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The Role of Pectinase in the Rotting of Fruits and Vegetables: A Review

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ABSTRACT

The fruits and vegetables are rich in nutritional value which make them an easy target of organisms causing spoilage and soft rot. The soft rot is one of the destructive disease of fruits and vegetables and occurs worldwide wherever fleshy storage tissues of fruits and vegetables are found. Such disease can occurred by some bacteria and fungi which are highly responsible for this soft rot causing spoilage and they are endowed with an important enzymological power, the main action of their pathogenicity. The enzyme pectinase has the power of degrading the cell wall of plant, causing the liquefaction of tissues which favour the development of phytopathogens. The understanding the role of enzymes synthesizing by pathogens causing spoilage and need to develop the new protection approach to deal with it. This review summarizes the main literature data on the different pathogens synthesizing the enzyme pectinase causing soft rot in fruits and vegetables.

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INTRODUCTION:

Fruits and vegetables are very important nutritional source for human beings, due to their rich source of water, vitamins and micronutrients. They are the part of daily human diet (Djellout Nadine et al, 2020). The consumption of these fruits and vegetables helps to combat many nutrition related diseases.

However, post-harvest diseases are a major cause of fruits deterioration and unprocessed vegetables (Nguyen-the C et al 1994).Physiochemical such as oxidation and post-harvest handling and biological agents are responsible for this changes, particularly those related to phytopathogenic microorganisms (Djellout Nadine et al, 2020).

Some enzymes that dissolve cellulose and pectin are already present inside the fruits as they age. When the fruit is infected with a fungus and fungal hyphae spread in intercellular spaces and inside parenchyma cells, the molds secrete enzymes that cause tissue maceration which eventually leads to corruption of the fruit (Elnaghy M.A et al 1989, Elkatatny M.S.T 1989, Srivastaval, D et al 2017). The degree to which fruits rot during infection depends on the ability of a pathogen to produce cellulolytic and pectinolytic enzymes. Various cell wall degrading enzymes have been shown to be secreted by phytopathogens (Yao et al 1996).

Enzymatic maceration of plant tissues resulting in soft rot is an important factor in plant pathogenesis. Among the pectic enzymes known to be active in maceration are endo-polygalaturonase, endo-pectate lyase and endo-pectin lyase (Samuel K C.Obi 1981).

Tissue degrading enzymes play a prominent role in diseases like bacterial and fungal soft rot of vegetables. Pectinases are enzymes that hydrolyze the pectin material of host tissue and play an important role in the development of soft rot (K.A Bhat 2012).The involvement of pectic enzymes in the degradation of pectic constituents of cell walls and of the middle lamella of plant tissues has been reported for diverse types of disease such as rot, dry rot, wilts, blights and leaf spots which are caused by pathogenic agents such as fungi, bacteria and nematodes (Bateman and Millar,1966).

The production of pectic enzyme *in vivo* is usually proven by the removal of infected tissue from the sick plant, purification of the crude enzymes and determination of its ability to reproduce disease symptoms in a healthy plant. Evidence of pectinase activity can sometimes be obtained microscopically as the invaded cells will be seen to separate along the line of the middle lamella and subsequent loss of their staining ability (Simbo Aboaba 2009).In this study, an attempt has been made to review the relevant available literature concerning the role of pectinase causing the soft rot in fruits and vegetables.

Pectin –its structure and properties

Pectin is a high molecular weight heterogeneous and acidic structural polysaccharide which is one of the major constituents of cereals, vegetables, fruits and fibers (O'Neil MA et al 1996). It is the component of middle lamella and primary cell wall in plant cell wall and within the wall forms a matrix in which a network of cellulose and hemicelluloses are embedded (Caffall KH et al 2009). Pectin has been characterized as having a backbone of D-galacturonic acid residues, linked by α (1–4) linkage with a small number of rhamnose residues in the main chain and a rabinose, galactose and xylose on its side chains (Gummadi SN 2002).

Pectin contributes to the mechanical strength and physical properties of primary cell walls there by maintaining structural integrity. Pectic substances represent between 0.5-4% of fresh weight plant material (Jayani RS et al 2005 and Sakai T et al 1993)). In addition to their role as cementing and lubricating agents in the cell walls of higher plants, they are responsible for the texture of fruits and vegetables during growth, maturation and their storage (Alkorta I et al 1998 and Caffall KH et al 2009). Furthermore, pectic substances are involved in the interaction between plant hosts and their pathogens (Collmer A et al 1986).

