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Review Paper

Potential of Bacterial Chitinases and Exopolysaccharides for Enhancing Shelf Life of Food Commodities at Varying Conditions

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Abstract

Many fruits and various other foods are decomposed by fungal attacks. Poor post harvest transport and storage managements result into great economic losses. In addition, consumption of fungi contaminated foods leads to further human health risk issues. The situation is especially prevalent in countries characterized with warmth and humid environments, where in fungal growth and insect attacks are very common. Chemical control measures increase health risks and environmental problems. Biological control promises a safe solution. Fungi possess chitin as integral part of their outermost boundaries, while insects besides their exoskeleton also have constitutional components of chitin if other organ system, especially gut. Fortunately chitin is not represented either structural or physiological component of human bodies. Thus a biological control strategy which can damage chitin, a vital component of fungi and arthropods may be believed to be non-toxic for humans. Several bacterial species are being reported as biofungicide. The chitinase producers appear potential candidates of biological control of fungi for enhancing shelf life of various food commodities. Several bacterial species are being reported as biofungicide. The chitinase producers appear potential candidates of biological control of fungi for enhancing shelf life of various food commodities. The present review highlights this aspect of bacterial chitinases. The information given provide insight for research efforts directed towards development of environmentally and human health friendly biological control strategies for fruits and other attackable foods deteriorating and damaging during post harvest storage. Implications of relevant extremophiles in this regard sound appealing, due to their non-pathogenic nature and high stabilities of the enzymatic products.

Keywords: Biological control, biopreservation, biofungicide, bacterial chitinases.

Introduction

Biopreservation, defined as the increased shelf life of food with the use of microbes and their antimicrobial, is innocuous and ecological approach to the problem of food preservation and has gained much attention in these years[1,2]. Preservation of food stuffs remained a necessity for humans because the durability of food is very limited, various foodstuffs are available only during a short season, transportation time from the production site to users are continually increasing and consumers in our society are involved in division of labor that insist him for buying durable products[3]. Preservative agents are needed to ensure that food will remain safe[4]. A large number of foods are being saved against spoilage in many countries and maintained the quality of food by application of different microbial systems. Because of improvement in quality in traditional fermented foods, extensive research in this field is being worked out with goals of improvement in quality of food products[1]. Due to negative effects on the human health as well as environment, chemical pesticides and various food preservatives has been widely criticized[5,6]. Various microorganisms and/or their activities have been taken into account to alternate chemicals. Antimicrobial enzymes are playing key role in defensive system of livings against microbes. The cell walls of bacteria and fungi the basic structural component are degraded by these hydrolytic enzymes produced by various microorganisms.

Different enzymes inhibit microbial growth and increase shelf life. A few number of enzymes are already being used but the cost in production has limited its use on large scale however, now gene cloning technique made possible the production of enzyme at low cost but hardly exploited. Shelf life of any food is starting from harvesting or industrial preparation during which it remains stable and maintains qualities. Consumers and manufacturers require fresh, additive free and food with natural taste[7]. Efforts are being carried out for identification of bacterial cultures whose direct/indirect antimicrobial effects may address pathogens and different spoilage microbes in a public health friendly way[8].

Post harvest damages of foods

Post harvest damages are a major expense in food production; reduce the availability of food for commercial purpose and result in food shortage in developing countries. Food spoilage is the process that causes foods to be unable for consumption. Spoiled foods may have pathogen and their toxins cause disastrous effects on health. Food loss from harvest to consumption generates considerable environmental and economic effects. The USDA economic research service pointed out that in U.S. food were lost in more than ninety six billion pounds in 1995. Fresh produce and fluid milk each accounted for 20% of this loss, while lower percentage were for grain products (15.2%), caloric sweetness (12.4%), processed fruits and vegetables (8.6%), meat, poultry and fish (8.5%), fat and oils (7.1%). If 20% of a crop is lost then 20% of the fertilizers and irrigation water used to grow that crop is also lost[9].

Microbial implication in food spoilage

The preservation of foods against microorganisms activity depicts both purpose to ensure food safety - that is, minimizing the risk of users facing food borne disease - and preventing or delaying microbial spoilage. Because the same microorganisms are rarely of concern as regards food safety and food spoilage, effective food preservation must generally rely on a combination of approaches. These approaches involve: i. minimizing the initial load of critical microorganisms through hygienic measures; ii. changing the environmental conditions in the food (temperature, pH, water activity, redox potential, etc.) so that they become unfavorable for growth; iii. inactivating the living microorganisms of concern, for example, by heat, radiation or the action of antimicrobial agents.

