STUDIES ON THE ANTAGONISTIC ACTIVITY OF *Trichoderma viride* AND *Bacillus* sp. AGAINST POTATO SOFT ROT PATHOGENS

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BENIN CITY

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ANTIPLAGIARISM TEST

I attest and declare that this thesis titled "**Studies on the Antagonistic Activity of *Trichoderma viride* and *Bacillus* sp. against potato soft rot pathogens"** has passed the anti-plagiarism test and does not violate any copyright regulation.

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This is to certify that this project was carried out by **Sarah Itohan ERIEWELO (Miss) (PG/LSC1817734)** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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APPROVAL

This is to certify that this work is approved in partial fulfillment of the requirements for the award of M.Sc. in Food and Industrial Microbiology in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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DEDICATION

This work is dedicated first to Almighty God, the maker of heaven and earth. May His name be praised forever. And to my late grandmother, Mrs Egubere Victoria Omuvi.

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ABSTRACT

The study was carried out to determine the antagonistic activity of *Trichoderma viride* and *Bacillus* sp. against microorganisms responsible for disease or spoilage of potato. Diseased/spoilt potato tubers were obtained from open markets in Benin City. Standard microbiological techniques were employed for isolation of bacteria and fungi from diseased/spoilt potato tubers. Bacteria and fungi were enumerated using pour plate method. Cultural, morphological and biochemical characteristics were employed for identification of the isolates. Pathogenicity tests were used to evaluate the involvement of the isolates in the spoilage or disease of potato tubers. Biocontrol or antagonistic activity was carried out for the spoilage-causing microbes which were *Trichoderma viride* and *Bacillus* sp. using Potato dextrose agar and Mueller Hinton agar. The results revealed that the heterotrophic bacterial counts for spoilt/diseased potato ranged from $\log_{10}$ 3.65±0.41 cfu/g (Uselu market) to $\log_{10}$ 4.02±0.25 cfu/g (Aduwawa Market), while the fungal counts ranged from $\log_{10}$ 3.57±0.30 cfu/g (Aduwawa market) to $\log_{10}$ 3.85±0.10 cfu/g (Uselu market). The cultural morphological and biochemical characteristics of bacterial isolates from spoilt potato revealed their identity as; *Erwinia* sp*, Pseudomonas aeruginosa, Bacillus* sp. and *Staphylococcus aureus* while the fungal isolates from spoilt potato were *Aspergillus* sp*., Rhizopus* sp. and *Penicillium* sp.. The pathogenicity or spoilage test of isolated microorganisms from spoilt to healthy potato tubers showed that only *Erwinia* sp. and *Aspergillus* sp. were found to induce disease or spoilage condition in potato tubers. The *in vitro* antagonism (percentage inhibition) of *Bacillus* sp. and *Trichoderma viridia* on growth of *Aspergillus* sp. and *Erwinia* sp. showed that *Trichoderma viridia* had more biocontrol effect on growth of *Aspergillus* (50%) and *Erwinia* (47%) after 5 days of culture on growth media. In conclusion, *Trichoderma viride* and *Bacillus* sp. exhibited significant antagonistic activity against potato soft rot pathogens. The study identifies *Erwinia* sp. and *Aspergillus* sp. as key contributors to potato spoilage. *Trichoderma viride* demonstrates a notable biocontrol effect, inhibiting the growth of *Aspergillus* sp. and *Erwinia* sp.. These findings reveals the potential of *Trichoderma viride* and *Bacillus* sp. as promising biocontrol agents for mitigating potato soft rot, offering sustainable and environmentally friendly alternatives for potato cultivation and storage.

CHAPTER ONE

INTRODUCTION

Plants are a major source of food, fibre, fodder, medicines and many other useful products for mankind. Various insects, bacteria, viruses, fungi and other pests attack plants at various stages of their development (Ashwani *et al.,* 2011). This reduces their productivity and leads to huge losses. The most common and popular method of disease control is the use of synthetic pesticides. However, due to the non-biodegradable nature of such pesticides and the development of resistance by pathogens, biological control offers an important alternative to synthetic chemicals for disease management (Rajendiran *et al.,* 2010).

The goal of biocontrol research is to provide additional tools for disease management. Biocontrol can be used in situations where no chemical control is available, where conventional pesticides cannot be used due to reentry or residue concerns, or where the product must be certified as organic (Ajith and Lakshmidevi, 2010). Biocontrol involves harnessing disease suppressive microorganisms to improve plant health. Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, pathogen, biocontrol agent, microbial community on and around the plant, and the physical environment (Dev and Dawande, 2010). Currently, several biocontrol agents have been recognized and are available as bacterial agents for example *Pseudomonas, Bacillus,* and *Agrobacterium* and fungal agents such as *Trichoderma, Aspergillus, Gliocladium, Ampelomyces* and *Candida* (Naher *et al.,* 2014).

There are a variety of fungal species that have been reported as biocontrol agents, although *Trichoderma* species clearly dominate, perhaps due to their ease of growth and wide host range (Whipps and Lumsden 2001). Several strains of *Trichoderma* have been reported to be effective biocontrol agents for various soil-borne, plant pathogenic fungi under greenhouse and field conditions (Ajith and Lakshmidevi, 2010; Dev and Dawande, 2010). The genus

Trichoderma is a common filamentous imperfect fungus (Deutromycetes), the most common saprophyte in the rhizosphere and is found in almost all soils. It is a very effective biological means for management of plant disease, especially for soil-home pathogens. It is highly interactive in root, soil and foliar environments. Several *Trichoderma* species were reported to restrict plant pathogenic fungi under laboratory and natural conditions. They secrete a variety of volatile and non-volatile compounds with antibiotic properties (Ajith and Lakshmidevi, 2010).

The sweet potato or sweetpotato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the bindweed or morning glory family, Convolvulaceae. Its large, starchy, sweet-tasting tuberous roots are used as a root vegetable (Woolfe, 1992). The young shoots and leaves are sometimes eaten as greens. Cultivars of the sweet potato have been bred to bear tubers with flesh and skin of various colors. The sweet potato is native to the tropical regions of the Americas. Of the approximately 50 genera and more than 1,000 species of Convolvulaceae, *I. batatas* is the only crop plant of major importance—some others are used locally (e.g., *I. aquatica* "kangkong"), but many are poisonous (Rolston *et al.,* 1987).

Sweet potato (*Ipomoea batatas*) has an enormous potential to be an effective and economic source of food energy (Oyeyipo, 2012). It is an important source of antioxidants and anthocyanidins (Oladoye *et al.,* 2013). Sweet potato is 76% water, 21% carbohydrates, 2% protein, and contains negligible fat. It can be incorporated with yam to make "Amala" and pounded yam. The production of sweet potato, especially vegetable potato, is seriously affected by rots (Oyeyipo, 2012). Soft rot can cause heavy losses in stored potatoes if not properly managed, creating a perception of poor quality in export seed potato markets. Non-emergence of plants, wilting, browning of plant tissues, stem desiccation and plant death have all been linked to infection by soft rot bacteria. Soft rot of potatoes has been caused by a range of bacteria around the world such as *Pectobacterium carotovorum, Pectobacterium*

atrosepticum and *Dickeya* species. Previously, these bacteria belonged to the genus *Erwinia* (Salami and Popoola, 2007). Soft rot disease of potato crops is commonly recognized by soft, wet, cream- to tan-colored flesh, normally surrounded by a dark brown to black ring.

Currently, the control of soft rot pathogens relies on integrated pest management, including meristem culture in the production of certified seed, rigorous inspections and seed testing schemes, good sanitation during harvest and grading of tubers and the requirement for farmers to use certified seed. Other methods, such as tuber pasteurization, chemotherapy and thermotherapy have been found to be effective in experimental settings, but are rarely used in practice (Toth *et al.,* 2001; Czajkowski *et al.,* 2011).

Sweet potatoes have been described as having thin, delicate skin that is easily damaged by cuts and abrasion during harvesting, transportation or distribution. Several researches have reported that the microorganisms responsible for the spoilage of sweet potato produce extra-cellular enzymes such as amylases, celluloses, galactouronidases, xylanases and pectin-methyl esterases and these enzymes degrade the cell wall components of the produce (Salami and Popoola, 2007; Oladoye *et al.,* 2013). Several rots that affect sweet potato after harvesting have been substantially reported (Onifade *et al.,* 2004; Oyeyipo, 2012). These rots are linked to a number of factors that are physiological, physical, and microbiological. During harvesting, storage or transportation, mechanical damage occurs and this damage has been known to be predisposing tuber to spoilage and storage rots (Oyeyipo, 2012).

1.1 Aim and Objectives of the Study

The aim of this study was to determine the antagonistic activity of *Trichoderma viridae* and *Bacillus* sp. against sweet potato spoilage bacteria.

The specific objectives were to;

1. isolate and identify organisms associated with the spoilage of sweet potato tubers.
2. isolate *Trichodera viride* and *Bacillus* sp. from agricultural soils.
3. determine the antagonistic activity of *Trichoderma* and *Bacillus* spp. against spoilage bacteria isolated from sweet potato tubers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bacterial Soft Rots Diseases

Inevitably, whenever fleshy plant tissues in storage or in field undergo rot, you can be sure that bacteria are always present. This is evident by the foul smell given off as a function of volatile substance released by the bacteria during disintegration of plant tissues. The tissues undergoing rottenness become soft, watery with a slimy mass of cellular debris. Bacterial cells are regularly discharged from the cracks in the tissues. In several soft rot cases, the bacteria involved could be saprophytes or parasites and not necessarily a pathogen (Agrios, 2005). They could be present in the soft rotten fruits because of the avenue already created by the pathogen or prevailing environmental conditions.

Globally, bacterial soft rot occur with a corresponding serious consequence in terms of losses to crops from the field, to during storage (Agrios, 2005). Bacterial soft rot has been reported to be more devastating in terms of reduction of yield and market value than any other known bacterial diseases. Most crops are usually susceptible to soft rot due to bacteria, which usually in the space of few hours may develop during storage as well as marketing. The disease may reduce the amounts and quality of crops available for sale as well as the market value of the produce. However, the preventative measures against bacterial soft rot are expensive to adopt (Gupta and Thind, 2006).

2.2 Ecology of the Soft Rot Bacteria (*Erwinia*)

Globally, amongst the most destructive vegetable diseases, soft rot due to *Erwinia* popularly called *Erwinia* soft rot is one of its most devastating and arguably the most destructive disease. According to Opara and Asuquo (2016), the disease occurs wherever plant tissues or

vegetables are found. Bacterial soft rot pathogens are not only found in infected plants but can take up habitation in soil, various water bodies and guts of insects (Perombelon, 2002).

