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Swapna
Department of Agricultural Microbiology, UAS, Raichur, Karnataka, India

Mahadevaswamy
Department of Agricultural Microbiology, UAS, Raichur, Karnataka, India

Udaykumar Nidoni
Department of Processing and Food Engineering. UAS, Raichur, Karnataka, India

Amrutha G
Department of Agricultural Microbiology, UAS, Raichur, Karnataka, India

PF Mathad
Department of Processing and Food Engineering. UAS, Raichur, Karnataka, India

Corresponding Author:
Swapna
Department of Agricultural Microbiology, UAS, Raichur, Karnataka, India

Coating fruits and vegetables with probiotic bacteria (LAB) to enhance food safety

Swapna, Mahadevaswamy, Udaykumar Nidoni, Amrutha G and PF Mathad

Abstract
After harvesting fruits and vegetables, due to bad storage and transportation conditions, lack of good quality control measures and preservation techniques against food contaminants impact food safety and security. The persistence of microorganisms on food contact surfaces, equipment and processing environments has been reported as a main contributing factor in food-borne outbreaks, in particular, *Salmonella, E. coli, B. cereus* and *L. monocytogenes* which even endanger consumer safety and contribute to food-borne illness (Number and others 2012). In addition, disease-causing microorganisms like *Aspergillus, Botrytis, Colletotrichum, Fusarium, Alternaria, Penicillium, Phomopsis, Rhizoctonia* and *Rhizopus* genera are responsible for reducing the shelf life of different fruits and vegetables. Though chemical fungicides proved to be highly effective against a wide range of pathogens in foods, their potential negative impact on human health and reduce the rate of antibiotic-resistant strains, has prompted research on the use of native probiotic bacteria to inhibit the growth of food contaminants and pathogens in order to prevent food-related illness to humans and to increase the shelf life of different fruits and vegetables, respectively.

Keywords: *Colletotrichum* sp, *E. coli*, food contaminants, food borne disease outbreak, fruits, probiotic bacteria, *Salmonella* sp, vegetables etc

Introduction
Among the most food pathogens worldwide due to the considerable human rates of illness reported, *Salmonella* and *E. coli* remains the wide species detected in the local food market. Despite the cleaning procedures they persist on food contact surface which leads to biofilm formation and microbial cross-contamination. They once attached produce resistant biofilms which consist of a reservoir of cells and once detached, continuously contaminate the foods. It has been reported that more than 99.9% of microorganisms live attached to surfaces in natural and man-made ecosystems and form a specific and complex structure called biofilm. In the past few decades, due to rise in the availability of fresh produce like fruits and vegetables and their consumption has also increased food borne illnesses (Berger *et al*. 2010; Byrne *et al*. 2014; Kozak *et al*. 2013) [2, 3, 12], making microbiologists develop strategies to reduce the occurrence of food borne outbreaks worldwide (Lynch *et al*. 2009) [14]. An outbreak of the bacterial food-borne disease was reported in India during 1980-2009 and indicated that in 24 outbreaks there were around 1130 persons involved. The important bacterial agents responsible for these outbreaks were *E. coli, Staphylococcus aureus, Vibrio, Salmonella* and *Yersinia enterocolitica.*

A serious concern in the food industry is the formation of biofilms, as food-borne pathogens and contaminants have the ability to attach and grow on food surfaces and other food contact surfaces. There are many reports which have shown the presence of pathogens with the ability to form biofilms is one of the major contributing causes of food contamination and spoilage. *S. enterica* contaminated soil affected the crops like carrots, lettuce, and radish and for more than 6 months persisted in the crops which were reported by Islam *et al*. (2004a, b) [9, 8]. The key factor involved in the food contaminants for biofilm formation is quorum sensing. Similar reports were documented by Smith *et al.* (2004) [22], that quorum sensing is a regulated system in which bacterial cells interact with each other such that it benefits an entire biofilm population. Complete removal of the risk of microbiological contamination from fresh fruits and vegetables sold in local markets cannot be done though with conventional sanitation techniques employed for cleaning and processing of fresh produces. Barak and Liang (2008) [1] reported that many agricultural crops including tomatoes are contaminated by *S. enteric*.

The high persistence nature of *Salmonella* in plant environments was reported by Teplitski *et al*. (2009) [24]. Therefore they can act as vectors of contaminants to different other foods present in the local market (Berger *et al*. 2010) [2]. So their removal or complete destruction in such cases is very difficult. Denis *et al*. (2016) [6] recently analyzed the trends and prevalence of bacterial contamination in fresh fruits and vegetables sold in local Canadian markets. Although with a considerably low prevalence of various pathogenic microorganisms were found with notable seasonal variations in their incidence. However, the scenario in India is likely to be different.

