

PRESERVATION OF MICROBIAL SPOILAGE OF FOOD BY BIOSURFACTANT-BASED COATING

VIKRANT SHARMA¹, MUSKAAN GARG¹, DEVISMITA TALUKDAR¹, PALLAVI THAKUR¹, MARIUS HENKEL²,
 DEEPANSH SHARMA^{3*}, GAURAV KUMAR^{1*}

¹Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India.

²Institute of Food Science and Biotechnology, Hohenheim University, Stuttgart, Germany. ³Amity Institute of Microbial Technology, Amity University, Jaipur, Rajasthan, India. Email: deepanshsharma@gmail.com/gauravkr@yahoo.com

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ABSTRACT

Objectives: Microbial spoilage of food is one of the leading causes of food scarcity worldwide, which could have devastating effect on the socioeconomic of any country. Along with classical food preservation methods, various innovative approaches can significantly increase the food safety. Biosurfactants are the amphiphilic microbial metabolites, while many of them have potential antimicrobial properties and therefore can be used for food preservation.

Methods: During this study, three biosurfactants obtained from *Bacillus subtilis* (two of them) and *Pseudomonas* sp. (rhamnolipid) were screened for their antifungal activity against *Aspergillus oryzae* (MTCC 1846), *Fusarium solani* (MTCC 350), and *Curvularia* sp. by various *in vitro* and *in vivo* methods.

Results: During this study, among three surfactants only *Pseudomonas* sp. biosurfactant (rhamnolipid) exhibited significant antifungal activity against *A. oryzae*, *F. solani*, and *Curvularia* sp. Further, the rhamnolipid coating (1 mg/ml) on lemon, potato and tomato protected them from fungal spoilage up to 15 days at room temperature in contrast to untreated samples which started spoiling in 6–7 days.

Discussion: Above findings emphasis on the potential use of biosurfactants for the preservation of food items, however, a detailed study to ensure the safety of biosurfactant is of prerequisite.

Keywords: Biosurfactant, Food spoilage, Rhamnolipid, Amphipathic, Food preservation.

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INTRODUCTION

Microbial spoilage of crops and stored food can be disastrous to the socioeconomic of a nation and fungus is one of the most prominent reasons of food scarcity globally. Controlling the fungal rot is a difficult task because once it is initiated, then it is difficult to contain. Around 20%–30% of the total fruit and vegetables produced each year gets lost because of food spoilage [1,2]. Extended use of chemical to control the diseases had led to the devastating effect on the environmental balance of microbes in the soil, resulting in the development of resistant strains, groundwater contamination, and risk to human health. The biggest ecological challenge for microbiologists is the development of environmental-friendly substitute for the currently used chemical pesticides for the food preservation. According to many researchers, biological control of the plant pathogens is becoming a trend due to increased pollution concerns because of extensive use of the pesticides, food preservation, and development of the resistant pathogenic strains [3]. Environment-friendly microorganisms are used and have proved to promote plant growth and disease control in modern agriculture. Microorganisms are known to suppress the soil-borne plant pathogens through the secondary metabolites including antibiotics. Several studies reported that a variety of microorganisms produces a wide range of surface-active compounds (SAC), known as biosurfactant with fungicidal properties for various fungal species [4].

Microbial surface-active compounds are diverse molecules produced by different microorganism and are classified on the bases of their chemical structure and microbial origin [5]. The major classes of SAC are surfactin, fengycin, iturin, lichenysin, and rhamnolipid compounds with potent antimicrobial, antiadherent, anti-inflammatory, anticancer, antifebrin clot formation, antiviral, antimycoplasma, and

hypcholesterolemic activities for a large spectrum of application in medical and pharmaceutical field [6]. They also have application in the field of agriculture as biocontrol, bioremediation, and metal sequestering, also in food processing industries of their emulsifying, foaming, and dispersing properties [7,8]. One of the widely studied biosurfactants over the years has been the rhamnolipids [9] and has also reported to show antimicrobial and antifungal properties [10]. One other study reported the application of rhamnolipid biosurfactant against *Fusarium sacchari* [11]. However, the use of biosurfactant as antifungal agent against several other fungal species has not reported. Therefore, the present study was carried out with the objective to assess the efficacy of biosurfactant as potential fungicidal agent against *Aspergillus oryzae*, *Curvularia* sp., and *Fusarium solani*.