2.3 Morphology of *Erwinia* species

Erwinia species is a rod-shaped, Gram-negative, non-sporulating and peritrichously flagellated bacterium, which lives together with and can aggregate into pairs and chains or possibly lives alone. It is facultatively anaerobic, oxidase negative and shows a positive result to catalase test according to Pérombelon (2002) and Agrios (2006). *E. carotovora* the typical standard species implicated to cause soft rot, extracellularly produces a plethora of plant cell wall degrading enzymes (PCWDEs) like the pectic enzymes (responsible for degradation of pectin), hemicellulases, arabinases, cyanoses, cellulases (responsible for the breakdown of cellulose) and proteases. As a mesophile, *Erwinia* thrives best in temperature range between 27^{o}C – 30^{o}C (Perombelon, 2002). The cultural characteristics of the bacterium on most cultures are round, smooth, slightly raised, glistering, greyish-white to creamy-white with proper visibility on culture plates (nutrient agar) after about 24 hr especially for colonies of *E. carotovora* pv. *carotovora* and pv. *atroseptica*. Species such as *E. chrysanthemi* according to Gupta and Thind (2006) appear macroscopically on most media as round, smooth, flat to slightly raised, with margins becoming undulate to feathery, greyish-white to creamy–white colonies.

2.4 Modes of Transmission of *Erwinia* species

Opara and Asuquo (2016) reiterated that *Erwinia* can be disseminated in a variety of ways and one of such is via plant-to-plant infection. The caulosphere of most infected tubers have some form of inoculum, which are capable of initiating upon injury, the infection ensues and quickly rots the entire tuber. Often, these spreads wild and infect several other tubers. For tubers like potato, during washing in the wash tanks, the inoculum of bacterium on the

surface of the tubers can gain entry into the lenticels with the aid of hydrostatic pressure in the water tanks.

E. carotovorum can also survive in the gut of insects for hours and these insects serve as vectors or carriers of the pathogen from one plant to another where they induce disease condition in healthy plant. Using insect as vectors, soft rot bacteria are easily transmitted from one plant to another even when the feeds are miles apart. Agrios (1998) reported that most plant pathogens (viruses, fungi, nematodes, bacteria and protozoa) can be transmitted by insects. Insects' transmission occur in three basic ways according to Agrios (2005);

1. Passive transmission: this involves movement via an infected plant part, which has plant pathogen(s) on its surface. Sticky spores or pathogen propagules may cling to the insects while it moves about, from whence they can be transferred to begin a new infection in other plants parts or plants.
2. During the course of feeding, pathogen propagules of viruses, bacteria, protozoa, fungi and nematodes can be transferred from diseased plants to other plant parts or new plants.
3. Specific pathogen propagules such as nematodes, phytoplasmas, protozoa, viruses, and fastidious bacteria (xylem-and phloem-inhabiting bacteria) can be transmitted via the process of sucking or ingesting the propagules along with the sap of the plant, when the insects feed.

Consequently, the insects act as vectors for the pathogen, which circulates in the insect (without or with further pathogen multiplication) until it reaches the salivary glands from whence it can be injected into new susceptible plants (Agrios, 2005). The relationship between insects and bacterial soft rot has been evaluated in the research of Leach (1926) who was credited to be the first to make a report in this regard. Leach (1926) revealed that

Hylemyia cili-crura, a seed-corn maggot, plays an important role in the development and spread of blackleg of potato in both field and under storage. Following the study by Leach (1926), there exists a symbiotic relationship between *Hylemyia cili-crura* and the phytopathogen of potato blackleg. The eggs of the seed corn maggot, may be contaminated with the phytopathogenic bacteria while being deposited in the soil and the inoculum of the phytopathogen may be introduced into pieces of planted potato by the young maggots. These pathogens and other microorganisms are present in the internal portion of the pupal and larval stages as well as on the surface of the eggs (Leach, 1930; 1933). In a related study, Leach (1927), found that two insects (*Scaptomyza graminum* and *Elachiptera costata*) are common agents of inoculation of celery heart rot also caused by *Erwinia carotovora,* the cause of blackleg and seed-piece decay in the potato. Bacterial pathogens as well as spores of other agents can also be transferred via aerosolized droplets during periods of rainfalls on either diseased plants or any other thing that is contaminated with *Erwinia*, an aerosol can be created where the bacteria is airborne in water. Around 50% can only survive as an aerosol for 5- 10 min, but this is long enough to travel many miles in a brisk wind. It has also been reported that *Erwinia carotovora* can be isolated or found in water as it has been isolated or recovered from "surface waters, reservoirs, streams, ditches, lakes, rivers and the sea (Perombelon, 2002).

2.5 Host Range of *Erwinia*

Numerous literatures (Garba *et al.* (2014), Howard and David (2007), Agrios (2006), Perombelon (2002) and Toth *et al.* (2003)) have reported that *Erwinia* and other phytobacteria implicated in soft rot have a wide assortment of host range, from several agricultural and scientifically significant crop species such as tomato, green peppers, African violets, carrot, onion, leafy greens, squash, potato and other cucurbits. Apeyuan (2000) opined that the soft rot bacteria are common with plants, which have fleshly storage tissues

such as tubers, tomato, root, stalk or leaves like lettuce and cabbage, and succulent stem. Wakil and Oyinola (2011) reported that *Erwinia* species can also induce rot on stored tubers as well as on the aforementioned plants species such as tomatoes, onions and pepper.

Arsenijevic (1978) reported that other plants such as pepper, pineapple, cabbage, cactus, tomato and cauliflower have been found to be infected by soft rot. More so, Tsuyama (1978) revealed that tobacco plant has been found to be susceptible to the bacterium hence confirming the pathogenicity of the bacterium. Robert and Blanchette (1994) revealed that the lignified walls of the world's most durable timber (*Eusideroxoylon zwageri*), which has known resistance to white and brown rot fungi, can be degraded by *Erwinia*. The phytopathogen has also been reported to infect the fruits of eggplant, carrot, sweet potato, apple, garlic, olive, lemon, apricot, onion, radish, squash, as well as turnip (Ismail *et al.*, 2012). The host range of *Erwinia* include several plant genera ranging from all families of fruits, ornamentals, root and tuber crops and vegetables (Opara and Asuquo, 2016). Rajeh and Khlaif (2000) revealed that *E. carotovora* was implicated as the major cause of soft rot in a survey of eighty-seven bacterial soft rot isolates recovered from several vegetable crops such as, pepper, potato, spinach, cabbage, lettuce, cauliflower, tomato, onion and sweet melon. Agrios (2006) reported that *E. carotovora* has been implicated as the cause of severe stem end decay as well as devastating postharvest soft rot, which give considerable losses to pepper. More so, it was also revealed that *Erwinia* is a pathogen of economic importance, which can cause a devastating postharvest loss in stored fruits and vegetables via disintegration/decay of plant tissues.

2.6 Disease Symptoms Caused by the Species *Erwinia*

Lumb *et al.* (1986) opined that the symptoms of the foliar parts of plants are common with infection by *Erwinia* and related species. Foliage symptoms are usually wilting at the top of the leaves with successive withering around the margins and by extension, the entirety of the

leaves. Foliage symptoms sometimes from the lower leaves do spread to the stem, and eventually the whole plant may dry out in extreme cases (Opara and Asuquo, 2016). Powelson and Franc (2001) described certain symptoms of bacterial soft rot on tubers which ranged from some vascular discoloration to total deterioration or spoilage of the tuber. Necrotic lesions develop first in the lenticels, at the attachment site of the stolon, or it could also be in the injury/wounds created on the tuber surface. Affected tuber tissues appear as cream- to tan-coloured and then becomes granular and soft. Elphinestone and John (2010) added that the decayed tissue usually develop pigments (brown to black in colour) at the margins.

2.7 Pathology of the Soft Rot *Erwinia*

E. carotovora usually results to eventual death of the plant via the disintegration or degradation of the fleshy succulent plant organs like the tubers, roots, thick leaves and stem cuttings through the creation of osmotically fragile cell (Opara and Asuquo, 2016). This degradation is possible via the ability to produce PCWDEs (Plant cell wall-degrading enzymes) (Bell *et al.,* 2004). PCWDEs include cellulase and extracellular pectic enzymes, which break down the cellulose and pectin respectively.

Amadioha (2012) and Oladoye *et al.* (2013) in detail, evaluated enzymes, which were able to induce hydrolysis of the middle lamella of certain tissues in the host plant following development of soft rot. They reported that extra-cellular enzymes are produced by spoilage organisms and these enzymes, which include cellulase, amylases, polygalacturanases (PG), zylanases as well as PMEs (pectin-methyl esterases) are able to degrade the components of the cell walls of susceptible plants. The enzymes are also able to induce dissolution of the middle lamella, (which functions as the bond between the adjacent cell walls thereby loosening the cells with a corresponding discharge of water and foul odor. The by-products released due to the growth of bacteria similarly set ex-osmosis of liquid, which contains salts

and sugar to the inter-cellular spaces from within the cells, where it functions as food source for further growth of bacteria. The continuance of this process explains the loss of consistency as well as the watery condition of the decayed tissues of plants. The direct involvement of cellulolytic as well as pectic or pectinolytic enzymes produced by pathogens implicated to cause soft rot have also been described by Garber *et al.* (1990) and Walker *et al.* (1994) respectively, cited in Opara and Asuquo (2016).

However, under artificial epiphytotics, there have been a report of yield losses up to 98.8% described by Thinda and Payakab (1985). The survivability of the bacterium in soil or plant debris has been confirmed and this guarantees the infection of a new plant may continue whenever opportunity arises. The decay or rot process is aggravated when factors such as high temperature (about 30 ^{o}C) is coupled with high relative humidity, which results in a faster of multiplication rate of the soft rot pathogens according to the report of Agrios (2006).