There is a number of mechanisms and different steps by which many food contaminants get close in contact with a surface, attach and promote interactions between cell to cell in order to grow and form biofilms. Among the different steps in biofilm formation, the first step is reversible adhesion or adsorption of bacteria to the surface, triggered when the bacteria approach the surface over 50 nm, through Vander Waals interactions. When the distance is between 12 to 20 nm, hydrophobic, acid-base and electrostatic interactions which are covalent forces get involved in the adhesion process. Adhesion becomes irreversible when the distance decreases and at less than 0.5 nm short-range interactions called specific interactions are needed for bacteria to attach to abiotic surfaces. Structural adhesins of bacteria *viz*., pili, fimbria and flagella which are a part of the cellular envelope enhance cellular adhesion. These structures create bridges between cells and surfaces in order to strongly anchor bacteria to abiotic surfaces and allow them to overcome unfavourable conditions. In the same context, as the old adage goes "an ounce of prevention is worth a pound of cure", it is wiser to act at the source of the problem by hindering bacterial adhesion to abiotic surfaces instead of lately fighting already established biofilm. In the present situation, an increasing problem is food contaminants like enteric bacteria are becoming resistant to bacteriocins. The emergence of these bacteria resistant to bacteriocins can undermine their use as antimicrobial agents. For example generation of nisin-resistant isolates from *C. botulinum, L. monocytogenes, Bacillus cereus, B. subtilis, B. licheniformis* and *S. aureus*. A mutant strain of *L. Monocytogenes* was obtained when Ming and Daeschel were evaluating Gram-positive pathogenic and food spoilage bacteria for their resistance to nisin (resistant to nisin at 2000 U/ml).

Regardless of the numerous probiotic bacterial strains presented in the market, there is an ongoing need for the improvement of probiotic strains to be used as starter cultures or to develop a new natural methods for biopreservation; thus, probiotic isolates isolated from their natural environment (e.g., native fruits and vegetables) might possess unusual/unique characteristics including phenotypic differences and intraspecific variability compared to the known ones. Siroli *et al*. (2015) [21], highlighted the importance of isolating and selecting biocontrol agents from the same environment where they will be reintroduced since it was determined that the highest antagonistic capacity of the probiotic strains tested was against pathogenic and deteriorative microorganisms present; this can be attributed to suitable strain competition for nutrients, colonization and space.

The present study, therefore, is aimed at food contaminants like *E. coli* and *Salmonella* sp, the two prominent members of *Enterobacteriaceae* family, known for their involvement in food-borne outbreaks and other pathogens like *Fusarium, Aspergillus, Colletotrichum, Botrytis* etc. which are contributing for the decrease in the shelf of different fruits and vegetables worldwide. In order to overcome all these problems, the use of native probiotic bacterial isolates has been taken into account for the study.

Lactic acid bacteria (LAB)

Probiotics are live microorganisms which when consumed provide health benefits to the host. Some native fruits and vegetables derived have been recently screened for the presence of probiotic LAB (Swain *et al*., 2014). To inhibit the colonization of pathogenic bacteria in food products the use of Lactic Acid Bacteria (LAB) is proposed among the different methods. LAB are generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) of the United States and have an antagonistic capacity against different pathogenic bacteria which makes them ideal for developing bioprotective agents in fresh fruits and vegetables (Trias *et al*., 2008; Jeddi *et al*., 2014; Li *et al*., 2015) [25, 11, 13].

The use of LAB has the following benefits

1. Increase in the shelf life of food due to the action of antimicrobial metabolites and the decrease of water activity (salting/drying);
2. Due to the reduction of pathogenic microorganisms and their toxins, increased food safety;
3. Improved sensory characteristics and nutritional value

Several mechanisms have been proposed by which probiotics act, these include

1. Competing for nutrients and epithelial attachment site;
2. Modifying the environment makes it unfavourable for pathogens; and
3. Producing antimicrobial peptides (Chichlowski *et al*. 2007) [4]

To food products LAB can be applied directly as a protective culture, considering that the food product will promote LAB growth, and competence with the target microorganisms will occur (Shafiur-Rahman, 2007) [20].

Probiotic microorganisms possess broad-spectrum antagonistic activity, especially by the production of bacteriocins and it is a well-accepted phenomenon as compared to an older concept where only closely related species can be inhibited by bacteriocin. Many studies have reported that the production of secretory antimicrobial components by LAB has broad range antagonistic activity which is independent of lactic acid and hydrogen peroxide against Gram-positive and Gram-negative organisms. Among the bacteriocin-producing bacteria, the major producers are LAB and endospore-forming *Bacillus spp*. Bacteriocins are potent antimicrobial peptides synthesized by ribosomes. Searching for desirable bacteriocins has gained more attention and become most significant since the emergence of bacteriocin-resistant strains is very rare (Willey and Van Der Donk 2007) [26].

