanced food security. In addition, terpenes and volatile aromatic chemicals, for example, are important EO categories that help food hygiene without affecting quality. For example, EOs might be utilized as an additional preservative to extend the shelf life of grains and cereals because of their numerous effects, including antioxidant and antibacterial properties [16].

The antifungal and anti-aflatoxigenic activities of 5′-hydroxy-aurapten (5′-HA) on *A. flavus* isolated from nuts (*Lotus lalambensis*) were investigated. In this study, 5′-HA demonstrated a higher antifungal potential against *A. flavus,* having a minimum inhibitory concentration of 62.5 mg/L. It was found that 5′-HA had reduced conidia germination for *A. flavus* by 60% at a dose of 40 mg/mL in the early (A, B, C), middle (L, M, N), and late (P, Q, W) stages of the aflatoxin biosynthesis pathway. Moreover, 5′-HA also inhibited the synthesis of aflatoxins, AFB1 and AFB2, by 50% and 23.3%, respectively. 5′-HA increased the efficacy of enzymatic antioxidants CAT (Catalase) and SOD (Superoxide dismutase) by 56.25% and 66.66%, respectively. The anti-aflatoxigenic mechanism of 5′-HA is thought to work by increasing the expression profile of the transcription factors *atfA* and *atfB* by 2- and 2.5-fold, respectively [17]. Sodium lignosulfonate was found to be an antifungal compound due to its fungistatic activity against *M. circinelloides*, *A. amoenus*, and *P. solitum.* These strains were obtained from spoiled alfalfa hay (*Medicago sativa*). Sodium lignosulfonate (NaL) had superior preservation properties for the ground high-moisture hay as a substrate [18]. In comparison to spoiled hay, sodium lignosulfonate and PRP had a protective effect against hay proteolysis at a concentration of 0.5%, as assessed by a decrease in ammoniacal nitrogen (NH_3-N). Preservatives can prevent plant proteins from deteriorating, retaining their biological worth, according to these studies.

Natamycin is an antifungal medicine with poor solubility that is used in food products to address the base of cheese and sausages. This use does not risk the customer’s safety. For beverage preservation, a highly soluble natamycin–cyclodextrin integral membrane was created. This approach results in high drug concentrations that are dangerously above the acceptable limit. In addition to assessing an adequate daily natamycin food intake, researchers must investigate natamycin’s impact on the intestinal bacteria as a reservoir for tolerance, which results from the amount of feces in one’s system to be abnormally high. Foods having natamycin, introduced and blended uniformly, such as yoghurt, and even the administration of cyclodextrin intercalation to drinks and wine, all contribute to natamycin levels and fecal *Candida* spp. drug exposure. *Candida* spp. have established natamycin tolerance in the bowels of persons who have been treated with natamycin for fungal diseases. As a consequence, it is impossible to figure out the likelihood that using natamycin to keep yoghurt and beverages promotes *Candida* spp. polyene tolerance [19].

The bioactivity of *Lactobacillus brevis* AM7 during fermentation with bread hydrolysate was evaluated against the fungus (20% to 70%). Using Liquid Chromatography, nine antifungal compounds (with 10–17 amino acid residues and masses spanning 1083.6 to 1980.7 Da) were investigated, all of which were expressed in wheat protein sequences. Bread hydrolysate fermented by *L. brevis* AM7, non-fermented bread hydrolysate, and a slurry composed of water and bread combination were all used to make bread and compared with conventional wheat bread. Compared to the other pieces of bread, those fermenting hydrolysate (18 and 22% of the dough weight) had the maximum mold-free shelf life, extending up to 10 days until mold appeared. Moreover, the fermentation hydrolysate had the fewest adverse influences on bread texture, demonstrating biotechnology’s beneficial impact and potential [20]. The essential oil of *Thymus algeriensis* was studied as a possible soft cheese preservative. We devised a novel method for determining the essential oil’s ability to preserve soft cheese. During 30 days of storage at 4 °C with 25 L of essential oil introduced, there was no contamination of *Penicillium aurantiogriseum*. Minimum inhibitory concentrations for antifungals varied from 0.01 to 0.04 mg/mL range. According to the data, the oil was active with a half-maximal inhibitory activity of 0.132 mg/mL. The volatile components in the oil were determined by using gas chromatography, gas chromatography-mass spectrometry, and nuclear magnetic resonance spectrometry. The most frequent constituent in the oil was discovered to be carvacrol, which made up 80.9% of the overall amount, followed by p-cymene (7.7%) [21].

Both people and the environment are put at risk by chemical preservatives and fungicides. Bio-preservatives, such as lactic acid bacteria (LAB), on the other hand, are efficient, secure, and biodegradable, as well as add adequate beneficial health effects. The antifungal activity of strain RM1 was the highest amongst 23 rod-shaped LAB isolates collected from Egyptian traditionally fermented milk (Rayeb). Strain RM1 was distinguished from genetically similar Lactobacillus species by 16S rRNA phylogenetic analysis and distinctive phenotypic traits, indicating that it is a distinct species whereby the name *Lactobacillus* sp. RM1 is suggested. *Lactobacillus* sp. RM1 cell-free supernatant (CFS) has considerable and broad antifungal effects, mostly against toxigenic fungi and pathogenic bacteria.

