THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE
Ikeyi Adachukwu P et al, The *Experiment, 2013, July, Vol.12. (4), 807-813*

ISOLATION AND CHARACTERIZATION OF BACTERIA ASSOCIATED WITH SPOILT IRISH POTATO (SOLANUM TUBEROSUM)

ABSTRACT

Irish potato is an edible tuber from the *Solanum tuberosum* plant, which is actually native to South America, not Ireland as many people think. Irish potatoes are named after Ireland because; they are closely associated with the Irish potato famine, a historical famine caused by a mold infection of the Irish potato crop. The isolation of bacteria responsible for the spoilage of Irish potato was done using Nutrient agar media. Four genera of bacteria which include *Bacillus spp.*, *Erwinia spp.*, *Pseudomonas pp.*, and *Corynebacterium spp*. were identified to be the causative microorganism responsible for potato spoilage. These microorganisms may cause low economic yield of potato. Good sanitary measures and planting of resistant varieties of potato should be used to prevent entrance of these microorganisms into potato tubers.

Keywords: *Solanum tuberosum*, Causative microorganism, Nutrient agar media and Irish potato

INTRODUCTION

In the quest for food and the struggle for human survival, Irish potato has historically played important role and is suitable in addressing the problems of food scarcity. This is due to their yield per area (hectare) per unit time and their nutritional value (i.e. ratio of protein to carbohydrate) (Tewe *et al*., 2003). African farmers are faced with constraints in the production of food and cash crops. Some of these constrains are poor soils, poor farm practices, use of local varieties, land tenure and damages by diseases and pests (FAO, 2008). Irish potato tubers like many other crops are attacked by many various pathogens which cause disease in them. Such pathogens are fungi, bacteria and viruses. The greatest of these constraints is that of post-harvest spoilage of farm produce, out of the several tons harvested, just a fraction is utilized while most of the amount is lost to post harvest diseases especially rot diseases (Alexandratos, 2000). These pathogens cause great losses and reduction in the values of these crops but with a good system of control, this can be eradicated.

Potato is a starch tuberous crop from the perennial S*olanum tuberosum* of the *solanaceae* family (also known as the nightshades). The word may refer to the plant itself as well as the edible tuber. In the region of the Andes, there are some other closely related cultivated potato species. Potatoes were introduced outside the Andes region four centuries ago, and have become an integral part of much of the world's cuisine. It is the world's fourth-largest food crops, following rice, wheat and maize (Hawkes, 2000). Long term storage of potato requires specialized care in cold warehouse (Lang, 2001). Wild potato species occur throughout the Americas, from the United States to Southern Chile (Langar, 2005). The potato was originally believed to have been domesticated independently in multiple location (McNeill, 2009), but later genetic testing of the wide variety of cultivars and wild mild species proved a single origin for potatoes in the area of present-day southern and extreme north western Bolivia (from a species in the *Solanum berevicaule* complex) where they were domesticated 7,000-10,000 years ago (McNeill, 2008). Following centuries of selective breeding, there are now over a thousand different types of potatoes (Afrios, 2008), of these sub-species, a variety that at one point grew in the Chile Archipelago (the potato's south central Chilean sub-center of origin) left its garn plasum on over 99% of the activated potatoes world-wide (Salaman, 2008). The animal diet of an average global citizen in the first decades of the 21st century included about 33kg (731b) of potato (Hawkes, 2000). However, the local importance of potato is extremely variable and rapidly changing. It remains an essential crop in Europe (especially eastern and central Europe), where per capital production is still the highest in the world, but the most rapid expansion over the past few decades has occurred in Southern and Eastern Asia. China is now the world's largest potato producing country, and meanly a third of the world's potatoes are harvested in China and India (Stevenso *et al*., 2001).

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE
Ikeyi Adachukwu P et al, The *Experiment, 2013, July, Vol.12. (4), 807-813*

Many people in poor countries, who cannot afford high-calorie diet such as milk products, meat and pulses use potatoes as their prime source of calories but microorganisms such as *Bacillus spp., Erwinia spp*., etc reduce the nutritional contents of Irish potato and this necessitated the curiosity of this study. Therefore, the aim of this research was to isolate and characterize bacteria that is associated with the spoilage of Irish potato

MATERIALS AND METHODS

Sterilization

Autoclavable materials such as agar were epically sterilized in an autoclave at 121ºC for 15 minutes. Properly washed Petri dishes, beakers, wash bottles, test tubes, pipettes, conical flasks, spatula, inoculating needles, and forceps were also sterilized using hot air oven at a temperature of 150ºC for 1 hours. The wire loops were sterilized by heating in the blue flame of the Bunsen burner until red hot and allowed area to cool before using. 70% alcohol is used to swab the working bench area to prevent contamination.