Pectinase and Classification of Pectinase Enzymes

Pectinase are the group of enzymes that catalyze the breakdown of substrates containing pectin. These are the one which hydrolyze pectic substances. Pectic enzymes are widely distributed in nature and are produced by bacteria, yeast, fungi and plants. In plants, pectic enzymes are very important since they play a role in cellular growth as well as in fruit ripening. Pectolytic activity of microorganisms plays a significant role, firstly, in the pathogenesis of plants since these enzymes are the first to attack the tissue (Collmer A et al 1986). In addition, they are also involved in the process of symbiosis and the decay of vegetable residues (Lang C et al 2000).

Pectinases are the heterogeneous group of enzymes that are classified into polygalcturonase (PG), pectinesterase (PE), and pectin lyase (PL) based on their mode of action on the substrate. These enzymes break the pectin polymer into smaller fragments by hydrolysis, trans-elimination and de-esterification reactions, respectively. There are two main enzymes reported, which able to hydrolyze glycosidic linkages. First is polygalacturonase (PG), also known as pectin depolymerise enzymes which act on α 1→4 glycosidic bond of the main chain of polygalacturonic acid and hydrolyse it by release of a water molecule. Second is polymethylgalacturonases (PMG) enzymes which catalyze the hydrolytic cleavage of α (1→4) glycosidic bonds.

Enzymes that involve in de-esterification reaction include pectinesterases (PE), also known as pectin methyl hydrolase. They catalyze de-esterification of the methoxyl group of pectin and yields pectic acid. Enzymes that cleave α(1→4) glycosidic linkage by transelimination reaction include polymethyl galacturonate lyases (PMGL) and catalyze trans-eliminative cleavage of pectin and produce unsaturated galacturonates (Heena Verma et al 2018).

Pectinolytic Enzyme Production

Pectinolytic enzymes are produced by many organisms like bacteria, fungi, yeasts, insects, nematodes, protozoan and plants. The microbial world has shown to be very heterogeneous in its ability to synthesize different types of Pectolytic enzymes with different mechanisms of action and biochemical properties (Anil RS 2018). Many pathogenic bacteria and fungi are capable of degrading pectin (Bateman DF 1972).a) Pectinolytic Bacteria: Bacteria like *Bacillus, Pseudomonas* and *Micrococcus* isolated from retting flax, jute, sisal and coir and *Erwinia* from coffee fruits have shown to possess the ability to degrade pectin by producing pectinolytic enzymes (Chesson A 1980).b) Pectinolytic fungi: Many fungal species are capable of degrading pectin by producing different pectinolytic enzymes. The study of Shinidia AA (1995) showed that temperature variation during garbage composting led to corresponding changes in the distribution of pectin degrading fungi in the compost and the most common pectinolytic fungi were *Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Penicillium chrysogenum, Fusarium moniliforme, Alternaria alternate, Cladosporium cladosporioids and Trichoderma reesei* and these fungi also formed the part of pectinolytic microflora of coffee fruits.

Pectinase enzyme involve in the soft rot of fruits and vegetables

The nutritional richness of fruits and vegetables makes them an ideal target for microorganisms, causing in particular soft rot. Certain research revealed that pectinase enzyme synthesizing by these pathogens causing the soft rots in fruits and vegetables.

The study of Tarek M et al (2019) showed the effectiveness of a biological Agent and fungicide on the tomato rotting activity of *Alternaria solani*. They determined the pectinolytic and cellulolytic activities of the causative agents of tomato fruit rot. Their investigation showed that *A. solani* produced both cellulolytic and pectinolytic enzmes in vitro and it is widely accepted that the production of these enzymes is a major means by which these fungi invade the host tissue, which agrees with the experimental findings of Ramchandran and Kurup (2013), who showed that pectinase-producing fungi cause spoilage of fruits and vegetables. The activity of these enzymes decreased with an increase in the *T harzianum* filtrate percentage and chemical fungicide concentration.