2.8 Virulence of Soft Rot Bacteria

Following the successful invasion of a plant, the bacterium finds habitation in the intercellular spaces or vascular tissues, from where it produces PCWDEs as described Perombelon (2002) Toth *et al.* (2003). Extracellular maceration enzymes, like the polygalacturonase (Peh), xylanase, pectate lyase (Pel), protease (Prt) as well as cellulase (Cel) are all significant pathogenic determinants or features of soft rot (Lee *et al.*, 2013). For *Erwinia,* the pectinases are enzymes of utmost importance with respect to bacterial virulence*,* meanwhile, a class of pectinases known as the endo-pectate lyase enzymes, play the most important role in the dissolution of the cell walls of plant. There are several evidences about how that the different pectate lyase enzymes differentially contribute to *Erwinia* virulence. A general secretory pathway secretes cellulase as well as pectinase in the soft rot *Erwinia* species, and this pathway is different from that used by the proteases. There is the wide distribution in most Gram-negative bacteria, implicated to cause plant and animal diseases.

Of these homologues, the protein components of the pectinase and cellulase pathways are often equally used for secreting *Erwinia* virulence (Lee *et al.*, 2013).

During infection by pathogens, plant structures, which are composed of cellulose, hemicellulose fibres as well as pectin, are degraded by these enzymes thus leading to necrosis of plant cell and maceration of tissues (Abbott and Boraston, 2008). For the pathogenesis of soft rot bacteria, a plethora of regulatory factors used for controlling these enzymes are also important. *P. carotovorum* uses quorum sensing (QS), defined as the communication system between bacterial cells, which are necessary for the regulation of several genes for pathogenesis (Lee *et al.*, 2013). For the QS of Gram-negative bacteria, the genes *ExpI/ExpR* are in charge of the production of AHL (acylhomoserine lactone), which in turn regulates the production of the aforementioned enzymes (Pirhonen *et al.*, 1991; Nasser *et al.*, 1998; Whitehead *et al.*, 2002; Henke and Bassler, 2004). Furthermore, *hexA, gacA* and *flhD* genes, participate in regulating the pathway for production of PCWDEs according to Cui *et al.* (2008). Numerous more genes such as *hel, trkA*, *yheL* as well as *cysQ,* are reported by Laasik *et al.* (2005) to be involved in producing/secreting PCWDE. In addition to factors mentioned above, there are several other factors required for the survivability of *P. carotovorum* in a host. Ability to take up nutrients as well as being motile are key for bacterial pathogenicity. In the year 1991, Pirhonen *et al.* recounted that mutant of *Erwinia carotovora*, which had impairment in motility-related genes, where observed to have reduced virulence in host plants. The constituents of the export of the organ of motility apparatus are related to T3SS (type III secretion system) evolutionarily (Aizawa, 2001) and T3SS are employed for the transportation of the factors of virulence into the host cells. More so, the developing organs of motility organ is directly associated with the secretion of the factors of virulence in soft rot bacteria as described by Chatterjee *et al.* (2009). Urbany and Neuhaus (2008) reported that the uptake of nutrient by phytopathogens from the tissues of the host plant, is also an integral

process in pathogenesis. More so, numerous reports in literatures about the relationship between pathogenicity in soft rot causing bacteria as well as other phytopathogens and the metabolism of purine and pyrimidine (Kim *et al.*, 2003; Chatterjee and Sonti, 2005). It has also been established that gluconate metabolism is essential for virulence in *P. carotovorum* according to Mole *et al.* (2010).

Soft rot phytobacteria for effective invasion and disease process must attach to the surface of the plant and resist the antibacterial chemicals produced by such plants. About a decade ago, it was reported that plant surface colonization is enhanced by the production of cellulose, which is essential for pathogen resistance to chlorine treatment in *Dickeya dadantii* as described by Prigent-Combaret *et al.* (2012). In the production or synthesis of the enzyme cellulase in *Dickeya dadantii*, the operon *Bcs* has been reported to be directly involved (Jahn *et al.*, 2011). However, in the same bacterium (*P. carotovorum*) the genes associated and responsible for attachment and survival are not well characterized in the necrotroph. Generally, the interactions between plants and this necrotrophic phytopathogen are not well understood as with biotrophs. Previous scholarly work by Laasik *et al.* (2005) and Cui *et al.* (2008) have respectively focused on PCWDEs production in *P. carotovorum,* but in this complex pathogenesis, several other factors are considered to be involved.

2.9 Control of the soft rot *Erwinia*

Several measures for effective control of soft rot bacteria are listed below and they include;

i. On first notice, all infected crops and rotted plants should be carefully and promptly destroy all infected crops and rotted plants, such as tubers, corms, roots, rhizomes, or other affected plant parts.
ii. Disease-free planting stock, which are devoid of bruises and cuts are to be planted only.
iii. Close planting and soils which are poorly drained should not be planted upon.

iv. Crop rotation for long duration should be practiced for crops such as grasses, corn and cereals when such crops are immune.

v. Insect vectors which are capable of transmitting the pathogen to initiate a new infection in a host plant or injure the host should be totally exterminated.

vi. Wounds on plants during the process of cultivation should be avoided as such, practices such as packing, digging, storing as well as handling during and after harvest should be done with utmost care.

vii. Crops should harvested only during the dry weather condition.

viii. Dry, plant produce and storage organs which are blemish-free, healthy, clean and mature should be kept or stored in a well-ventilated, clean and dry area at the recommended humidity level as well as temperature.

In the event that infected plant materials had previously been stored, such storehouses ought to be thoroughly sanitized and fumigated from floor to ceiling with an active disinfectant solution such as sodium hypochlorite (1000 to 1900 ppm). More so, tools should be disinfected by dipping in 70% solution of alcohol or 1% bleach solution (Prigent-Combaret *et al.*, 2012; Jahn *et al.*, 2011).

2.10 Other Postharvest Diseases of Sweet Potatoes

Black Rot (Caused by *Ceratocystis fimbriata*):

Ceratocystis fimbriata induces black rot, characterized by dark, sunken lesions exhibiting concentric rings and resulting in a dry and firm decay. This disease thrives in warm and humid conditions. Effective management strategies include stringent sanitation practices and the implementation of proper sweet potato curing processes (Vinayaka *et al.,* 2012).

Rhizopus Soft Rot (Caused by *Rhizopus stolonifer*):

Rhizopus stolonifer is responsible for rhizopus soft rot, presenting with cottony, white mycelium and tissue softening. High humidity and warm temperatures create an environment

conducive to its development. Adequate ventilation and maintaining appropriate storage temperatures are crucial for preventing the onset of rhizopus soft rot (Vinayaka *et al.,* 2012).

Fusarium Root Rot (Caused by *Fusarium* spp.):

Fusarium root rot is identified by brown lesions on the sweet potato roots, leading to wilting and eventual plant collapse. This disease is favored by moist soil and poor drainage. Implementing crop rotation and opting for disease-resistant sweet potato varieties are effective measures in managing Fusarium root rot (Vinayaka *et al.,* 2012).

Silver Scurf (Caused by *Helminthosporium solani*):

Helminthosporium solani induces silver scurf, evidenced by silver-gray lesions on the sweet potato surface. Cool and humid storage conditions foster the development of this disease. To combat silver scurf, proper curing practices and the application of fungicides during storage are recommended (Vinayaka *et al.,* 2012).

White Mold (Caused by *Sclerotinia sclerotiorum*):

White mold is caused by the fungus Sclerotinia sclerotiorum, resulting in white, fluffy mycelium and the formation of sclerotia. Cool and moist conditions promote its growth. To prevent white mold, it is essential to ensure good air circulation and avoid overwatering during storage (Vinayaka *et al.,* 2012).

Botrytis Rot (Caused by *Botrytis cinerea*):

Botrytis rot, attributed to the fungus Botrytis cinerea, is characterized by grayish-brown lesions with a fuzzy appearance. This disease thrives in conditions of high humidity and cool temperatures. Managing botrytis rot involves maintaining proper ventilation and reducing moisture levels during storage (Vinayaka *et al.,* 2012).

Bacterial Soft Rot (Caused by various bacteria):

Bacterial soft rot is a result of various bacteria and is recognized by slimy decay and a foul odor. Warm and humid storage conditions create a conducive environment for bacterial growth. Prevention involves rigorous sanitation practices and careful handling to minimize opportunities for wounds, which serve as entry points for bacterial infection (Vinayaka *et al.,* 2012).

Effectively managing these postharvest diseases requires a comprehensive approach, including proper curing, storage conditions, and disease control measures. By implementing these strategies, losses due to postharvest diseases can be minimized, ensuring the high quality and extended storage life of sweet potatoes (Vinayaka *et al.,* 2012).

2.11 Control of Postharvest Diseases

Hongyin *et al.* (2011) reported that postharvest diseases of fruits and vegetables result in considerable economic losses globally. The primary or main method for control of postharvest diseases has been through the use of synthetic fungicide treatment (Holliday 1980; Eckert and Ogawa 1998). However, the possible harmful effects on human health by these synthetic fungicides have raised global concerns over its indiscriminate usage (Norman, 1998) and emergence of phytopathogens which are resistant to fungicide (Holmes and Eckert, 1999) have made the situation even worse. Thus, better or novel replacements or alternatives to fungicides for the control of postharvest diseases, are in urgent demand. These alternatives are expected to low residues, with little or no toxicity to man at large or non-target organisms. For effective control of postharvest diseases, it must begin with pre-harvest processes in the field if losses are to be curtailed. Several strategies and approaches have been adopted for controlling postharvest disease and consequent losses to fruits and vegetables, which include but not limited to usage of bioactive compounds, heat treatment, biological control measures (Mari *et al.*, 2007), waxing, irradiation, disinfection and physical treatment as well as fumigation (Wilson *et al.*, 1991).

2.12 Biocontrol of Plant Diseases

The term “biological control” and the shortened synonymous term “biocontrol” have been applied in diverse areas of natural science (biology), with plant pathology (phytopathology) and entomology being the most notable microcosm. In entomology, it describes or entails the use of entomopathogenic nematodes, microbial pathogens or even live predatory insects, to suppress different population of insect pests. In phytopathology, it entails the usage of microbial antagonists (either bacterial or fungi) to suppress the development of disease condition in plant as well as entails using host-specific pathogen for effective control of weed population. Looking at both aforementioned microcosms, the agent, which suppresses the population of pests/pathogen(s) is recognized as the biocontrol agent according to Pal *et al.* (2006).