Packaging could play important role in food quality assurance for perishable and susceptible to contamination foods. Conventional food packaging enhances shelf life with quality maintenance add safety assurance. Different types of food including fruits, vegetables, milk, meat, grains etc are attacked by microbes. Of these the sugar rich foods support rapid microbial metabolism and growth. Fruits and vegetables pass along a long route from production farms up to the consumer. Similarly baking during procedure due to application of non-sterile equipments and air for cooling the food commodities face contaminations[10]. Consequently, the produce bears high risk of exposure to damaging factors including microbial attack and physico-chemical deteriorations. Murthy *et al.*[11] for example have described major marketing channels for fresh grapes in Bijapur (India) which can be summarized as;

Farmers → commission agents (local) →wholesalers (local/distance) → retailers (local/distance) → consumers (local/distance)

Due to humid and warm environment, lack of proper packaging for less efficient transport, chances of microbial contamination following injuries and cracks in the peels of such fruits cannot be ruled out. More or less same route is followed for the fruits marketing in Pakistan. This practice exposes the produce to physical and biological attacking agents which together pave for microbial attack. Sugar rich fruits become more vulnerable to fungal attack. Doyle[9] mentioning comprehensively factors which cause deterioration of food highlighted insects foods infesting and rodents chewing on foods; microbes growing on foods in addition to several physico-chemical and other biological agents. The author also stressed the importance of presence of proper moisture as too little can cause cracking, crumbling, or crystallization whereas its excess causes sogginess, stickiness, or lumping.

The problem of food spoilage is prevailing especially in the developing countries[12]. In most of the regions, low temperature is practiced to increase the shelf life of food. In many countries including Pakistan ambient temperature remain high in most part of the year. In the present scenario of disrupted and expensive supply of electricity provision of low temperature for storage purpose has become out of range for most of the people. In such situations food become more susceptible to bacterial and fungal attack. The latter organisms are known to add afflatoxin, causing drastic health effects including cancer. Fungi may also cause off-flavor formation and mycotoxins production[13]. Antimicrobial agents are available in variety but focus on the legal preservatives and marketing considerations have generated a new trend that use of preservations present are diminishing instead the use of minimal processing and prolonged food shelf life is increasing desired effective means of antimicrobial agents ensuring food safety[14,15].

Food losses are serious problems in tropical countries like Pakistan where ambient of temperature and humidity etc is suitable for microbial growth in conjunction with and food spoilage. Losses in developing countries run high because of poor storage and food handling technologies. Favorable growth conditions for microbes result in deterioration of food in large quantity ranging from 80-85% damage of crop[16,17]. It is also known post harvest managements decide the food quality, its market and profits. In developing countries especially the small scale farmers are facing many problems. Losses in pre- and post harvest crops due to various mentioned reasons result in low yields, income and food availability[18].

Methods of food preservation

Various physical and chemical methods capable of controlling/influencing microbial growth have been implicated in food preservation processes. Temperature, water activity, high energy radiation and pH etc represent physical treatments of different procedure for saving them from microbial deterioration. Similarly chemical preservatives have long history of their use. Common salts, smoke or sulfur dioxide have been used since long. Chemical antimicrobial agents included organic acids, fungicides, alcohols and antibiotics. Benzoic acid has been introduced in last century while propionic acid and sorbic acid are in practice[3]. Ethanol causes strong undesirable chemical odor in most food products while use of antibiotics as packaging additive is not approved as such practices would increase development of resistance in microorganisms. Application of chemical fungicides has been an effective method for the control of post-harvest diseases. But no need to argue is that application of chemical control urgently needs to be replaced by biopreservative strategies in a health conscious society. Health risks concerned with chemical preservatives have made it necessary to develop alternatives for controlling post-harvest diseases and hazards[19].