Studies on pectinase activities of isolates of *Erwinia carotovora* and *Rhizopus sp*. causes soft rot in cabbage was studied by K.A.Bhat et al (2012) and found that crude pectinase enzymes obtained in the form of culture filtrate from different isolates and were assayed by cup-plate method. Pectin lyase and hydrolase activity of *Erwinia* isolates was also carried out using viscometric assay where the percent decrease of viscosity gives the measure of chain splitting enzymes in the test samples.

Wakil and Oyinlola (2011) determined the diversity of pectinolytic organisms causing soft rot disease of five different vegetables marketed in Ibadan Nigeria. They found that the microbial load of each vegetable sample showed pectinolytic activity. Their results indicated that soft rot of vegetables is caused by a wide range of pectinolytic organisms that vary with different vegetables andlocations of purchase. *Erwinia species* were the dominating organisms although, *Pseudomonads* and *Xanthomonas* strains were also reported.

The fungi that cause decay and rotting of cucumber, lemons and tomatoes in some markets was studied by SMN Moustafa (2018). The role of CMC-ase and polygalacturonase (PG) enzymes and its role in corruption of these fruits were also studied. *A.alternata, P.italicum* and *R. stolonifer* were isolated from fruits of rotten tomato, lemon and cucumber respectively. Enzyme production from three tested fungi were studied and stated that enzymes play an important role in infecting fruits and vegetables with fungi and causing its corruption and spoilage. Cellulase enzymes cause softening and dissolution of primary cell walls of fruits, which are mainly composed of cellulose and leads to easy entry of penetration begs and haustoria of fungi into parenchymatous cells. Poly galacturonase (PG) play an important role in the decomposition and disintegration of plant cells of fruits as a result of dissolution of the middle lamella in the cell walls of the cells. This, in turn, causes cells to disintegrate and then to decompose and lose their cohesion until they become rotten. Results of this study showed that *P.italicum* and *R. stolonier* have a high ability to produce cellulose (CMC-ases) and PG enzymes.

The study of Samuel K.C Obi et al (1981) revealed that there is a pectic enzymes activities of bacteria associated with rotted onion. Six bacterial genra consisting of seven species were isolated from rooted onions and these were a *Vibrio sp*.(PS1), *Micrcoccus epidermidis* (PS2), *P. cepacia* (PS3,PS6), an *Acinetobacter sp*.(PS4), a *Xanthomona sp*. (PS5), *Bacillus poymyxa* (PS7) and *Bacillus megaterium* (PS8). The enzyme extracts from the onion tissue cultures exhibited some hydrolase activity for three isolates, *P. cepacia*,(PS3), *P.cepacia* (PS6) and *Bacillus polymyxa* (PS7).All the isolates produce both lyase and pectinesterase enzymes and the highest activity was recorded in *Vibrio Sp*. Their isolates examined demonstrated considerable pectin lyase activity and suggested that it may be prudent to inhibit pectic enzymes rather than at that whole organism.

Ishii S (1976) has observed the rapid maceration of onion tissue by endo-pectin lyase from *Aspergillus japanicus*. There is a role of pectinase enzymes in the development of soft rot caused by *Pseudomonas fluorescens* in the purple variety of onion was studied by Simbo Aboaba (2009) and found the ability of *P. fluorescens,* implicated in the soft rot of onion bulbs to produce pectic enzymes *in vitro* and *in vivo*. The viscometic assay confirmed this and suggests role for the pectic enzyme in soft rot development in plants.

Determination of pectic enzyme and proteolytic enzyme inhibitors which are known to accumulate in tomato plants whether it also found in tomato fruits, and if their specificity might be correlated with the rate of rot development by three fungi *G. cingulate, C. atramentarium* and *B. cinerea* pathogenic on tomato fruits was reported by Brown A.E.et al (1982).

Conclusion

The various organisms such as bacteria and fungi are responsible for soft rot of fruits and vegetables and represent a category of phytopathogens that are very dangerous, and this due to their ability to synthesize a plant cell wall degrading enzymes. From these the most important enzyme is pectinase which play important role in rotting the fruits and vegetables. This enzyme allow pectinolytic organism to cross the protective barrier of fruits and vegetables, causing soft rot in them. This is why it is also necessary to inhibit such enzyme action causing soft rot rather than only whole organisms.

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