Lactobacillus spp. RM1 has antifungal capabilities and the ability to prolong the shelf life of wheat grains, implying that it could be used as a natural food preservative [22]. Antimicrobial substances generated or expelled by LAB can counteract foodborne illnesses, making it a possible alternative to artificial preservatives [23]. Natural preservatives such as LAB are effective, safe, and biodegradable, with added health advantages. LAB is also frequently used as a bio-preservative to increase the shelf life of food products while in storage [24][25]. Organic acids, short-chain fatty acids, hydrogen peroxide, reuterin, diacetyl, bacteriocins, and bacteriocin-like inhibitory compounds are some of the antifungal substances produced by LAB [26][22][27], *Lactococcus lactis* spp. *lactis* ATCC 19435 inhibits fungal growth and ochratoxin A synthesis in fungal growth conditions [28][29]. Antifungal compounds found in LAB have been proven effective in decreasing yeasts and molds that degrade food [30]. Therefore, to eliminate toxic fungus and increase the quality, safety, and shelf life of food and agricultural products, it is critical to look for natural, food-grade antifungal chemicals from LAB.

References

1. Li, J.; Wang, W.; Xu, S.X.; Magarvey, N.A.; McCormick, J.K. Lactobacillus reuteri-produced cyclic dipeptides quench agr-mediated expression of toxic shock syndrome toxin-1 in staphylococci. Proc. Natl. Acad. Sci. USA 2011, 108, 3360–3365.
2. Engelhardt, T.; Albano, H.; Kiskó, G.; Mohácsi-Farkas, C.; Teixeira, P. Antilisterial activity of bacteriocinogenic Pediococcus acidilactici HA6111-2 and Lactobacillus plantarum ESB 202 grown under pH and osmotic stress conditions. Food Microbiol. 2015, 48, 109–115.
3. Rhoads, D.M.; Umbach, A.L.; Subbaiah, C.C.; Siedow, J.N. Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol. 2006, 141,

357–366.

4. Desbois, A.P.; Smith, V.J. Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. Appl. Microbiol. Biotechnol. 2010, 85, 1629–1642.
5. Rajanikar, R.V.; Nataraj, B.H.; Naithani, H.; Ali, S.A.; Panjagari, N.R.; Behare, P. V Phenyllactic Acid: A green compound for food biopreservation. Food Control 2021, 128, 108184.
6. Prema, P.; Smila, D.; Palavesam, A.; Immanuel, G. Production and characterization of an antifungal compound (3-phenyllactic acid) produced by Lactobacillus plantarum strain. Food Bioprocess Technol. 2010, 3, 379–386.
7. Kadyan, S.; Pradhan, D. Antifungal Lactic Acid Bacteria (LAB): Potential use in food systems. In Novel Strategies to Improve Shelf-Life and Quality of Foods; Mishra, S.K., Goyal, M.R., Eds.; Apple Academic Press: New York, NY, USA, 2020; pp. 73–94. ISBN 100301027X.
8. Svanström, Å.; Boveri, S.; Boström, E.; Melin, P. The lactic acid bacteria metabolite phenyllactic acid inhibits both radial growth and sporulation of filamentous fungi. BMC Res. Notes 2013, 6, 464.
9. Singh, O.; Khanam, Z.; Misra, N.; Srivastava, M.K. Chamomile (Matricaria chamomilla L.): An overview. Pharmacogn. Rev. 2011, 5, 82.
10. Liu, L.-Y.; Li, Z.-H.; Ding, Z.-H.; Dong, Z.-J.; Li, G.-T.; Li, Y.; Liu, J.-K. Meroterpenoid pigments from the basidiomycete Albatrellus ovinus. J. Nat. Prod. 2013, 76, 79–84.
11. Gupta, P.; Gupta, S.; Sharma, M.; Kumar, N.; Pruthi, V.; Poluri, K.M. Effectiveness of phytoactive molecules on transcriptional expression, biofilm matrix, and cell wall components of Candida glabrata and its clinical isolates. ACS Omega 2018, 3, 12201–12214.
12. Zillich, O.V.; Schweiggert-Weisz, U.; Eisner, P.; Kerscher, M. Polyphenols as active ingredients for cosmetic products. Int. J. Cosmet. Sci. 2015, 37, 455–464.
13. Ivanova, A.; Ivanova, K.; Tzanov, T. Inhibition of quorum-sensing: A new paradigm in controlling bacterial virulence and biofilm formation. In Biotechnological Applications of Quorum Sensing Inhibitors; Kalia, V.C., Ed.; Springer: Singapore, 2018; pp. 3–21.
14. Tjia, J.A. Journey into C. albicans Biofilms: Proteomic and Functional Genomic Approaches to Uncovering Mechanisms of Adherence; University of Toronto: Toronto, ON, Canada, 2016.
15. Krishnamoorthy, R.; Gassem, M.A.; Athinarayanan, J.; Periyasamy, V.S.; Prasad, S.; Alshatwi, A.A. Antifungal activity of nanoemulsion from Cleome viscosa essential oil against foodborne pathogenic Candida albicans. Saudi J. Biol. Sci. 2021, 28, 286–293.
16. Bhavaniramya, S.; Vishnupriya, S.; Al-Aboody, M.S.; Vijayakumar, R.; Baskaran, D. Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain Oil Sci. Technol. 2019, 2, 49–55.