Globally, phytopathogens are not just a major threat to plant health but also a chronic threat to ecosystem stability and food security as described by Compant *et al.* (2005). Over the past decades, food producers became accustomed to the usage of agrochemicals and even more depended on it as a reliable method of producing crop with economic returns. However, the increased usage of agrochemicals have caused several negative effects, such as the development of resistant pathogens to the agents used for control as well as their broad spectrum nature in that they affect non-target organisms with huge environmental impacts (Gerhardson, 2002). Besides, the cost-effective nature of pesticides, predominantly in regions where poverty rules as well as consumer preference and demand for pesticide-free food has necessitated a search for alternatives of these agrochemicals. Biocontrol of plant disease is the usage of natural enemies of pathogens or pests for the effective control or eradication of their population (Compant *et al.*, 2005). It also could take the form of inducing the host plant resistance using non-pathogenic or incompatible microbes. The term has also generally been used or employed in the usage of extracted products of natural origin or fermented products

obtained from several microbes against a target pest or pathogens. These extracted natural products may be a compound containing complex or relatively simple mixtures of natural ingredients, having multiple or specific effects on the target pest or pathogen respectively. The numerous definitions of biocontrol offered in several studies have been controversial. A notable example is with, members of the USNRC (U.S. National Research Council), who defined biocontrol as "the use of natural or modified organisms, genes, or gene products, to reduce the effects of undesirable organisms and to favour desirable organisms such as crops, beneficial insects, and microorganisms". The above definition caused several debates and it was rather often considered to be too elaborate by several scholars who carried out field work. Published delineations of biocontrol vary subject to the target of suppression; source of biocontrol agents, their numbers as well as type; and lastly, the degree and timing of human intervention as described by Pal *et al.* (2006).

Broadly, biological control is defined as the process where the damaging activities of one pathogen(s) is suppressed by the action of one or more other agents (microbes), often known as their natural enemies. Concerning diseases of plant, the suppressing activity of the biocontrol agent can be obtained in numerous ways. When the activity of the grower(s) are considered as important, the cultural practices like crop rotation and planting of disease resistant varieties (whether it is genetically engineered or naturally selected) would be included in biocontrol definition. For the purpose of the fact that plant hosts respond to many biological factors, which could be non-pathogenic as well as pathogenic, induced systemic resistance in the host might also be considered as a form of biocontrol (Pal *et al.*, 2006). In a narrow sense, biocontrol refers to the purposeful usage of resident or introduced microbes, other than disease-resistant host plants, to suppress the population as well as activities phytopathogen(s). It may comprise of the usage of microbial inoculants for the suppression of plant disease(s). Alternatively, it may also involve the management of soils for the promotion

of the combined activities of plant-associated microbes and the native soil, which contribute to the general suppression of disease(s). In a much narrower sense, biocontrol of epiphytotics refers to the usage of a singular antagonist in a moncropping system to suppress a singular pest or pathogen (Sadfi *et al.*, 2002).

2.13 History of Biological Control

William Roberts was the first person credited to have first introduced the term 'antagonism' after he demonstrated the antagonistic action between bacteria and *Penicillium glaucum* in a liquid medium. This was in the year 1874 when he introduced the term antagonism (Junaid *et al.*, 2013). It was not until the year 1914 when for the first time, Von C.F. coined the term "biological control" as a plant disease management strategy or a plant disease control method. The earliest efforts of biological control as a plant disease management strategy directly involved the introduction of bacterial antagonists (*Pseudomonas cepacia* and *Bacillus subtilis*) as well as fungi (*Rhodotorula* and *Trichoderma*) in plant–pathogen interactive environment (Sadfi *et al.*, 2002). From that time, several microbial antagonists have been recognized and used for the control of diverse diseases of plants.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

Samples of decaying sweet potatoes and healthy sweet potatoes were obtained at Uselu and Aduwawa markets, Benin City, Edo State, Nigeria. A total of twenty (20) potato samples were purchased from both markets consisting of ten (10) samples per market. The ten samples composed of five (5) fresh samples and five (5) spoilt samples. They were collected in a sterile polythene bag, labelled properly and transported to the laboratory for analysis.

3.3 Preparation of Culture media

Nutrient agar medium

The laboratory preparation of Nutrient Agar Medium was a systematic process conducted to create a versatile growth medium suitable for cultivating bacteria. In the initial steps, 28 grams of nutrient agar were meticulously dissolved in 1000 ml of distilled water, creating a solution that served as the foundation for the medium. This mixture was then gently heated, allowing the agar to dissolve completely. Once the agar was fully dissolved, the medium was sterilized by autoclaving at 121°C for 15 minutes, ensuring the elimination of any potential contaminants.

After the sterilization process, the nutrient agar medium was cooled to a temperature ranging between 45-50°C, making it suitable for handling. The molten medium was then poured into sterile petri dishes and left undisturbed to solidify. This solidification resulted in a smooth and consistent surface, ideal for the growth and isolation of bacteria isolates.

Potato dextrose agar medium

The laboratory preparation of Potato Dextrose Agar (PDA) involved a series of steps to create a versatile medium suitable for the cultivation of fungi and molds. Commencing with the peeling and chopping of 200 grams of potatoes, these were boiled in 500 ml of distilled water

until achieving a softened state. The resultant potato extract underwent straining to eliminate solid particles, and to this extract, 20 grams of dextrose (glucose) were added. Augmenting the medium with 15-20 grams of agar, the mixture was carefully boiled to dissolve the agar thoroughly. Sterilization was then executed through autoclaving at a temperature of 121°C for 15 minutes. The cooled medium, now ranging between 45-50°C, was poured into petri dishes to solidify. The completed Potato Dextrose Agar provided a nutrient-rich substrate, facilitating the growth, isolation, and maintenance of various fungal species in laboratory settings.

3.3 Enumeration and isolation of bacteria from spoilt sweet potatoes

Based on the technique of Harrigan and McCance (2005), ten gram (10 g) of spoilt/diseased potato sample was dispensed into 90ml of sterile distilled saline water and the tube was shaken gently to ensure thorough mixing. After mixing, a serial dilution was carried out by transferring 1 ml from the first test-tube into the second test-tube which contained 9 ml of distilled water. The second tube was mixed gently and 1ml taken from the second tube into the third test-tube, and so on till the fifth test-tube.

Isolation of organisms was done using the pour plate method (Harrigan and McCance, 2005). One milliliter (1ml) each from the test-tubes (10^{-1} to 10^{-4}) was pipetted using a new sterile pipette into sterile Petri-dishes for nutrient agar and potato dextrose agar. Thereafter molten nutrient agar and potato dextrose agar were poured in the respective labelled plates. Nutrient agar was used for bacterial isolation while potato dextrose agar was used for fungi. The plates were incubated at 28±2°C for 24 hr (only NA plates) and 3-5 days for PDA.

3.4 Isolation of *Trichoderma viride* and *Bacillus* sp. from Agricultural soils

The antagonistic bacteria in this study were isolated from agricultural soil in the Faculty of Agriculture, University of Benin. Typical bacteria phenotype corresponding to Gram-positive aerobic short rod, capable of producing straw colour on *Bacillus cereus* agar (supplemented

with polymyxin B) was regarded as suspected *Bacillus subtilis* colony (Bridson, 2006). More so, the samples were screened for using the method of Okigbo and Emoghene (2004). The soils samples that were analyzed for presence of the biological control agent were serially diluted using a dilution factor of 10 and pour plated to obtain bacterial colonies where were subcultured to *Bacillus cereus* agar supplemented with polymyxin supplement.

Trichoderma species was isolated via pour plate method from soil samples by using potato dextrose agar (PDA) medium. PDA was prepared and autoclaved at 121 °C at 15psi for 15 min and antibiotics (streptomycin at 0.2g/L) was added to sterilized stock media just before pouring to inhibit bacterial growth. The plates were incubated at 26°C for 4-7 days. The fungal colonies were selected, purified by streaking and incubated at 26 °C for 7-8 days. Green conidia forming fungal bodies were selected and microscopic observations were carried out.

3.5 Identification of Bacterial Isolates

Following pour plate culture of bacterial isolates from the samples, single colonies were subcultured on tryptone soya agar and incubated for 24 hr. at environmental temperature (28 ± 2^0C). Differential Media (Oxoid) such as Chromogenic *Bacillus cereus* agar with chromogenic *Bacillus cereus* selective supplement, Sorbitol MacConkey agar with Cefixime-Tellurite Supplement, Eosine methylene blue agar, *Pseudomonas* cetrimide agar (supplemented with glycerol), *Salmonella Shigella* agar, Mannitol salt agar, and triple sugar iron agar slants were used for successful isolation and culture of bacterial isolates from samples. Further confirmation of bacteria identity were carried out using the following morphological characteristics and biochemical tests and identified using standardized cultural and biochemical techniques as stipulated by Bridson (2006) in Oxoid manual.

3.5.1 Morphological test

Gram Stain

Thin smears of the isolates were made on glass slides using a wire loop and were heat-fixed and allowed to cool. The smears were stained with crystal violet stain for a minute before washing off immediately with potable water. Then the smears were covered with Lugol's iodine for 30-60 sec and immediately washed off with water. The smears were rapidly decolorized with acetone or alcohol and washed rapidly with clean water after 5 sec. Then the smears were stained with slaframine for 60 sec and immediately washed off. The stained smears were allowed to air-dry after which a few drops of oil immersion were dropped on the smears after which they were viewed under the optical microscope using the 100x objective lens. The Gram-positive organisms were viewed as purple cells while the Gram-negative organisms were viewed as pink or red cells.

3.5.2 Biochemical Tests

These tests were conducted to determine the ability of the bacterial isolates to produce enzymes such as catalase, oxidase, and urease. Other biochemical tests were carried out to determine the ability of the bacteria to either utilize a sugar or substrate sources.

Catalase (Hydrogen peroxide; H_2O_2) Test

The biochemical test was carried out to assess and detect if the enzyme catalase is present. Catalase is an enzyme that catalyzes the release of oxygen from hydrogen peroxide with a resultant effervescence. Catalase catalyzes the breakdown of toxic H_2O_2 (hydrogen peroxide) into water and oxygen, which are harmless. The enzyme is produced or expressed by all aerobic organisms and thus it is a useful test in differentiating members of the aerobic and anaerobic organisms.

Methodology: A drop of H_2O_2 (3 %) was placed on a grease free slide to which a loopful of the bacterial isolate is applied. Positive catalase activity was shown by effervescence, while no effervescence indicates absence of the enzyme.

Oxidase Test

The biochemical test is basically carried out to identify the presence of the artificial electron acceptor (cytochrome-c-oxidase), which is able to reduce oxygen. It is used to detect the presence of the enzyme in bacteria. That is, if certain oxidases which are required for the transportation of electrons between tetramethyl-p-phenylene-diamine (the redox dye) and electron donors in the bacteria are present or not.