METHODS

Chemicals

For the *in vitro* study of the antifungal efficiency (sodium dodecyl sulfate) was used as positive control and procured from Loba Chemie, India. Fungal culture mediums including potato dextrose broth (PDB) and potato dextrose agar (PDA) were procured from Hi-Media Laboratories Pvt., Ltd., India. The biosurfactant isolated in earlier study (data unpublished) from *Bacillus subtilis* (two of them) and *Pseudomonas* sp. (rhamnolipids) was used for carrying out the studies.

Phytopathogenic fungal strains

The phytopathogens *A. oryzae* (MTCC 1846) and *F. solani* (MTCC 350) were procured from microbial type culture collection and gene bank (MTCC), Chandigarh. The obtained cultures were revived in the PDB, and then after growth came in PDB, the cultures were transferred on the PDA. *Curvularia* sp. were isolated from the spoiled chili on the PDA

plate and purified by repeated subculturing. The identification of the isolated fungi was performed based on its culture characteristics and microscopic structure [12]. The fungus was maintained in PDA and glycerol stocks for further use.

In vitro antifungal activity of the biosurfactant

The targeted fungal samples including *A. oryzae* (MTCC 1846), *Curvularia* sp., and *F. solani* (MTCC 350) were treated with three biosurfactants including *B. subtilis* 10T, *B. subtilis* 3285, and *Pseudomonas* sp. rhamnolipid. The experiment was carried out in the 2 ml sterilized centrifuge tube, where 500 µl of biosurfactant solution (1 mg/ml in distilled water) was directly inoculated with the mycelium and spores of targeted fungi. Further, the setup was incubated at 28°C for 24 h and the 100 µl of the mixture was transferred to the PDA plate from each treatment. The plates were incubated at 28°C for 72 h and were observed for the presence of fungal growth.

In vivo antifungal activity of the biosurfactant

Vegetable host selection

Fungicidal activity of the biosurfactant was checked against specific fungi on their suitable host vegetable. During the study, lemon was used for *A. oryzae* (MTCC 1846), tomato was used for *Curvularia* sp., while potato was used for *F. solani* (MTCC 350). The selected vegetables were free from wounds and rots, also the selection was based on the homogenous maturity and size.

Antifungal activity of the biosurfactant

Mature tomato and potato tubers were selected carefully on the absence of any disease or wound and also on the basis their size. The fruits and vegetables were surface disinfected by dipping in sodium hypochlorite 2% for 10 min followed by rinsing 3 times by sterilized distilled water to eliminate the saprophyte pathogen present on the surface and residual sodium hypochlorite. Then, they were surface dried under the filter-sterilized air flow. Further, one lemon was coated with the biosurfactant solution (1 mg/ml in water) while the other one was kept uncoated. Both lemons were then stabbed with sterilized wooden stick (Diameter 1.5 mm) and the area was sprinkled with spores of *A. oryzae* (MTCC 1846) and incubated at 28°C for 15 days. After the incubation, the surface of the lemons was observed for any abnormalities and fungal growth. Later, these lemons were sliced in two equal half and observed for the spread of fungal infection. Similar setup was made to study the antifungal activity of biosurfactant toward *F. solani* (MTCC 350) and *Curvularia* sp., while potato and tomato were used, respectively, as host material.

In another setup, biosurfactants coated vegetables (1 mg/ml in water) were sprinkled with fungal spores without stabbing the surface of vegetables. Above setup was also incubated for 15 days and observed for any abnormalities and fungal growth on the surface. Later, these vegetables were sliced in two equal half and observed for the spread of fungal infection.