Collection of Sample

Ten (10) samples of spoilt Irish potato tubers were bought from Ogbete Main Market Enugu, Enugu State, Nigeria, using sterile stomacher bags. The samples were immediately transported to microbiology laboratory department of IMT Enugu for analysis.

Preparation of Sample

The potato tubers were grouped and labeled A to E with two potato tubers in a group. Each of the groups were mashed aseptically in a sterile stomacher bag and 10.0g of the mashed potato was suspended in 90ml of diluents (water) and serially diluted in same diluents. One milliliter of appropriately diluted sample was pour plated in nutrient agar and the poured plated were incubated at 37ºC for 48 hours. The viable bacteria were enumerated and expressed as colony forming units (C.F.U) per gram sample. Representatives of the different colonies were selected according to their morphological characteristics, purified by successive sub-culutring on nutrient agar and identified using the standard method of (Harrigan and McCance, 2007).

Preparation of the Culture Media

The media for culturing was aseptically prepared when needed according to the manufacturer's instructions, and autoclaved at 121ºC for 15mins.

Identification of Bacterial Organism

The bacteria organism isolated from spoiled Irish potato was subjected to Gram staining and biochemical test for proper identification.

Gram Staining

The slide containing the heat fixed smear was laid across a staining rack and placed over a sink. The smear is flooded with 0.5% aqueous crystal violet for 30 seconds, excess stains were washed off with lugols iodine and allowed to react for 30 seconds, while the slid was laid across the staining racks, the iodine was carefully rinsed out with distilled water. Then the smear was rinsed briefly for 3 seconds with 50-50 acetone alcohol until blue colour ceases to come out, this is the decolorizing step which if prolonged will interfere with the result, thus, the slid was quickly rinsed out with distilled water to avoid excessive decolorization. The slide was dried between

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY
ISSN 2319-2119

fold of blotting paper, the slid was again laid across the staining rack and flooded with 1% aqueous safranin for 60 seconds after which the stain was washed off with distilled water. The slid dried between folds of filter paper, the slid was place with a drop of immersion oil and examine under oil immersion objective of the microscope. Bacterial stain blue-black are said to be gram positive while the one stained red are said to be gram negative.

Biochemical Characteristics

The following biochemical tests were carried out for the identification of bacteria isolates.

Catalase Test

This test is used to differentiate those bacteria that produce the enzyme catalase.

A small amount of the culture was gotten from agar slopes, using a clean sterile platinum wire loop that was inserted in drops of H_2O_2 on a clean microscopic slide. The production of gas bubbles indicates positive reaction.

Indole Test

This is used to detect the production of indole by bacteria growing on media containing amino acid.

The peptone water media was inoculated and incubated for 48 hours at 37ºC. The tubes were further allowed to stay for more than 48 hours in the incubator for the accumulation of indole. After this period, 0.5ml kovas' reagent was added separately to each tube and shaked gently. The appearance of a red colour in the alcohol layer indicates a positive reaction.

Motility Test

This is used to test whether the organism are motile, i.e., the presence of flagella.

About 2-5 drops of peptone water with growth of the organism was placed on a clean slide with a loop. The cover slip was placed over the slide. The slide was left for sometimes and then examine microscopically with high power objective. Motile organism would be seen swimming around.

Citrate Utilization Test

This is used to test whether the organism can use the compound citrate as its only sources of carbon and energy.

Using Simon's citrate agar, the sterile media was inoculated from a saline suspension of the test organism and incubated for 96 hours at 37ºC. A blue colour and streak of growth indicates a positive reaction, while the original colour and no growth indicates a negative reaction.