Procedure: A whatman filter paper was soaked with a solution of 1% tetramethlyphenylene diamine hydrochloride. A 24 hr culture of the test isolate(s) was smeared onto the impregnated filter paper. The presence of a purple colour, indicated a positive result.

Test for Urea Hydrolysis (Urease Test)

This was performed to show the capability of some bacteria to form an alkaline product (ammonia) via splitting urea under the influence or action of the enzyme urease.

Procedure: Urea was added to urease agar base before it was inoculated with the test organism in a slant. At optimum temperature incubation was done (37 ^{0}C) for 24-48 hr. The development of an intense pink/red color is indicative of a positive results while negative results show no colour.

Indole Formation Test

This biochemical test was performed to evaluate the capability of bacteria to produce indole via the hydrolysis of tryptophan. The spot indole test was used in this study to detect rapid indole producing organisms. This test is used to detect the presence of tryptophanase, an enzyme which catalyze the breakdown of tryptophan to release indole on reaction with cinnamaldehyde to produce a blue-green compound. When the enzyme is absent, there would be no colour production (indole negative).

Procedure: The filter paper was saturated with a 1% paradimethylaminocinnamaldehyde reagent. A colony of the culture to be tested was removed from the agar surface using a loop and then transferred to the surface of the filter paper that had already been saturated with the reagent. A positive result was confirmed when a blue color developed within 30 seconds. In most cases, indole-producing organisms turned blue within 30 seconds to one minute. The development of a slightly pink coloration or no color change at all was indicative of a negative result.

Citrate Utilization Test (Simon Citrate Agar (SCA) Slant)

SCA slants were used for this biochemical testing procedure. It is usually performed to evaluate the capability of the bacterium to utilize citrate as its sole carbon source. The biochemical medium contains sodium citrate (sole carbon source), bromothymol blue (indicator) as well as ammonium dihydrogen phosphate (nitrogen source).

Procedure: The medium was prepared as a slant using a test tube, and the bacteria isolates to be tested were cultured. They were allowed to stand for 24 hours in an incubator. The development of a blue color indicated a positive reaction to citrate, while no color change or the retention of the green color of the medium indicated a negative reaction.

Triple Sugar Iron Agar Test

This test is used to evaluate the efficacy of bacterial isolates (particularly those of Gram-negative group) to fermentatively utilize glucose, lactose and/or sucrose as well as produce hydrogen sulfide (H_2S) gas. The composition of the medium include 1 part of glucose and peptone: 10 parts of sucrose: 10 parts of lactose. Phenol red and ferrous sulphate serves as an indicator for acidification of medium and H_2S production respectively. The medium was prepared according to manufacturer's instruction and it was prepared in tubes and placed in a slant position at an angle of about 60^0. The medium was allowed to solidify before it was

inoculated with the test bacterium of interest. The bottom part of the tube (butt) was first inoculated by stabbing through the medium to the base and then the slant portion of the medium is inoculated next by streaking. The medium was incubated for 18-24 hr. before the results were read using standard chart. Results for TSI is either by fermentation of glucose which could turn the entire medium to yellow (acidic) within 8 to 12 hr. The butt of the TSI agar will remains acidic even after 18 to 24 hr incubation period because of the presence of organic acids resulting from the fermentation of glucose under anaerobic conditions in the butt of the tube. The slant reverts to alkaline state that is indicated by red color as the fermentation products gets oxidized to carbon dioxide (CO_2) and water (H_2O) and peptone in aerobic condition the slant undergoes oxidation releasing alkaline amines (Phenol red in alkaline pH turns red while in acidic pH turns yellow). The results and possible interpretation of the test is shown below.

Other Sugar Fermentation test:

Mannitol Fermentation was confirmed by growth on mannitol salt agar with ability to turn the pink medium into yellow.

3.6 Identification of Fungal isolates

After successful enumeration, the fungal isolates were subcultured on PDA and the molds and yeasts were morphologically characterized after being stained with Lactophenol cotton blue. The results obtained were then compared with standard references for proper identification of the isolates (Cheesbrough, 2004).

A drop of lactophenol blue stain was placed on a clean grease free sterilized glass slide and after this a sterile inoculating wire loop was used to pick the mycelium unto the glass slide from the mold culture. The mycelium was spread evenly on the slide and then covered with cover slips gently and then allowed to stay for some seconds before observing under x40 under the microscope. The colonial and morphological characteristics of each isolate were

determined, appearance of special structures including the nature of spore/ascospores if produced. The growth, the appearance of the colony from initial to the time of maturity was also taken into consideration as well as the presence or absence of septate hyphae (Cheesbrough, 2004).

3.7 Pathogenicity test

Fresh and healthy tubers of potatoes were washed with tap water and surface sterilized with 70% ethanol. Cylindrical cores were removed from the tubers with the help of a 5mm cork borer. Four-millimeter (4 mm) agar discs containing 7days old cultures of the isolates was introduced into the holes and sealed with the sterile Vaseline. Controls was be set up. All the treated tubers was placed singly into sterile polythene bags and incubated at 28±2 °C for 10 days. The tubers were cut through and examined for rot at the end of the incubation period (Pinchuk *et al.,* 2001).

3.8 Antagonistic Activity of *Trichoderma* soft rot pathogens

The antagonistic properties of *Trichoderma viridae* was investigated against the spoilage organisms isolated from potato. Assay was performed in Petri plates (90 mm) containing 20 ml of PDA, allowed to dry for 3 days. An entire pure culture, 4 mm in diameter obtained from a day old culture of *Trichoderma viridae* and pathogenic organisms were cut using a cork-borer. Pathogens disks were transferred 10 mm from the edge of each Petri dish. Each *Trichoderma* sp. inoculant was placed 10 mm from the other edge, opposite to the pathogens. The inoculated plates were incubated upside-down at 25°C, and was observed for inhibition or otherwise of their growth for 8 days. The control Petri dishes was inoculated each alone with pathogens. The radial growth of the pathogens in the control and the treated Petri dishes was measured every 24 h, and the inhibition percentage of the antagonism was calculated according using the following formula:

The percentage inhibition = Rl - R2 / Rl

Where Rl is the value of radial growth of pathogen in control plates and R2 is the radial growth of the pathogen in the treated plates (Odebode, 2006).

3.9 *In vitro* biological control potential of *Bacillus subtilis* against bacterial isolates

The *in vitro* biological control efficacy of the antagonist (*B. subtilis*) against the bacterial pathogens capable of inducing soft rot was carried out on Mueller-Hinton agar plates. The antagonist and pathogen were standardized using McFarland turbidity standard (1.5 $x10^8$ cells/ml) and cultured by streaking across the entire Petri dishes separately. Briefly, a cork borer of 8 mm size was used to excised a definite portion of media containing bacterial cells (antagonist), which was transferred to a cultured bacterial medium containing the pathogen. The excised portion of media containing the antagonist and the culture to which it was transferred were incubated simultaneously overnight. Visible zones of inhibition was seen on the pathogen as a function of the presence of the antagonist. Control plates (positive and negative) were prepared using only the pathogen and antagonist in isolation.

3.10 Statistical Analysis

Data were subjected to descriptive statistics and were analyzed using Microsoft Excel version 2019. Mean and standard deviation of data were taken and used for analysis.

CHAPTER FOUR

RESULTS

The heterotrophic bacterial counts for spoilt/diseased potato ranged from $\log_{10}$ 3.65±0.41 cfu/g (Uselu market) to $\log_{10}$ 4.02±0.25 cfu/g (Aduwawa Market), while the fungal counts ranged from $\log_{10}$ 3.57±0.30 cfu/g (Aduwawa market) to $\log_{10}$ 3.85±0.10 cfu/g (Uselu market).

The cultural, morphological and biochemical characteristics of bacterial isolates from spoilt potato were *Erwinia* sp.*, Pseudomonas* sp.*, Bacillus* sp. and *Staphylococcus aureus* while the fungal isolates from spoilt potato were *Aspergillus* sp.*, Rhizopus* sp. and *Penicillium* sp.. Similar isolates save for spoilage causing bacteria (*Erwinia* sp.) were obtained from agricultural soils. The pathogenicity or spoilage test of isolated microorganisms from spoilt to healthy potato tubers showed that only *Erwinia* sp. and *Aspergillus* sp. were found to induce disease or spoilage condition in potato tubers.

The *in vitro* antagonism (percentage inhibition) of *Bacillus* sp. and *Trichoderma viride* on growth of *Aspergillus* sp. and *Erwinia* sp. showed that *Trichoderma viride* had more biocontrol effect on growth of *Aspergillus* sp. (50%) and *Erwinia* sp. (47%) after 5 days of culture on growth media.

Table 4.1: Cultural, morphological and biochemical characteristics of isolated bacteria from soils and spoilt potato

Cultural				
Elevation	Low convex	Low convex	Low convex	Convex
Margin	Smooth	Entire	Smooth	Smooth
Colour	Cream	Cream	Cream	Cream
Shape	Circular	Circular	Circular	Circular
Morphological				
Gram stain	+	-	+	-
Cell type	Cocci	Rod	Rod	Rod
Arrangement	Clusters	Single	Single	Single
Spore staining	ND	ND	+	ND
Biochemical				
Catalase	+	+	+	+
Indole	ND	-	+	-
Citrate	+	+	+	+
Urease	ND	+	ND	+
Oxidase	ND	+	ND	-
Gr. Diff. Agar	MSA	PCA	BCA	MCC
Identity	*Staphylococcus aureus*	*Pseudomonas* sp.	*Bacillus* sp	*Erwinia* sp.