RESULTS

In vitro antifungal activity of biosurfactant

The antifungal assay of the biosurfactant was done through challenge method and was checked against *F. solani* (MTCC 350), *Curvularia* sp., and *A. oryzae* (MTCC 1846). The targeted fungal mycelium and the spores were treated directly with the biosurfactant solution (1 mg/ml) for 24 h and later inoculated on PDA plates. The assay was carried out using three biosurfactants including *B. subtilis* (two varieties) and *Pseudomonas* sp. rhamnolipid. Among three biosurfactants, only rhamnolipid exhibited antifungal activity and completely inhibited the growth of all three test fungi on PDA plate. Results of the same are summarized in Fig. 1. Since rhamnolipid showed the desirable activity, it was selected for the further studies.

In vivo antifungal activity on stabbed vegetables

The *in vivo* analysis of antifungal potential of the biosurfactant was checked by infecting the fresh vegetables with their respective phytopathogen. The study was carried out on lemon, tomato, and potato where lemon was infected by *A. oryzae* (MTCC 1846), tomato was infected by *Curvularia* sp., and potato was infected by *F. solani* (MTCC 350). The vegetables were stabbed before dusting the fungal spores on them. The experiment was carried out in normal room condition where one fruit sample was surface coated uniformly with the rhamnolipid solution of concentration 1 mg/ml, while the other was kept uncoated. The observations were made after 15 days, and it was observed that the sample which was coated with the biosurfactant solution shown no sign of visible spoilage on surface. The transverse section of the samples was also observed; internally, the coated fruit sample was healthy while the uncoated one was spoiled internally (Figs. 2-4).

In vivo antifungal activity on unstabbed vegetables

The study was carried out on lemon, tomato, and potato where tomato was infected by *Curvularia* sp., and potato was infected by *F. solani* (MTCC 350). The vegetables were not stabbed before dusting the fungal spores on them. The experiment was carried out in normal room condition where one fruit sample was surface-coated uniformly with the rhamnolipid solution of concentration 1 mg/ml, while the other was kept uncoated. The observations were made after 15 days, and it

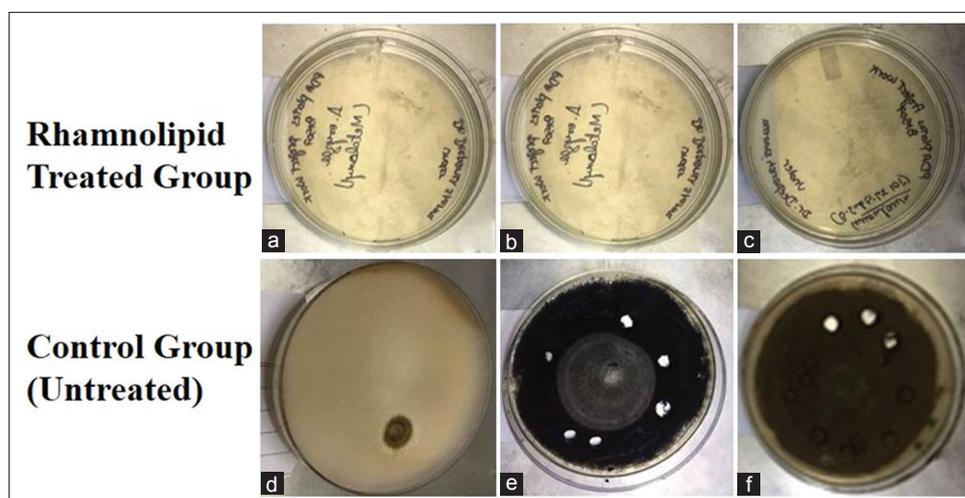


Fig. 1: *In vitro* antifungal activity of *Pseudomonas* sp. rhamnolipid. Here, (a-c) are rhamnolipid-treated plates of *Fusarium solani* (MTCC 350), *Curvularia* sp., and *Aspergillus oryzae* (MTCC 1846), respectively; (d-f) are untreated plates of *F. solani* (MTCC 350), *Curvularia* sp., and *A. oryzae* (MTCC 1846), respectively

