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DETECTION OF EXTRACELLULAR ENZYMES IN MICROBIAL ISOLATES FROM DISEASED CARROT (*DAUCUS CAROTA*) FRUITS

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ABSTRACT

A research work was carried out on diseased carrots obtained from the main Sango Ota market to isolate microorganisms associated with post harvest deterioration of carrots. Small portions (2 x 5mm) of the diseased parts of carrots were aseptically inoculated on Nutrient agar and Saboraud agar incubated at 37^{O}C and 26^{O}C respectively. The fungal species isolated were *Aspergillus niger, Penicillium notatum, Mucor spp.* and *Fusarium* spp. and they were identified using morphology and microscopy. The bacterial species isolated were *Bacillus spp., Leuconostoc spp., Xanthomonas spp* and *Klebsiella spp* and they were identified using microscopy, morphology and various Biochemical tests. Extracellular enzyme production was carried out on the isolates to test for their ability to elaborate Amylase and Protease. All the isolates except *Aspergillus niger* produced Amylase. *Leuconostoc spp., Xanthomonas spp., Klebsiella spp. and Aspergillus niger* elaborated Protease. The results of this present investigation with further studies on carrots can be utilized in carrot juice production which is not commonly available in Nigeria.

Key words: Carrots (Daucus carota); Extracellular enzymes; Bacterial isolates, fungal isolates; Pathogenicity; Post harvest deterioration

INTRODUCTION

Carrot is a root vegetable usually orange, purple, red white or yellow in color with a crisp texture when fresh (1). The edible part of a carrot is a taproot and it is a domesticated form of the wild carrot which is native to Europe and Southwestern Asia (2). Carrots (*Daucus carota*) are very rich in vitamin C as well as Carotene (3). Carotene is the orange coloring matter of the root and it is a prolific source of pro vitamin A (4). Carotenes have been associated with the ability to prevent the appearance of certain cancers especially lung or mouth ones or impede the development of cancerous cells (5). Carrots can be eaten in a variety of ways but raw carrots must be thoroughly washed because they may carry harmful bacteria or parasites (6). The greens are edible as a leaf vegetable but are rarely eaten by humans as they are mildly toxic (7). Despite all the benefits derived from carrots and carrot juices, a large percentage of carrots are lost annually to post-harvest deterioration caused by microorganisms (8, 9, 10). These plant pathogens have virulence factors which are responsible for the disease causing process in the host (11, 12). The pathogens must first produce enzymes that would degrade or break down the plant cell wall which is the first line of defense of a plant cell wall before colonizing the plant (13). These enzymes produced at the initial stage to begin the infection process are cell wall degrading enzymes or extra cellular enzymes (14, 15, 16). This study therefore describes microbial isolates and the production of extracellular enzymes from diseased carrot fruits.

Materials and Methods

Sources of Carrot Samples

The diseased carrots used for this research work was the orange species and it was obtained from the Sango Ota main market, Ota, Ogun State. They were taken to the Microbiology Laboratory of the Department of Biological Sciences, Covenant University, Ota for analysis.

Isolation and Identification of microbial isolates

The diseased carrots were surface sterilized using 10% (v/v) sodium hypochlorite solution for 10 minutes. They were then rinsed with several changes of sterile distilled water to remove the residual effect of the Sodium Hypochlorite solution. Small portions,

about 4 – 6mm in diameter of the diseased carrots were cut and aseptically inoculated on sterile agar plates using sterile scalpels and forceps. Nutrient agar and Saboraud Dextrose agar were then poured into separate plates and incubated at 37^{o}C for bacteria and 26^{o}C for Fungi for a period of 24 hours and 48 hours. The cultures were subcultured until pure cultures of the microbial isolates were obtained. This was done using morphological, biochemical and cultural characteristics of bacteria according to the methods of Finegold and Martin (17). These include observing colonial morphology of the isolates on Nutrient agar, Gram staining and Different biochemical tests.