***Bacillus* sp**

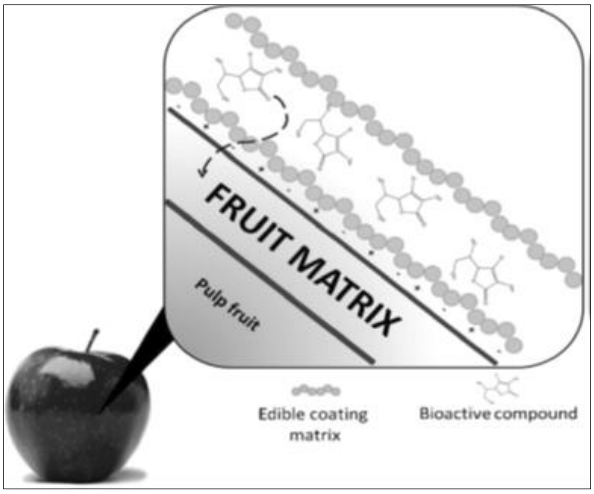
Bacillus sp is an endophytic bacterium which colonizes the same ecological niches as pathogenic microorganisms and is also found in diverse environments. They have a significant advantage over epiphytic microorganisms being present in the endosphere, rhizosphere and also in phyllosphere because of stable pH, the flow of nutrients, humidity and the lack of competition from other spoilage microorganisms. It has a broad spectrum of activity against different fruit pathogens namely *Aspergillus, Botrytis, Colletotrichum, Fusarium, Alternaria, Penicillium, Phomopsis, Rhizoctonia* and *Rhizopus* genera. Their bioprotective activities were demonstrated *in situ* in varieties of fruits including pomegranates, tomatoes, apples and grapes. *B. subtilis* strains are eco-friendly and have the potential to be bioactive for controlling post-harvest lost due to food pathogens and contaminants and their generally recognized status as safe microorganisms to use in the food industry. *B. subtilis* has been shown to increase the resistance of a wide range of stored fruits and vegetables to various diseases and abiotic stresses (drought, salinity, extreme temperatures, toxic metals, *etc*.) during handling, transportation, and storage, with the effect of protecting stored food from postharvest decays and prolonging its shelf-life.

Since consumers increasingly demand foods free of synthetic preservatives, it is necessary to identify and study new alternatives. These new approaches of using a consortium of probiotic bacteria *Bacillus subtilis* and LAB as an edible coating on fruits should be useful for controlling food-borne pathogens and contaminants which are found in the local market and for extending the foods' shelf-life.

Application of mixture of LAB and *B. subtilis* bacteria as an edible coating

To search for natural antimicrobials, it must be cost-effective from an economic point of view and the mixture of several natural antimicrobials combined with food preservation techniques would be one alternative approach. Studies on these represent promising advances in the search for novel applications of edible coatings as carriers of diverse probiotics and open novel possibilities for the development of novel food with probiotic products. Many research groups have recognized the potential of edible coatings as an alternative to conventional packaging and to enhance food protection. The main advantage of using edible coatings is that several bioactive compounds such as antimicrobials, antioxidants, flavour and probiotics in the polymer matrix and consumed with the food, enhancing safety or even better nutritional and sensory attributes. In most fruits according to literature data, increased concentration of bacterial strains elevated the biocontrol activity and reduced the level of pathogens. 10^7-10^8 CFU/ml concentration is generally considered the most effective in controlling post-harvest fruits diseases.

The effectiveness of antagonists mainly depends on their ability to outperform pathogens based on their capacity for rapid growth and survival under unfavourable conditions and is strongly dependent on their initial concentration when applied to the wound site. Antimicrobial components can be incorporated during the process or sprayed over the food.



Fruit Martrix

Alginate is the most used matrix for the microencapsulation of probiotic bacteria. It is a GRAS substance, economical, reproducible, and biocompatible and requires mild gelation conditions, has the property of ionotropic gelation, presents low immunogenicity and can be a good carrier of bioactive natural compounds (Islam *et al*., 2010; Chun *et al*., 2014; Heydari *et al*., 2015; Raeisi *et al*., 2016) [10, 5, 7, 18]. In addition, it has been shown to increase the survival of bacteria and it is easy to use alginate (Ruíz-Martínez *et al*., 2009; Parra, 2010) [19, 17]. It has been reported that increasing the concentration of alginate leads to an increase in colony counts of *Bifidobacterium longum* (Özer *et al*., 2009) [16].

Conclusions

Antimicrobial activity of probiotic bacteria or their bacteriocins against frequently found food contaminants and pathogens causing disease outbreaks in fruits and vegetables have been reported. Nevertheless, studies in the field of natural food biopreservation are conducted to an increasing extent. As consumers are more concerned about food quality along with their refusal of chemical additives, there is a growing demand for alternative antimicrobial treatments and probiotic bacteria (LAB) are well-accepted natural means of selective microbial inhibition. On the other hand, microencapsulation in a biodegradable matrix protects probiotic bacteria against unfavourable environmental conditions, which maintains their viability for a longer period. Therefore, the use of microencapsulated probiotic bacteria promises to be an effective technique to guarantee safety and to extend the useful life of fruits and vegetables during post-harvest.

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