Sugar Fermentation Test

This is used to know whether the organism can ferment sugar and produce acid. Fermentation tests were carried out using the following sugars: Glucose, sucrose, lactose, maltose, and mannitol. To each of 10ml of peptone water in the test tube, 1.5g of each sugar was separately dissolved into and labeled, 3 drops of 0.01% phenol red was added. Durham tubes were inserted in it and

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

inverted position into the tubes for detection of gas production. The tubes were plugged with non-absorbent cotton wool and sealed with aluminium foil before being sterilized in an autoclave at 121ºC for 15 mins at 151 boiling pressure. The tubes were then aseptically inoculated with small bacterial colonies using sterile wire loop. The tubes were incubated for 24 hours at 37ºC and un-inoculated tube served as controls.

RESULTS

Table1: below shows that Potato sample A had the highest colony counts of 0.73 CFU/g (10^2) while Potato sample G had the lowest colony counts of 0.35 CFU/g (10^2)

S/N	Potato Samples	Colony Count	Mean of Total Count CFU/g (10^2)
1	A	73	0.73
2	B	67	0.67
3	C	57	0.57
4	D	65	0.65
5	E	63	0.63
6	F	45	0.45
7	G	35	0.35
8	H	58	0.58
9	I	46	0.46
10	J	58	0.58

Table1: Total bacteria population and their occurrence in spoilt Irish Potato samples

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE
Ikeyi Adachukwu P et al, The *Experiment, 2013, July, Vol.12. (4), 807-813*

Cell Morphology	Gram reaction	Catalase	Citrate	Indole	Glucose	Sucrose	Lactose	Maltose	Mannitol	Probable identified organisms
Rod	+	+	+	-	-	-	-	-	-	*Bacillus spp.*
Rod	-	+	+	-	-	-	-	-	-	*Pseudomonas spp.*
Rod	-	+	+	+	+	+	+	+	+	*Erwinia spp.*
Club-shaped	+	+	-	-	+	-	+	+	+	*Corynebacterium spp.*

Table 2: Above shows that all the micro-organisms were reactive to Catalase while only *Corynebacterium spp.* was positive to gram reaction.

Table 2: Characteristics of bacteria strains isolated from spoilt potato

Colonial characteristics after incubation	**Gram reaction**	**Presumptive organisms**
Cream dull surface with irregular shape	Gram positive cocci, some occurs in chains arrange in chains and squarer end	*Bacillus spp.*
Greenish colonies	Gram negative rod shaped in pairs with one or more polar flagella	*Pseudomonas spp.*
Yellow, flat round colonies 1.0um -15um in diameter	Gram negative rod shape, cells is arranged in pairs and chains	*Erwinia spp.*
Blue-green hue	Gram positive club shaped appear in clusters	*Corynebactrium spp.*

Table 3: Cultural and morphological characteristics of bacteria isolate

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

The results obtained in this study revealed that the spoilt Irish potato samples contained four bacteria strains belonging to four genera. The bacteria isolates were *Bacillus spp., Pseudomonas spp., Erwinia spp.,* and *corynebacterium spp*. All the spoilt potatoes bought from Ogbete main market Enugu, were reported to be associated with *Erwinia spp., Bacillus spp.* and *corynebactenium spp.* which

was also reported to be associated with bacterial soft rot in potatoes (Olivleri *et al*., 2004; Mahmoud *et al.*, 2008). Adisa (2006) reported Erwinia spp. as one of the most important bacteria causing spoilage of potatoes. Bacteria soft rot is one of the causes of microbial spoilage of potatoes and is the most important disease that can spread extensively during potato storage. Losses due to this disease may range from 0-100% depending upon the method of potato handling.

Although, the production of enzymes by the isolated bacterial strains was not investigated in this study. Bacteria causing rots in potato have been reported to produce a wide range of hydrolytic enzymes such as cellulases, pectinases, xylanases and proteases (Olivier *et al*., 2004). These enzymes are responsible for tissue maceration and cell death, after which the microorganisms have access to the nutritional resources of the dead plant tissues (Avekamp *et al*., 2008). *Erwinia* and *Bacillus species* have been found in soil and they gain entrance through wounds and natural openings such as lenticels. Although *Pseudomonas spp*. has never been reported to be associated with potato spoilage, its isolation from this study was however not surprising, since *Pseudomonas species* is commonly found in soil and are mainly plant pathogens (Collins *et al*., 2004).
The effects of these organisms to man when consumed may be harmful to the body which can cause dangerous diseases to man.