17. Ali, E.M.; Alkuwayti, M.A.; Aldayel, M.F.; Abdallah, B.M. Coumarin derivative, 5′-hydroxy-auraptene, extracted from Lotus lalambensis, displays antifungal and anti-aflatoxigenic activities against Aspergillus flavus. J. King Saud Univ.-Sci. 2021, 33, 101216.

18. Reyes, D.C.; Annis, S.L.; Rivera, S.A.; Leon-Tinoco, A.Y.; Wu, C.; Perkins, L.B.; Perry, J.J.; Ma, Z.X.; Knight, C.W.; Castillo, M.S.; et al. In vitro screening of technical lignins to determine their potential as hay preservatives. J. Dairy Sci. 2020, 103, 6114–6134.

19. Dalhoff, A.A.H.; Levy, S.B. Does use of the polyene natamycin as a food preservative jeopardise the clinical efficacy of amphotericin B? A word of concern. Int. J. Antimicrob. Agents 2015, 45, 564–567.

20. Nionelli, L.; Wang, Y.; Pontonio, E.; Immonen, M.; Rizzello, C.G.; Maina, H.N.; Katina, K.; Coda, R. Antifungal effect of bioprocessed surplus bread as ingredient for bread-making: Identification of active compounds and impact on shelf-life. Food Control 2020, 118, 107437.

21. Bukvicki, D.; Giweli, A.; Stojkovic, D.; Vujisic, L.; Tesevic, V.; Nikolic, M.; Sokovic, M.; Marin, P.D. Cheese supplemented with Thymus algeriensis oil, a potential natural food preservative. J. Dairy Sci. 2018, 101, 3859–3865.

22. Shehata, M.G.; Badr, A.N.; El Sohaimy, S.A.; Asker, D.; Awad, T.S. Characterization of antifungal metabolites produced by novel lactic acid bacterium and their potential application as food biopreservatives. Ann. Agric. Sci. 2019, 64, 71–78.

23. Nebbia, S.; Lamberti, C.; Lo Bianco, G.; Cirrincione, S.; Laroute, V.; Cocaign-Bousquet, M.; Cavallarin, L.; Giuffrida, M.G.; Pessione, E. Antimicrobial potential of food Lactic Acid Bacteria: Bioactive peptide decrypting from caseins and bacteriocin production. Microorganisms 2021, 9, 65.

24. Shehata, M.G.; Badr, A.N.; El Sohaimy, S.A. Novel antifungal bacteriocin from Lactobacillus paracasei KC39 with anti-mycotoxigenic properties. Biosci. Res. 2018, 15, 4171–4183.

25. Ahmad Rather, I.; Seo, B.J.; Rejish Kumar, V.J.; Choi, U.; Choi, K.; Lim, J.H.; Park, Y. Isolation and characterization of a proteinaceous antifungal compound from Lactobacillus plantarum YML 007 and its application as a food preservative. Lett. Appl. Microbiol. 2013, 57, 69–76.

26. Yang, E.J.; Chang, H.C. Purification of a new antifungal compound produced by Lactobacillus plantarum AF1 isolated from kimchi. Int. J. Food Microbiol. 2010, 139, 56–63.

27. Ryu, E.H.; Yang, E.J.; Woo, E.R.; Chang, H.C. Purification and characterization of antifungal compounds from Lactobacillus plantarum HD1 isolated from kimchi. Food Microbiol. 2014, 41, 19–26.

28. Badr, A.N.; Abdel-Fatah, S.M.; Sree, Y.H.A.; Amra, H.A. Mycotoxigenic fungi and mycotoxins in Egyptian barley under climate changes. Res. J. Environ. Toxicol. 2017, 11, 1–10.

29. Badr, A.N.; Nada, F.; Shehata, M.G.; Amra, H.A. Anti-mycotic and anti-mycotoxigenic properties of Egyptian dill. J. Appl. Sci. 2017, 17, 184–195.

30. Schnürer, J.; Magnusson, J. Antifungal lactic acid bacteria as biopreservatives. Trends Food Sci. Technol. 2005, 16, 70–78.