Colony Morphology on Nutrient Agar

This includes the type of pigment where present, size of colony, texture (opaque, translucent or transparent), adherence to agar, edge (undulating, round, dentate).

Biochemical Tests

Biochemical tests carried out were Catalase test, carbohydrate fermentation, Urease Test, Indole production Test, Citrate Utilization Test, Methyl Red and Voges-Proskauer test.

Fungal Identification

The morphological and cultural characteristics with reference to sporulation of the fungi isolates were used in identifying the organisms. The methods used were direct observation of the plates and the slide culture technique.

Extracellular Enzyme Detection

The enzymes assayed for were Amylase and protease. The Solid medium technique (18) was employed for the extracellular enzyme detection. The ability of the various isolates to degrade starch after the production of amylolytic enzyme was the criterion used to detect the enzyme produced by the isolates. The medium used for proteolytic enzyme production contained Gelatin as substrate.

Results

The result of this investigation revealed bacterial isolates and fungal isolates from diseased carrots. The isolates were identified based on cultural, microscopic and biochemical characteristics of the isolates (Table. 1; 2; 3).

Table. 1 IDENTIFICATION OF ISOLATES FROM DISEASED CARROTS

ISOLATES CODE	IDENTIFICATION
C1	*Aspergillus niger*
C2	*Penicillium chrysogenum*
C3	*Mucor species*
C4	*Fusarium species*
D1	*Bacillus species*
D2	*Leuconostoc species*
D3	*Xanthomonas species*
D4	*Klebsiella species*

Table. 2 THE MORPHOLOGICAL AND GROWTH CHARACTERISTICS OF BACTERIAL ISOLATES

Morphological and Growth Characteristics	D1	D2	D3	D4
Cell Morphology	Rods in chains	Rods	Rods singly and in pairs	Rods singly
Gram's reaction	+	-	-	-
Form on Agar plate	Circular	Circular and scattered	Circular	Circular
Pigmentation	Creamy	Glistering white	White	Creamy white
Growth	Abundant	Moderate	Abundant	Abundant
Edge	Entire	Undulate	Entire	Entire
Surface	Mucoid	Smooth	Smooth	Smooth
Motility	+	+	+	-
Elevation	Raised	Slightly raised	Slightly raised	Slightly raised
Surface growth	Pellicle	No pellicle	Pellicle	Pellicle
Turbidity	+	+	+	+
Sediment	+	+	+	+

Keys: + Positive - Negative

Table 3: IDENTIFICATION OF BACTERIAL ISOLATES USING BIOCHEMICAL TESTS

BIOCHEMICAL TESTS	D1	D2	D3	D4
Indole	-	-	+	+
Citrate	+	+	+	+
Catalase	+	+	+	+
Methyl red	-	-	+	+
Urease	+	+	+	+
Glucose	A+ve G-ve	A+ve G+ve	A+ve G+ve	A-ve G+ve
Maltose	A+ve G+ve	A+ve G+ve	A-ve G-ve	A-ve G-ve
Sucrose	A+ve G-ve	A-ve G-ve	A+ve G-ve	A-ve G-ve
Lactose	A+ve G-ve	A+ve G+ve	A+ve G+ve	A+ve G+ve
Galactose	A+ve G-ve	A+ve G+ve	A-ve G-ve	A-ve G-ve
Identification	*Bacillus spp*	*Leuconostoc spp*	*Xanthomonas spp*	*Klebsiella spp*

KEYS:

	G+ve	GAS PRODUCTION	A-ve	ACID NOT PRODUCED
+ POSITIVE	A+ve	ACID PRODUCTION	G-ve	GAS NOT PRODUCED
- NEGATIVE				

TABLE 4: CHARACTERIZATION OF FUNGAL ISOLATES ON SABORAUD DEXTROSE AGAR

ISOLATES CODE	**CULTURAL CHARACTERISTICS**	**MICROSCOPIC EXAM OF SLIDE CULTURE X 400**	**IDENTIFICATION**
C1	Fluffy white growth of colonies with elevated mycelia growth that turned black after 32 hours	Single –celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophores, the vesicle, long conidiophores arise from	*Aspergillus niger*

		septate hyphae.	
C2	The mature colonies were observed after 48 hours and were greenish in color.	Single –celled spores (conidia) in chains develop at the end of the sterigma arising from the metula of the conidiophores; branching conidiophores arise from a septate mycelium.	*Penicillium chrysogenum*
C3	White entire growth of single colonies with raised center, early stages of mycelia growth around the edges.	Spores are oval, non-septate mycelium gives rise to single sporangiophores with globular sporangium containing a columella	*Mucor* spp
C4	The spores give off orange pigmentation, outgrowth from the centre producing fluffy mycelia growth with orange pigmentation	Multicelled spores (conidia) are oval or crescent-shaped and attached to conidiophores arising from a septate hyphae	*Fusarium spp*

Bacterial isolates, Penicillium chrysogenum (**C2**) and Mucor spp **(C3)** produced amylase while *Fusarium spp* and *Aspergillus niger* **(C1)** did not produce amylase. All the bacterial isolates, *Bacillus spp, Leuconostoc spp, Xanthomonas spp, Klebsiella spp* produced amylase enzyme. *Aspergillus niger* was the only fungi that produced protease enzyme. *Leuconostoc spp, Xanthomonas spp and klebsiella spp* all produced protease (Table. 5)

TABLE 5: PRODUCTION OF EXTRACELLULAR ENZYMES OBTAINED FROM DISEASED CARROTS

ENZYMES	C1	C2	C3	C4	D1	D2	D3	D4
Amylase	-	++	++	+	++	++	++	++
Protease	+	-	-	-	-	+	++	++

KEYS: + Positive - Negative

DISCUSSION

The results of this present investigation revealed bacterial isolates Bacillus *spp, Leuconostoc spp, Xanthomonas spp and kliebsiella spp* from diseased carrots. The fungal isolates obtained were *Aspergillus niger, Penicillium chrsogenum, Mucor spp and Fusarium spp.* Other researchers have reported carrots in pathogenicity with other causative organism. These differences in isolates obtained which varied from researcher to researchers can be attributed to the fact that a different type or variety of carrots used as well as different experimental procedures (6). Extra cellular enzymes function by breaking down plant cell materials (19). The middle lamella of the plant cell wall is mainly made up of cellulose therefore a good or highly pathogenic organism should be able to produce amylase and cellulase which would break down the starch and cellulose component of the fruit thereby causing the fruit to deteriorate and cause disease (20). All the bacterial species isolated produced amylase in substantial quantities. *Penicillium spp* and *Mucor* spp produce amylase in large quantities, while *Fusarium spp* also produced amylase but not as substantial. *Aspergillus niger* barely produced amylase. The organisms also produced protease. Protease is an enzyme that breaks down protein into amino acids and amines which can be easily utilized. This can lead to increased proliferation and virulence (21). Proteases attack and hydrolyse the peptide bonds of proteinous materials such as protein and peptide bonds (22). *Aspergillus niger, Leuconostoc spp, Xanthomonas spp and klebsiella spp* were found to produce protease. It shows that they are serious pathogens of carrots and therefore causes fast proliferation and leads to damage of plant tissues. This research work therefore revealed *Xanthomonas spp, Leuconostoc spp, Klebsiella spp and Aspergillus*

niger as the most pathogenic organisms because they elaborated the two enzymes tested for.

CONCLUSION

Carrots consumed in Nigeria are mostly from the Northern part of Nigeria and it is possible that these carrots are infected by some of these microorganisms during transportation, handling as well as during storage before they are finally purchased and consumed. Specific and proper handling as well as optimum conditions for the storage of these carrots in this part of the world should be studied extensively. The results of this research work add to existing reports on pathogenicity of carrots. It will also help in further studies in determining possible physiological control measures to lessen, if not prevent, microbial deterioration of carrots fruits post harvest.

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