Biopreservation and chitinolytic bacteria: In actual the problem is little knowledge of the effectiveness of the use of antimicrobial agents in comparison with other sources of food preservation. Biological control agents are safer, human health compatible and environmentally friendly[20]. Microbial control agents provide safer alternative of chemical insecticides because of their efficiency and harmless nature for non-target organisms[21,22]. Enzymes are versatile tool sustainable development in a variety of industries as they have important environmental benefits. Enzymes are biodegradable, show improved use of raw materials and decreased amount of waste products[23]. Microbial enzymes are important in this regard. The hydrolases and the oxidoreductases are playing relevant role. Cell walls of microorganisms act as the substrates for the hydrolases. Inactivation of cell happened by the degradation of the cell wall components. The oxidoreductases affects by destroying vital proteins in cell[12]. Bacteriocins and small protein antibiotics have appeared as qualified molecules for controlling bacterial contaminations[24]. Lactic acid bacteria (LAB) through bacteriocins, play important role in biopreservation of fermented food[25]. But bacteriocin control bacteria and not fungi. Antifungal compounds enlist as fatty acids[26] and phyenyllactic acid[27]. Knowledge of bacterial metabolism has explained various ecological roles of diverse kinds of enzymes. In this regard chitinolytic bacteria are pivotal for recycling the second biggest biocarbohydrate reservoir of chitin[28]. Chitin, the polymer is structural component of fungal cell wall and arthropods exoskeleton. Fungi and insects both synthesize and deposit chitin in their protective structural components[29].

Thus biological control strategy involving chitinolytic activities may address preservation of foods simultaneously from arthropods and fungal attacks. Fungi represent a major problem as food spoilage organisms, human pathogen and air contaminant. Fungal cell wall is mainly composed of polysaccharides, constitutes 80-90% of total dry weight of the cell walls, can be used as destructive agent against various fungi. One of the major examples is chitinases enzyme. Fungal cell walls contain almost 22% to 44% chitin[27]. Fungal cell walls contain chitin composed of N-acetyl-D-glucosamine molecules cross linked with other by beta (1-4) glycosidic linkages which attains a highly insoluble crystalline structures organized into microfibrils[30]. Most of the fungal cell wall hydrolyses have chitinolytic activity. Potential application of chitinases in biocontrol of unwanted fungi is promising. The chitinase producing strains could be used for biological control of fungi under diverse sets of condition[31]. The antifungal activity of chitinases was first discovered in plants produced by them as defense mechanism against fungal pathogen.

Many chitinolytic bacteria can play important role in biocontrol of fungi. Major genera containing chitinolytic species are Pseudomonas[32], Aeromonas[33], Xanthomonas, Serratia[34], Cytophagia, Arthrobacter[35], Bacillus[36], Streptomyces[37], Cellvibrio[38], Alteromonas[39], Enterobacter[40], Vibrio[41] and several thermophiles[42]

Chitinases a group of enzymes capable of degrading chitin to low molecular weight products[43] Endochitinases and exochitinases degrade chitin by hydrolyzing glycosidic bonds to low molecular weight products[30]. Endochitinases cleave chitin randomly at internal sites, generating low molecular mass multimers of N-Acetylglucosamine (GlcNAc), such as chitotetraose, chitoriose, and diacetylchitobiose. Exochitinase can be divided into two subcategories; chitobiosidases which catalyze the progressive release of diacetylchitobiose starting with the non reducing end of chitin microfibrils and β-(1,4) N-acetylglucosaminidase which degrade the oligomeric products of endochitinases and chitobiosidases, generating monomers of GlcNAc[44]. Fortunately, chitin is not a constituent biological part or metabolite of vertebrates. Thus bacteria producing chitinases have a great potential in controlling fungi and insects under select conditions[28]. Several workers have reported antifungal activities of chitinase producing bacteria. For instance, Sadfi *et al.*[45], reported 100% control of *Fusarium oxysporum* by antifungal activity of *Bacillus thuringiensis*. Kamil *et al.*[46] described antifungal potential of *Bacillus licheniformis* for controlling various pathogenic fungi (*Rhizoctonia solani, Macrophomia phsiolina, Fusarium culmorum, Pythium spp., Alternaria alternate* and *Sclerotium rolfsii*) responsible of destroying various food materials.