Legend: MSA= Mannitol salt agar, MCC= MacConkey agar, PCA= *Pseudomonas* cetrimide agar; BCA= Bacillus cereus agar, ND=Not determined, Gr. Diff. Agar = growth on differential agar, + = positive, - = negative

Table 4.2: Cultural, morphological and microscopic properties of identified fungal isolates from spoilt potato tubers and soil

Colour of mycelium on agar plate	Dark brown colored growth	Green mycelium	Army green and entire, non-luxuriant with concentric ring	Initially white, with age turning gray and developing black dots
colour of plate culture reverse	Dark	Pale yellow	Orange	light gray
Microscopic characteristics				
Nature of hyphae	Septate	Septate	Septate	Non- septate
Type of Spore	Conidiospore	Conidiospore	Conidiospore	Sporangiophores
Spore structure/Attachment	consists of a smooth and colorless conidiophores and spores.	Conidia size and shape are similar to Penicillium and Aspergillus but Trichoderma forms sticky clumps of conidia with a distinctive green pigment rather than in chains. Typical green spore clumps are identified as Trichoderma.	clear (not pigmented) hyphae with smooth-walled conidiophores, stipes are rather long and is biverticillate	single and unbranched sporangiophore
Rhizoids	Absent	Absent	Absent	Present
Appearance of special structure	Conidial heads radiate, becoming columnar when mature; conidiophores are long and smooth-walled; biseriate; two rows of phialides cover the entire vesicle.	Conidiophores hyaline and loosely branched at right angles. Phialides flask-shaped and inflated at the base, with very short collarettes	Conidiophore stipes smooth-walled; phialides mono- or biverticillate, flask-shaped. Phialides do not show long pointed extensions at the tips	Rhizoids occur at the junction of stolon and sporangiophore
Class of fungi	Ascomycetes	Ascomycetes	Ascomycetes	Zygomycetes
Possible Identity	*Aspergillus* sp.	*Trichoderma* sp.	*Penicillium* sp.	*Rhizopus* sp.

Table 4.3: Distribution of microbial isolates from soil and spoilt potato tubers

Bacteria	Spoilt potato tuber	Soil sample
Erwinia sp.	+	-
Staphylococcus aureus	+	+
Bacillus sp.	+	+
Pseudomonas isp.	+	+
Fungi		
Rhizopus sp.	+	+
Trichoderma viride	+	+
Aspergillus sp.	+	+
Penicillium sp.	+	+

Key: + = present, - = negative

Table 4.4: Pathogenicity test of microbial isolates from spoilt and healthy potato tubers

Bacteria	24 hr	48 hr	72 hr	96 hr	Spoilage Signs
Erwinia	-	+	+	+	Necrosis around inoculation point
Staphylococcus	-	-	-	-	**No sign of spoilage**
Bacillus	-	-	-	-	**No sign of spoilage**
Pseudomonas	-	-	-	-	**No sign of spoilage**
Fungi					Spoilage Signs
Rhizopus	-	-	-	-	**No sign of spoilage**
Trichoderma	-	-	-	-	**No sign of spoilage**
Aspergillus	-	+	+	+	Necrosis around inoculation point, soft tissues
Penicillium	-	-	-	-	**No sign of spoilage**
Control	-	-	-	-	**No sign of spoilage**

Key: + = positive for disease sign, - = negative

CHAPTER FIVE

DISCUSSION

Bacteria and fungi are known to be the major cause of postharvest disease or spoilage of potato accounting for huge losses. The high density of microbes observed for diseased or spoilt tubers could be attributed to the nature, and quality of the tubers since the integrity of the plant cell wall integrity has been compromised, thereby causing the microbes (including saprophytes, spoilage or pathogens) to gain easy access to the tubers (Agrios, 2005). For infection, deterioration, degradation or spoilage of the fruit to occur, the layer of protection must be breached thus giving grounds to saprophytes and pathogens to carry out their activities. This was also evident in this study as some of the microbes obtained in the spoilt/diseased tubers were found to be saprophytes as they could not initiate disease in healthy tubers (typified by necrosis of cells and tissues). Most plant pathogenic fungi and bacteria are also able to colonize plants once there are openings on them and this explains why there might be more fungal and bacterial density in spoilt/diseased tubers.

Bacterial isolates obtained from deteriorated potatoes exhibited cultural, morphological, and biochemical characteristics indicative of *Erwinia viride, Pseudomonas* sp*., Bacillus* sp*. and Staphylococcus* sp. Conversely, fungal isolates derived from spoiled potatoes demonstrated characteristics associated with *Aspergillus* sp*., Rhizopus* sp *and Penicillium* sp. This result was consistent with the work of Ogundipe *et al.* (2012) and Agbabiaka *et al.* (2015) who reported that *Salmonella, Escherichia, Bacillus* and *Pseudomonas* can be isolated from diseased/spoilt tomato fruits. More so, Guo *et al.* (2001) reported the presence of a plethora of bacterial and fungal isolates associated with diseased/spoilt potatoes amongst which were *Rhizopus, Aspergillus, Bacillus* and *Pseudomonas* also found in this study.

Basic Koch postulates involving four major rules were followed to determine the pathogenic or spoilage microbial isolates associated with potato tubers. This test differentiates and separates pathogens from non-pathogens or saprophytes associated with tomato fruit. Amongst all microbial isolates evaluated in the study, only two potential pathogens were implicated in to cause rot (*Erwinia viride* and *Aspergillus* sp.). Their wide host range set them apart from several other plant pathogens known to have a narrow host range (Agrios, 2005). They have been implicated to cause disease in both root and tuber crops, vegetables and certain fruit crops. Their involvement in rot is nothing less of a surprise when compared to the other saprophytes found in this study.

The ability of the bacteria isolate in the study to cause disease in sweet potatoes could be due to the presence of certain enzymes known to disrupt the integrity of plant cell walls. These eextracellular maceration enzymes, such as pectate lyase, cellulase, polygalacturonase, xylanase and protease, are important pathogenic factors of soft rot bacteria (Lee *et al.*, 2013). The enzyme of greatest significance in *Erwinia* virulence is reported to be the pectinases, and within this class, the endo-pectate lyases play a major role in the dissolution of the plant cell wall. This could be attributed to the dissolution of potato cell wall. There are also reports that the different pectate lyases differentially contribute to *Pectobacterium* virulence. Previous studies have focused on PCWDE enzyme production in *P. carotovorum* (*Erwinia*) (Cui *et al.*, 2006; Laasik *et al.*, 2005), but many more factors are considered to be involved in its complex pathogenesis.

The findings in this study were consistent with the several reports in literature about the ability of *Bacillus subtilis* to control a plethora of plant pathogens. Etebu *et al.*, (2013) gave a report which was consistent with the findings in this study that *B. subtilis* is able to control bacterial soft rot of tomato and vegetable crops. It has also been reported that deployment of *B. subtilis* as a biological control agent has been found to cause a systemic change in the

physiology of the plant during metabolism as reported in 2006 and 2005 by Abd-Allah *et al.* and AbdAllah and Ezzat respectively.

Conclusion

In conclusion, the investigation into the antagonistic activity of *Trichoderma viride* and *Bacillus* sp. against potato soft rot pathogens yields valuable insights. The study identifies *Erwinia* sp. and *Aspergillus* sp. as significant contributors to potato spoilage, with only these microorganisms inducing disease in healthy potato tubers. The in vitro biocontrol experiments demonstrate the substantial inhibitory effects of *Trichoderma viride*, particularly exhibiting a 50% and 47% reduction in the growth of *Aspergillus* sp. and *Erwinia* sp., respectively, after 5 days of culture. These findings underscore the potential of *Trichoderma viride* and *Bacillus* sp. as effective biocontrol agents, offering promising and sustainable alternatives for managing potato soft rot in cultivation and storage practices.

REFERENCES

Abbott, D.W. and Boraston, A.B. (2008). Structural biology of pectin degradation by Enterobacteriaceae. *Microbiology and Molecular Biology Review* **72**:301–316.

Abd-Allah, E.F. and Ezzat, S.M. (2005). Role of lipid metabolism through bioremediation of fusaric acid in germinating peanut seedlings. *Phytoparasitica* **32**:38-42.

Abd-Allah, E.F., Ezzat S.M. and Tohamy, M.R. (2007). *Bacillus subtilis* as an alternative biologically based strategy for controlling fusarium wilt disease in tomato: A histological study. *Phytoparasitica* **35**: 474-478.

Abd-Allah, E.F., Ezzat, S.M., Sarhan, M.M. and Metteleb, A.A.A. (2003). Biocontrol of peanut southern blight (*Sclerotium rolfsii*) by *Bacillus subtillis*. *Egypt Journal of Microbiology* **38**:207-216.

Abd-Allah, E.F. and EL-Didamony, G. (2007). Effect of seed treatment of Arachis hypogaea with Bacillus subtilis on nodulation in biocontrol of southern blight (*Sclerotium rotfsii*). disease. *Phytoparasitica* **55:**8-12.

Abd-Allah, EF, Hashem, A. and Ezzat, S.M. (2006). Lipid metabolism in tomato and bean as a sensitive monitor for biocontrol of wilt diseases. *Phytoparasitica* **34**: 516-522

Abeer, A. and Fawzi, A. (2013). Biological control of *Erwinia carotovora* Subsp *carotovora* by *Pseudomonas fluorescens*, *Bacillus subtilis* and Bacillus *thuringiensis. International Journal of Chemical, Environmental & Biological Sciences* **1**: 771-775.

Agbabiaka, T.O., Saliu, B.K., Sule, I.O., Oyeyiola, G.P. and Odedina, G.F. (2015). Microbial deterioration of tomato fruit (*Lycopersicum esculentum*) sold in three popular markets in Ilorin, Kwara State, Nigeria. *Fountain Journal of Natural and Applied Sciences* **4**(1): 10 – 18.

Agrios, G.N. (1998). *Plant Pathology (3rd Edition).* Academic Press, San Diego 803 pp.

Agrios, G.N. (2005). *Plant Pathology (5th Edition).* Academic Press, San Diego 896 pp.

Agrios, G.N. (2006). *Bacterial Soft Rots* (5th Edn.). Academic Press, San Diego 321pp.

Aizawa, S.I. (2001). Bacterial flagella and type III secretion systems. *FEMS Microbiology* **202**:157–164.

Ajith. P.S. and Lakshmidevi, N. (2010). Effect of volatile and non-volatile compounds from *Trichoderma* spp. against *Colletotrichum capsici* incitant of anthracnose on bell peppers. *Nature and Science* **8**: 265-269.

Amadioha, A. C. (2012). *Reducing Food Losses Through Sustainable Methods of Plant Disease Management: An Imperative for Actualization of Food Security in Nigeria.* A paper presented at the 13th Inaugural Lecture of MOUAU, June, 2012 25pp.

Apeyuan, K.D. (2000). *Comparative Susceptibility of Plant Storage Organs to Soft Rot Bacteria* (*E. carotovora*). Ahmadu Bello University, Zaria, 86pp.

Ashwani, T., Upender, S., Jaime, A.T., Gurpreet, S., Shipra G. and Rajesh K, (2011). *In Vitro* antagonism *of Trichoderma viride* against five phytopathogens. *Pest Technology* **5**(1): 59-62.