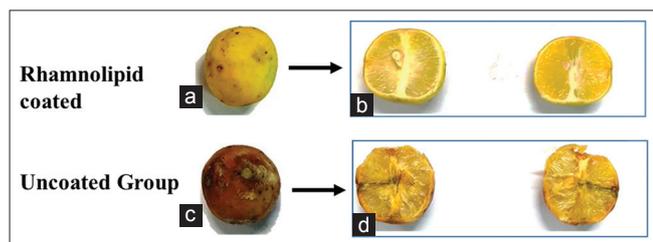


Fig. 2: *In vivo* antifungal activity of rhamnolipid by surface coating of lemon against *Aspergillus oryzae* (MTCC 1846) after 15 days. Here, (a) is the rhamnolipid-coated lemon after 15 days; (b) is showing the interior of the rhamnolipid-coated lemon after 15 days; (c) is the uncoated lemon after 15 days; (d) is showing the interior of the uncoated lemon after 15 days

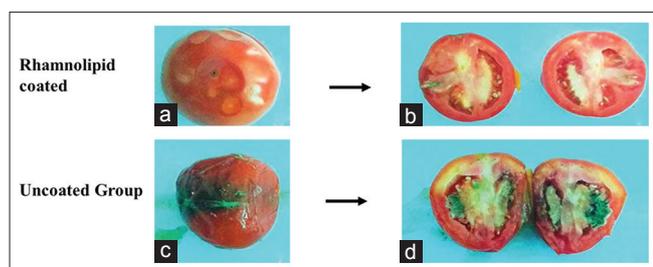


Fig. 3: *In vivo* antifungal activity of rhamnolipid by surface coating of tomato against *Curvularia* sp. after 15 days. Here, (a) is the rhamnolipid-coated tomato after 15 days; (b) is showing the interior of the rhamnolipid-coated tomato after 15 days; (c) is the uncoated tomato after 15 days; (d) is showing the interior of the uncoated tomato after 15 days

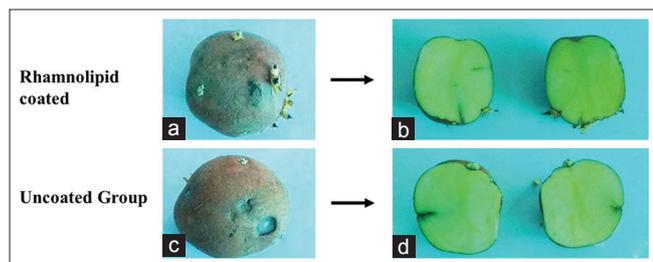


Fig. 4: *In vivo* antifungal activity of rhamnolipid by surface coating of potato against *Fusarium solani* (MTCC 350) after 15 days. Here, (a) is the rhamnolipid-coated potato after 15 days; (b) is showing the interior of the rhamnolipid-coated potato after 15 days; (c) is the uncoated potato after 15 days; (d) is showing the interior of the uncoated potato after 15 days

was observed that the sample which was coated with the biosurfactant solution shown no sign of visible spoilage on surface. The transverse section of the samples was also observed; internally, the coated fruit sample was healthy while the uncoated one was spoiled internally (Figs. 5 and 6).

It was also observed that the life span of the fruit sample is also increased, lemon and tomato get easily spoiled within 4–5 days when kept in normal conditions but after the application of the biosurfactant solution, the life span increased to 15 days.

DISCUSSION

Food spoilage by microorganisms is one of the most prominent regions being the shortage of food worldwide. Studies say that by 2050, we will be requiring an additional of 70% food and it will continue to upsurge due to increase in population. Currently, a large number of