Conclusion

The result of this study has shown that spoilt Irish potato were contaminated with pathogenic microorganisms which are harmful to man and may also lead to huge economic losses of potato yield. This could be prevented by practice of good sanitary measures and the use of resistant varieties.

Recommendation
Having known the nutritional value of Irish potato and the cause of its spoilage, it is therefore recommended that:

Irish potato should be used by many people especially those in poor countries, who cannot afford high calorie diet such as milk products, meat and pulses.

Consumption of spoilt Irish potato should be avoided because it contains pathogenic microorganisms which may be harmful to human health.

Farmers should practice good sanitary measures and also use resistant varieties of potato in order to prevent the entrance of these microorganisms inside the potato tubers.

REFERENCES

1. Ademolu, A.K. (2009). “Distribution, cellular extracellular enzymes and control of fungi associated with post harvest rot of cassava in South-western Nigeria”. University of Ibadan, Nigeria, pp.203-305.
2. Adisa, V. A. (2006). “Microbial spoilage *of salanum tuberosoum L*. tubers in Nigeria’. Dienahrung Press Ibadan, Nigeria, pp. 30, 709 and 712.
3. Agriso, G. N. (2008). “Plant pathology”. Academic Press, New York, p.703.
4. Ajiboye, E. O. (2004). “Effects of different nutrient sources on the growth of some post harvest pathogens of cassava tubers. University of Ibadan Press, Nigeria, pp.205-210.
5. Alexandratos, N. (2000). “World Agriculture: Towards 2010”. An FAO Study. New York, pp.560-610.
6. Anderson, C. A. (2005). “Post harvest microbial deterioration of Irish potato tubers, Thesis University of Ibadan, Nigeria, p.50.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE
Ikeyi Adachukwu P et al, The *Experiment, 2013, July, Vol.12. (4), 807-813*

7. Aveskamp, M. M., De Gruyter, J. and Crous, P. W. (2008). "Biology and Recent development in the systematic of phoma, a compose genus of major quarentive, significance fungi diversity". Arnold Publishers, London, pp.31 and 118.
8. Collins, C.H., Lyne, P.M., Grange, J.M. and Falkinham, J.C. (2004). "Microbiological methods". Arnold Publishers, London, p.456.
9. Dianne, O. (2006). "Whole foods companion". Chelsea Green Publishing Company, New York, pp.50.
10. F.A.O. (2008). "Food and Agriculture organization of the United Nations, Nutrition and Consumer Division" pp.60.
11. Hawkes, J.G. (2000). "The potato evolution, biodiversity and genetic resources". Smithsonian Institution Press, Washington, D.C., p.250.
12. Lang, J. (2001). "Notes of a potato watcher". Texas A and M. University Press, America, pp.61-70.
13. Langer, W.L. (2005). "American foods and Europe's population Growth. *Journal of Social History*, 8 (1975): 51-66.
14. Mahmoud, A.D.R., Mahmoud, A.A. and Gomma, A.M. (2008). "Antagonistic activities of potato associated bacteria via their production of hydrolytic enzymes with special reference to pectinases". *Journal of Agric. Biological Science*, 4(5): 575-584.
15. McNeill, W. (2008). "How the potato changed the world's history". *Journal of Social Research*, 66(1):67-83.
16. McNeill, H. (2009). "The introduction of the potato into Ireland". *Journal of Modern History*, 21(3): 218-21.
17. Mary, D. (2002). "The potato book". Published by William Morrow and Company, New York, pp.70-71.
18. Olivieri, F.P., Maldonada, S., Tonon, C.V. adnLasalongue, C.A. (2004). "Hydrolytic activities of *Fusarium solani* and *Fusarium solam F. spp.* associated with infection process of potato tubers". *Journal of Piytopathology*, 152(6):237-344.
19. Reader, J. (2008). "Propitious esculent: potato in world history". CRC Publishers, London, p.315.
20. Salaman, R. N. (2009). "The History and social influence of the potato". Cambridge University Press, London, pp.90-100.
21. Stevenson, W.R., Loria, R., Franc, G.D. and Weingartner, D. P. (2001). "compendium of potato diseases". Arnold Publishers, London, pp.250-255.

***Ikeyi Adachukwu P., Ogbonna Ann O. and Okolo Ifeoma P.**

Department of Science Laboratory Technology, Institute of Management and Technology (IMT), Enugu, Nigeria.

Email oky9992000@yahoo.com