Bridson, E.Y. (2006). *The Oxoid Manual* (9th Edition). Oxoid Limited, Basingstoke Hamsphire, England 623 pp.

Chatterjee, A., Cui, Y. and Chatterjee, A. K. (2009). RsmC of *Erwinia carotovora* subsp. *carotovora* negatively controls motility, extracellular protein production, and virulence by binding FlhD and modulating transcriptional activity of the master regulator. *Journal of Bacteriology* **191**:4582–4593.

Chatterjee, S. and Sonti, R. V. (2005). Virulence deficiency caused by a transposon insertion in the purH gene of *Xanthomonas oryzae*. *Canadian Journal of Microbiology* **51**:575–581.

Cheesebrough, M. (2004). District laboratory practice in tropical countries. Part 2. Cambridge University Press; p.357.

Compant, S., Duffy, B., Nowak, J., Cle´ment, C. and Ait Barka, D. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology* **71**(9): 4951-4960.

Cui, Y., Chatterjee, A., Hasegawa, H. and Chatterjee, A. K. (2006). *Erwinia carotovora* subspecies produce duplicate variants of *ExpR, LuxR* homologs that activate rsmA transcription but differ in their interactions with N-acylhomoserine lactone signals. *Journal of Bacteriology* **188:**4715–4726.

Czajkowski, R., Perombelon, M. C. M., Van-Veen, J. A. and Van-Der-Wolf, J. M. (2011). Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. *Plant Pathology* **10:** 1365–3059.

Dev, N. and Dawande, A.Y. (2010). Biocontrol of soil borne plant pathogen *Rhiozoctonia solani* using *Trichoderma* spp. and *Pseudomonas fluorescens. Asiatic Journal of Biotechnology Resources* **1**:39-44.

Eckert, J.W and Ogawa, M.J. (1998). The Chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root tuber crops. *Annual Review of Phytopathology* **26**:433-469.

Eckert, J.W. and Ogawa, J.M. (1985). The chemical control of postharvest diseases: subtropical and tropical fruits. *Annual Review of .Phytopathology* **23**:421–454.

Elphinestone, E. and John, G. (2010). *Soft Rot and Blackleg of Potato.* Technical Informational

Emoghene, A.O. and Futughe, A.E. (2016). Fungi as alternatives to agrochemicals to control plant diseases. **In:** *Fungal Applications in Sustainable Environmental Biotechnology*. Purchase, D. (Editor). Springer, Switzerland, 43-62 pp.

Erinle, I.D. (1989). Present status and prospect for increased production of tomato and pepper in Northern Nigeria Procedure of international symposium. Integrated Management Practices. AVRD, Tainan, Taiwan

Etebu, E. and Bawo, D.D.S. (2012). The effect of treatment methods and storage conditions on postharvest disease and fungal quality of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke). *Food Science Quality Management* **10**:7-27.

Etebu, E. and Enaregha, E. (2013). Postharvest quality of commercial tomato (*Lycopersicon esculentum* Mill.) Fruits Brought into Yenagoa Metropolis from Northern Nigeria. *Journal of Biology, Agriculture and Healthcare* **3**: 49-55.

Etebu, E., Nwauzoma, A. and Bawo, D. (2013). Postharvest spoilage of tomato (*Lycopersicon esculentum* Mill.) and control strategies in Nigeria. *Journal of Biology, Agriculture and Healthcare* **3**:51-61.

Food and Agriculture Organization of the United Nations (2003). Summary of Food and Agricultural Statistics. Rome, Italy. 56p.

Garba, H., Danladi, S. M., and Sule S. M. (2014). Isolation and identification of bacteria and fungi associated with rots of *Citrullus lanatus* and *Capsicum frutescence* in Sokoto markets. International Conference on Advances in Agricultural, Biological and Environmental, 12th July, 2014.

Gerhardson, B. **(**2002). Biological substitutes for pesticides. *Trends in Biotechnology* **20:**338–343.

Guo, X., Chen, J., Brackett, E. and Beuchat, L.R. (2001). Survival of Salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Applied and Environmental Microbiology* **67**: 4760-4764.

Gupta, S.K. and Thind, A. (2006). *Diseases of Cruciferous Vegetables*. Scientific Publishers,

India, 286p.

Henke, J.M. and Bassler, B. L. (2004). Bacterial social engagements. *Trends In Cell Biology* **14**: 648–656.

Holliday, P. (1980). Fungal Disease of Tropical Crops. Cambridge University Press. 340pp

Holmes, G.J and Eckert, J.W. (1999). Sensitivity of *Pencillium digitatum* and *Penicillium italicum* to postharvest citrus fungicides in California. *Phytopathology* **89**:716-721.

Hongyin, Z,. Renping, L., and Weimin, L. (2011). Effect of chitin and its derivative chitosan on postharvest decay of fruits. *International Journal of Molecular. Science* **12**:917-934.

Howard, F.S. and David, H.G. (2007). Onion bacterial soft rot**;** high plains IPM guide, a cooperative effort of the University of Wyoming, University of Nebraska, Colorado State University and Montana State University.

Ismail, M. E., Abdel-Monaim, M. F. and Mostafa, Y. M. (2012). Identification and pathogenic bacteria associated with soft rot disease of girasole tuber in Egypt. *Journal of Bacteriology Research* **4**(1*):*1-8.

Janisiewicz, W.J. and Korsten, L. (2002). Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology* **40**:411–412.

Junaid, J.M., Dar, N.A., Bhat, T.A., Bhat, A.F. and Bhat, M.A. (2013). Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *International Journal of Modern Plant and Animal Sciences* **1**(2): 39-57.

Kader, A.A. (2004). Increasing food availability by reducing postharvest losses of fresh produce. *Acta Horticulture* **682**: 2169-2176.

Laasik, E., Ojarand, M., Pajunen, M., Savilahti, H. and Ma¨e, A. (2005). Novel mutants of *Erwinia carotovora* subsp. *carotovora* defective in the production of plant cell wall

degrading enzymes generated by Mutransposososome-mediated insertion mutagenesis. *FEMS Microbiology* **243:**93–99.

Leach, J. G. (1926). The relation of the seed corn maggot *Phorbia fusciceps* zett to the spread and development of potato blackleg in Minnesota. *Phytopathology* **16**: 149-176.

Leach, J. G. (1930). Potato blackleg: the survival of the pathogen in the soil and some factors influencing infection. *Phytopathology* **20**:215- 228.

Leach, J.G. (1925). The seed corn maggot and potato blackleg. *Science* **61**:120.

Leach, J.G. (1927). The relation of insects and weather to the development of heart rot of celery. *Phytopathology* **17**:663- 667.

Leach, J.G. (1933). The method of survival of puparia of the seed corn maggot (*Hylemyia cilicrura* Rond.). *Phytopathology* **20**: 150-161.

Lee, .J.T., Bae, D.W., Park, S.H., Shim, C.K., Kwak, Y.S. and Kim, H.K. (2001). Occurrence and biological control of postharvest decay in onion caused by fungi. *Journal of Plant Pathology* **17:**141–148.

Lee, D.H., Lim, J.A., Lee, J., Roh, E., Jung, K., Choi, M., Oh, C., Ryu, S., Yun, J. and Heu, S. (2013). Characterization of genes required for the pathogenicity of *Pectobacterium carotovorum* subsp. *carotovorum* Pcc21 in Chinese cabbage. *Microbiology* **159**: 1487–1496.

Lumb, V.M., Perombelon, M.C.M. and Zutra, D. (1986). Studies of a wilt disease of the potato plant in Israel caused by *Erwinia chrysanthemi*. *Plant Pathology* **35**:196-202.

Lund, B.M. (1992). Ecosystems in vegetable foods. *Journal of Applied Bacteriology Symposium Supplement* **73:**115-126.

Mari, M., Neri, F. and Bertolini, P. (2007). Novel approaches to prevent and control postharvest diseases of fruits. *Stewart Postharvest Review* **3**:1-7.

Mole, B., Habibi, S., Dangl, J. L. and Grant, S. R. (2010). Gluconate metabolism is required for virulence of the soft-rot pathogen *Pectobacterium carotovorum*. *Molecular Plant Microbe Interaction* **23**:1335–1344.

Naher, L., Yusuf, U., Ismail, A. and Hossain, K. (2014). *Trichoderma* spp: A biocontrol agent for sustainable management of plant diseases. *Pakistan Journal of Botany* 46(4):1489-1493.

Nasser, W., Bouillant, M. L., Salmond, G. and Reverchon, S. (1998). Characterization of the *Erwinia chrysanthemi* expI-expR locus directing the synthesis of two N-acyl-homoserine lactone signal molecules. *Molecular Microbiology* **29:** 1391–1405.

Odebode, A.C. (2006). Control of postharvest pathogens of fruits by culture filtrates from antagonistic fungi. *Journal of Plant Protection Research* 46(1): 200.

Ogundipe, F.O., Bamidele, F.A., Adebayo-Oyetoro, A.O., Ogundipe, O.O. and Tajudeen, O.K. (2012). Incidence of bacteria with potential public health implications in raw *Lycopersicum esculentum* (tomato) sold in Lagos State, Nigeria. *Nigerian Food Journal* **30**(2): 106 – 113.

Okigbo, R. N. and Emoghene, A. O. (2004). Antifungal activity of leaf extracts of some plant species on *Mycosphararella fijiensis* Morelet, the causal organism of black sigatoka disease in banana *(Musa acuminata*). *Science Journal* **4**: 20-31.

Oladoye, C.O., Olaoye, O. A. and Cornnerton, I.F. (2013). Isolation and identification of bacteria associated with spoilage of sweet potatoes during postharvest storage. *Internation*al *Agricultural Food Science* **3**(1):10-15.

Oladoye, C.O., Olaoye, O.A. and Cornnerton, I.F. (2013). Isolation and identification of bacteria associated with spoilage of sweet potatoes during postharvest storage. *International Journal of Agriculture and Food Science* **3**(1): 10-15.

Onifade, A., Atum, H.N. and Adebolu, T.T. (2004). Nutrition enrichment of sweet potato *(Ipomoea batatas* L.) by solid substrate fermentation using four fungal species. *Global Journal of Pure and Applied Science* **10**(60): 31-36.

Opara, E.U. and Asuquo, A.A. (2016). An overview of characterization and identification of soft rot bacterium *Erwinia* in some vegetable crops. *Greener Journal of Biological Sciences* **6**(3):046-055.