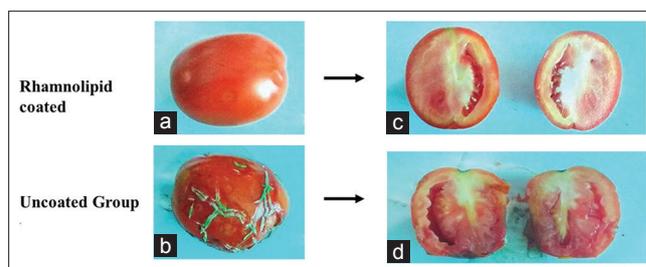


Fig. 5: *In vivo* antifungal activity of rhamnolipid by surface coating of tomato against *Curvularia* sp. after 15 days. Here, (a) is the rhamnolipid-coated tomato after 15 days; (b) is showing the interior of the rhamnolipid-coated tomato after 15 days; (c) is the uncoated tomato after 15 days; (d) is showing the interior of the uncoated tomato after 15 days

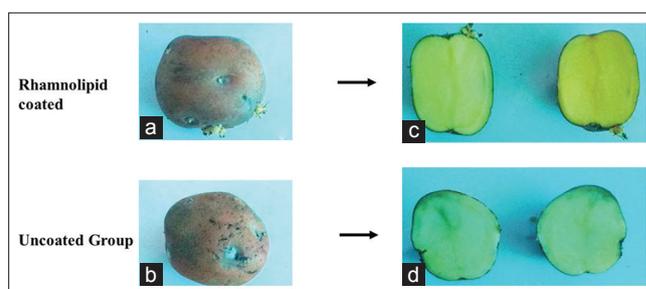


Fig. 6: *In vivo* antifungal activity of rhamnolipid by surface coating of potato against *Fusarium solani* (MTCC 350) after 15 days. Here, (a) is the rhamnolipid-coated potato after 15 days; (b) is showing the interior of the rhamnolipid-coated potato after 15 days; (c) is the uncoated potato after 15 days; (d) is showing the interior of the uncoated potato after 15 days

methods are being followed by agriculture practitioners to reduce the pre-harvesting and post-harvesting losses; however, their side effects, energy requirements, non-eco-friendly nature, and overall feasibility left the researcher to look for alternative methods.

Biosurfactants are amphiphilic molecules with some very useful properties which possess the great potential to be used in industries. In recent past, biosurfactants have been widely reported to exhibit significant antimicrobial activity against a variety of bacteria and fungi [13,14]. These reports greatly emphasize on the possible application of these biosurfactants in the development of various antimicrobial preparations. In past, biosurfactants have been reported to inhibit the growth of a variety of fungal species in various *in vitro* and vegetable-based *in vivo* models, thus suggesting its possible use in controlling the fungal spoilage in fruits and vegetables [6,7]. Therefore, this study was targeted to screen the bacterial biosurfactants for their potential to control fungal spoilage of vegetables. During this study, bacterial rhamnolipid biosurfactant exhibited significant antifungal activity in *in vitro* methods and its thin coating protected the vegetables from fungal spoilage for 15 days, thus increasing the life span of the vegetables significantly.

Similar kind of observations has been reported by other researchers where *B. subtilis* SPB1 lipopeptides biosurfactant controlled the dry rot potato tubers caused by *F. solani* in potato [6]. In addition to antimicrobial properties, in recent past, biosurfactants have also been reported to exhibit antiadhesive properties against a large number of microorganisms, thus also controlling the microbial attachment and growth on any surface over which they have been applied. One of the study reported the antiadhesive property of biosurfactant produced by lactobacilli against *Candida albicans* biofilm while in other study biosurfactant Lunasan produced by *Candida sphaerica* UCP 0995

exhibited significant antiadhesive property against *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, and *Streptococcus sanguis* [15,16].

CONCLUSION

During this study, *Pseudomonas* sp. rhamnolipid biosurfactant exhibited significant antifungal activity against phytopathogen *A. oryzae* (MTCC 1846), *F. solani* (MTCC 350), and *Curvularia* sp. rhamnolipid coating (1mg/ml) of the suitable vegetable protected them from fungal spoilage for up to 15 days at room temperature and thus increased their life span. Based on the above finding, it could be concluded that the *Pseudomonas* sp. rhamnolipid biosurfactant possesses significant antifungal activity and can be used for the development of coating material for the protection of vegetables from microbial spoilage.

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