Chitinolytic enzymes /Extremoenzymes as safe and low cost biological control agents: The considerations which are necessary for enzyme's application in foods are legal aspects, the health aspects, the possible side effect of enzyme and stability of enzyme. Following is a brief description highlighting the potential for vast application of enzymes and other exoproducts of extremophiles in food preservation science. Besides being non-pathogenic in nature and yielding highly stable and applicable exoproducts, knowledge of specificity of mechanisms through which a bacterial pathogen exerts selective toxicity in a pest and does not harm to non-target organisms, which certify it a safe biological control, is important not only for academic reasons but is required for actual application in the field[20]. Application of extremophilic bacteria of choice can render the process of biological control safe regarding damage to humans on one hand. While on the other hand they yielded more effective preservation of foods out spanning the biological control targets. Erbeznik[47] realized that extremophiles are diverse, esoteric and typically non-pathogenic. While Nicolaus *et al.*[48] documented that extremophilic microorganism and their exopolysaccharides (EPSs). Thus besides beneficial searching of relevant extremozymes, other products from extremophiles may widen their application scope. In this regard Poli *et al.*[49] have mentioned that among the extremophiles belonging to the Archaea domain showed significant importance in the field of biotechnology as they possess unique characters that enable us a new insights into their biology and evolution. One of the major type of biopolymers produced by these microorganisms are extracellular polysaccharides (EPSs), considered as a protection against their desiccation and predation as well. Thus such application of archael EPSs may help in retaining freshness of fruits during post-harvest storage and extend the scope of biopreservation. To achieve above mentioned biopreservation targets provision of low cost chitinases is more important. Bioconversion of shrimp and crab shell powder of marine waste by using various chitinolytic microorganisms for production of biofungicide has been investigated[50]. Extremophiles are valuable resources because of their unusual properties that have fulfilled the gap between biological and chemical processes. Extremophiles are adapted to living at high temperature in volcanic hot springs may be at 100°C and at low temperatures even in the cold polar seas, at high pressure in the deep sea at very low and high pH values (pH 0-1 or pH 10-11) or at very high salt concentrations (35%) as well[51].

A suitable approach may employ thermophilic/thermoduric bacterial chitinases capable of inhibiting fungal/insect pests attack without the requirements of refrigeration. Thermophilic extremophiles have attracted most attention for industrial application and their enzymes are suitable at elevated temperatures. Similarly hyperthermophiles have eliminated the risk of contaminations and their enzymes show high tolerance to various denaturing conditions[52]. Relevant enzymes and other products from psychrophiles may find applications in controlling fungal food deterioration under cold storage conditions. Extremophiles have attraction for biopreservation science due to other reasons too. Low temperature application is a well practiced strategy for food preservation from attack of biological agents. While in less affluent countries characterized with higher temperature climate provision of refrigeration becomes out of focus. Even while during cold storage in areas of cold climate fungal attacks of perishable items are not an uncommon observation. Psychrophiles show high catalytic activity and low thermal stability at moderate temperatures, exhibiting its increased flexibility. They are well reported for its suitability in chitin degradation[53]. Halophiles are also important in this regard working in extreme hypersaline habitat and their enzymes have adapted such environment pressure. They are also efficient in producing chitinases and hydrolysis of chitin which can be employed in fungal control[54]. Consequently for degradation of polymer, extremophiles are preferred that are and resistant to high temperatures. It is believed that extremophiles and genetic engineering of microbes will offer novel opportunities for biocatalysis, biotechnology and biotransformation.

Besides the biological deterioration of foods, their natural waters' evaporation lessened the shelf life. Preservation of the water contents of fruits, vegetables and processed/semi processed food is also central focus of food preservation strategies. Cold storage serves this function for suitable time frame while exoploysaccharides from extremophiles are being documented for this aspect of biopreservation.

Conclusion

Biopreservation may extend shelf life of fruits and foods while monitoring their natural tastes without the addition of health warranting chemicals. Post-harvest and storage management of fruits and foods can be improved by applying biofungicides. Shelf life of prepared/natural food commodities can be increased by the application of bacterial chitinases stable under given set off physicochemical conditions. Major involvement of such chitinases with diverse range of their characteristics may emerge in controlling fungal attacks of different perishable commodities meant to be kept without refrigeration other proper storage conditions at high temperature and abnormal pH conditions. Chitinases while capable of selectively controlling growth of fungi and insects are completely safer for humans and other non-target organisms. Abundance of chitinous waste makes isolation of chitinase producing bacteria ubiquitous. Then chitinases of choice can be incorporated into prepared foods rendering them fungi resistant. While in other select situation broth cultures of bacteria actively yielding chitinases can be coated on the surfaces of fruits especially to be stored in humid environments whereas, chances of fungal control and maintenance of growth/metabolism of the chitinolytic bacteria may favor the biological control agents to overweigh the situation. This not applies specifically for countries like Pakistan where warmth and humid environments prevail in most of the part of country while future research involving extremophiles for those endeavors is likely to develop new horizons of biological control. In addition complete study on preservation system may show promising synergistic effects between antifungal methods. Cost and health problems can also be solved by such investigations.

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