Oyeyipo, O.O. (2012). Bio-deterioration of sweet potato *(Ipomoea batatas* Lam.) in storage, inoculation-induced quality changes, and control by modified atmosphere. *Journal of Applied Science and Environmental Management* **16**(2): 189-193.

Pal, K. K. and McSpadden, B. G. (2006). Biological Control of Plant Pathogens. *The Plant Health Instructor*.1-25pp.

Pérombelon M.C.M. and Kelman, A., (1980). Ecology of the soft rot *Erwinia*. *Annual Review of Phytopathology* **18**:361– 387.

Perombelon, M.C.M. (2002). Potato diseases caused by soft rot *Erwinia*: An overview of pathogenesis. *Plant Pathology* **51**: 1-12.

Pinchuk, I.V., Bressollier, P., Verneuil, B., Fenet, B. and Sorokulova, I.B. (2001). *In vitro* anti-*Helicobacter pylori* activity of the probiotic strain *Bacillus subtilis* is due to secretion of antibiotics. *Antimicrobial Agents and Chemotherapy* **45**: 3156-3161.

Pirhonen, M., Saarilahti, H., Karlsson, M.B. and Palva, E.T. (1991). Identification of pathogenicity determinants of *Erwinia carotovora* subsp. *carotovora* by transposon mutagenesis. *Molecular and Plant Microbe Interaction* **4**:276–282.

Powelson, M.L. and Franc, G.D. (2001). Blackleg, aerial stem rot and tuber soft rot. *In*: *Compendium of Potato Diseases (*2nd edition). Stevenson, W.R., Loria, R., Franc, G.D. and Weingartner, D.P. (eds.). APS Press, St Paul, Minnesota, USA. 10-11pp.

Prigent-Combaret, C., Zghidi-Abouzid, O., Effantin, G., Lejeune, P.,Reverchon, S. and Nasser, W. (2012). The nucleoid-associated protein Fis directly modulates the synthesis of cellulose, an essential component of pellicle-biofilms in the phytopathogenic bacterium, *Dickeya dadantii. Molecular Microbiology* **86**:172–186.

Rajendiran, R., Jegadeeshkumar, D., Sureshkumar, B.T. and Nisha, T. (2010). *In vitro* assessment of antagonistic activity of *Trichoderma viride* against post-harvest pathogens. *Journal of Agricultural Technology* **6**: 31-35.

Rolston, L. H., Clark, C. A., Cannon, J. M.; Randle, W. M.; Riley, E. G., Wilson, P. W., Robbins, M. L. (1987). Beauregard' Sweet Potato. *HortScience,* **22**(6): 1338–1339

Sadfi, N., Che´rif, M., Fliss, I., Boudabbous, A. and Antoun, H. (2001). Evaluation of bacterial isolates from salty soils and *Bacillus thuringiensis* strains for the biocontrol of *Fusarium* dry rot of potato tubers. *Journal of Plant Pathology* **83**:101–118.

Salami, O.A. and Popoola, O.O. (2007). Thermal control of some posthan-est rot pathogens of Irish potato *(Solatium tuberosum* L.). *Journal of Agricultural Science* **52**(1): 17-31.

Thinda, B. S. and Payakab M. M. (1985). A review of bacterial stalk rot of maize in India. *Tropical Pest Management* **31**:311-316.

Toth, I. K., Bell, K.S., Holeva, M. C. and Paul, R. J. (2003). Soft rot Erwiniae from genes to genomes. *Molecular Plant Pathology* **4** (1): *17–30.*

Toth, I.K., Avrova A.O. and Hyman L.J., (2001). Rapid identification and differentiation of the soft rot erwinias by 16S-23S intergenic transcribed spacer-PCR and restriction fragment length polymorphism analyses. *Applied and Environmental Microbiology* **67**:4070-4076.

Vinayaka, H., Misra, R. S. and Jeeva, M. L. (2012). Sweet Potato Diseases: Diagnosis and Management. *Fruit, Vegetable and Cereal Science and Biotechnology* **6**(1): 65-78.

Wakil, S.M. and Oyinlola, K.A. (2011). Diversity of pectinolytic bacteria causing soft rot disease of vegetables in Ibadan, Nigeria. *Journal of Applied Biosciences* **38**: 2540 – 2550

Whipps, J.M. and Lumsden, R.D. (2001). *Commercial Use of Fungi as Plant Disease Biological Control Agents - Status and Prospects: Fungi as Biocontrol Agents - Progress, Problems and Potential,* CABI Publishing, Wallingford, Pp. 9-22.

Whitehead, N. A., Byers, J. T., Commander, P., Corbett, M. J.,Coulthurst, S. J., Everson, L., Harris, A. K., Pemberton, C. L. and Simpson, N. J. (2002). The regulation of virulence in phytopathogenic *Erwinia* species: quorum sensing, antibiotics and ecological considerations. *Antonie Van Leeuwenhoek* **81**: 223–231.

Willey, L.M., Sherwood, L.J. and Woolverton, L. (2008). *Microbiology* (7th Edition). Mc-Graw Hill, New York, 966 pp.

Wilson, CL, Wisniewski, ME, Biles, CL, McLaughlin, R, Chalutz, E and Droby S. (1991). Biological control of postharvest disease of fruits and vegetables: alternative to synthetic fungicide. *Crop Protection* **10**:172-177.

Woolfe, J.A. (1992). Sweet Potato: An Untapped Food Resource. Cambridge, UK: Cambridge University Press (CUP) and the International Potato Center (CIP).

APPENDIX

APPENDIX I

MEDIA COMPOSITION AND STERILIZATION

POTATO DEXTROSE AGAR
Code: CM0139
A medium recommended for the detection and enumeration of yeasts and moulds in butter and other dairy and food products.

Formula	*gm/litre*
Potato extract	4.0
Glucose	20.0
Agar	15.0
pH 5.6 ± 0.2	

Directions

Suspend 39 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring. In order to suppress bacterial growth it is sometimes desirable to acidify the medium to pH 3.5. This can be done by adding 1 ml of Lactic Acid 10% SR0021 to each 100 ml of sterilised medium at 50°C. The medium must not be heated after the addition of the acid, this would result in hydrolysis of the agar and destroy its gelling properties.

Description

A suitable medium for the isolation and count of yeasts and moulds in dairy products1 or those occurring on the surface of fresh meats, cured meats and sausage products and other foods2. This medium is suitable for the detection and enumeration of heat-resistant moulds in thermally processed fruits and fruit products2. Work carried out in cooperation with CSIRO Melbourne had shown that the minerals present in agar could influence the pigment formation of certain fungi. Where pigment production is a critical part of the identification of the fungus it is clearly important to stabilise this characteristic. The agar used in Potato

Dextrose Agar is carefully screened to ensure correct pigment production by fungi such as *Fusaria* species.

Positive control:	***Expected result***
Aspergillus fumigatus ATCC® 9197*	White mycelium, blue-green spores
Negative controls:	
Uninoculated medium	No change
At pH 3.5 *Bacillus subtilis* ATCC® 6633*	No growth

STERILIZATION OF MATERIALS

The glasswares used for this study were thoroughly washed with detergent and rinsed with distilled water. The glasswares such as beakers, conical flasks, test tubes and pipettes were wrapped with aluminium foil and appropriately sterilized in hot air oven at 160 °C for 1 hour. The culture media were sterilized in an autoclaved at 121 °C for 15 minutes at a pressure of 15 pounds per square inch (psi). Inoculating wire loop was sterilized by dipping in 70% ethanol and then flamed in Bunsen flame.

GRAM STAINING AND BIOCHEMICAL REAGENTS

STAIN AND REAGENT

Gram stain

The Gram stain was prepared using two stains (crystal violet and safranin or carbol fuchsin), Gram's iodine, and a decolorizing agent (ethyl alcohol).

A. Gram crystal violet

Solution A

Crystal violet - 2.0 g

Dissolved in ethanol (95%) - 20.0 ml

Solution B

Ammonium oxalate - 0.8 g

Distilled water - 80.0 ml

Gram iodine

Iodine (crystalline) - 1.0 g

Potassium - 2.0 g

Distilled water - 300.0 ml

3.0g of medium was dissolved in 300.0 ml of distilled water.

It is very important to note that; crystalline iodine, potassium and distilled water were combined to produce iodine solution and that Gram's iodine solution was stored in a dark bottle and protected from light so that it does not degrade.

Decolorizer

95 % ethyl alcohol was used.

Gram safranin

Safranin-O (certified) - 0.25 g

Ethyl alcohol (95 %) - 100.0 ml

Working solution:

Safranin stock solution – 10.0ml

Distilled water – 90.0 ml

Biochemical reagents

Indole medium

Peptone – 20.0 g

Sodium chloride – 5.0 g

Distilled water – 1000 ml

pH – 7.4

25.0 g of indole medium was dissolved in 1000 ml of distilled water and autoclaved for 15 min at 121 ºC and dispensed aseptically into sterile test tubes.

Oxidase reagent (Kovac's oxidase)

Amul-alcohol – 15.0 ml

p-dimethyl-aminobenaldehyde – 0.5 ml

Concentrated HCl – 50ml

Small quantity of Kovac's reagent was prepared by dissolving the aldehyde into alcohol and adding the acid slowly and then kept inside the refrigerator.

Catalase test

3% Hydrogen peroxide

APPENDIX II

Table 3.1: Possible Scenarios for Triple sugar iron test

S.N.	Result (slant/butt)	Symbol	Interpretation
1	Red/Yellow	K/A	Glucose fermentation only, peptone catabolized.
2	Yellow/Yellow	A/A	Glucose and lactose and/or sucrose fermentation.
3	Red/Red	K/K	No fermentation, Peptone catabolized under aerobic and/or anaerobic conditions.
4	Yellow/Yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation, Gas produced.
5	Red/Yellow with bubbles	K/A,G	Glucose fermentation only, Gas produced.
6	Red/Yellow with bubbles and black precipitate	K/A,G,H_2S	Glucose fermentation only, Gas produced, H_2S produced.
7	Yellow/Yellow with bubbles and black precipitate	A/A,G,H_2S	Glucose and lactose and/or sucrose fermentation, Gas produced, H_2S produced.
8	Red/Yellow with black precipitate	K/A,H_2S	Glucose fermentation only, H_2S produced.
9	Yellow/Yellow with black precipitate	A/A,H_2S	Glucose and lactose and/or sucrose fermentation, H_2S produced.
10	Yellow/Red	A/K	