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In vitro analysis of the infection of Dasheen (*Colocasia esculenta* (L.) Schott) plantlets by *Pseudomonas fulva*

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ABSTRACT: This study reports the plant–microbe association of *Pseudomonas* sp with Cocoyam or dasheen (*Colocasia esculenta* (L.) Schott) *in vitro* derived plant matter. The phytopathogen, isolated from infected dasheen leaves obtained from the field, was identified by 16S rRNA gene sequence similarity as *Pseudomonas fulva*, and its partially sequenced genome also confirmed by GenBank. *Pseudomonas* sp have not been implicated as a bacterial plant pathogen of the edible aroids. *In vitro* dasheen plantlets developed chlorotic and necrotic symptoms over the 18 days of the infection study which lead to the loss of structural coherence and the disintegration of vital plant organs. The findings of this investigation facilitate the introduction of *Pseudomonas* sp as a phytopathogen of *in vitro* derived dasheen (*Colocasia esculenta* (L.) Schott) plantlets, and will provide valuable information for creating an awareness for stakeholders involved in the cultivation and export of this tuber crop. This study indicates the first approach towards further investigating the means for control of *Pseudomonas fulva* infection of *Colocasia esculenta* plants in the field, with a view to increasing the production of this fresh produce.

Key words: Dasheen; *Colocasia esculenta* (L.) Schott, necrosis, *Pseudomonas* sp.

Introduction

Colocasia esculenta (L.) Schott, a member of the Araceae family (aroids), is a perennial herb largely cultivated throughout the humid tropics and sub-tropics for its edible corms and leaves. This plant is commonly known in the Caribbean and Tropical America as dasheen, taro, eddoe or old cocoyam. Dasheen corms are rich in starch and may be eaten in a manner similar to potatoes, that is, boiled, baked, roasted, fried, and the leaves and petioles used as a vegetable in soups (Kay, 1987). In addition, dasheen has attained the status of a commercial crop in some Caribbean Islands, Hawaii, Egypt, the Philippines and some Pacific Islands. However, diseases affecting Colocasia esculenta (L.) Schott cause significant economic losses, and have occasionally resulted in the abandonment of dasheen cultivation. Corn rots cause considerable damage but viral diseases and that of leaf blight are the most devastating in some areas (Centre for Overseas Pest Research, 1982).

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Dasheen leaf blight caused by *Phytophthora colacasiae* is characterized by leaves with small circular, water-soaked lesions which become rapidly enlarged, irregular in shape, zoned, dark brown with a yellow margin and contain yellow to red droplets, which later becomes hard (Jackson, 1977). These lesions produce secondary colonies which cause the collapse of leaves and subsequent reduction in numbers. The viral diseases of dasheen include those mainly caused by the Dasheen Mosaic, Alomae and Bobone viruses. Dasheen Mosaic Virus infection results in a feathery mosaic pattern adjacent to the veins which is intermittently expressed, and the severity and persistence of expressed symptoms vary according to plant genotype (Jackson, 1980; Jackson, 1982; Shaw *et at*, 1979; Zettler *et al*, 1978).

The Alomae virus causes a systematic necrosis which leads to the death of the plant. The characteristic symptom first includes a feathery mosaic followed by an inability of the leaves to open. Young leaves that are affected fail to open displaying a crinkled appearance, and become abnormally thickened with prominent veins. The necrosis eventually spreads from the tips of the leaves, down the petiole, finally killing the entire plant (Gollifer *et al*, 1977; Jackson, 1980). The Bobone virus infection of dasheen plants is not as lethal as the Alomae. The affected plants tend to be more stunted and the leaves twisted and curled. Leaves of these plants become puckered, distorted, brittle and thickened, does not become necrotic but remain green. The plants will eventually recover (Gollifer *et al*, 1977; Jackson, 1980). Incidences of bacterial diseases of the araceae family have largely been associated with *Xanthomonas campestris*.

Chase *et al*, 1992, indicated that one of the most serious diseases affecting C. e*sculenta* and other members of the Araceae family (aroids) is caused by strains of *Xanthomonas campestris. Xanthomonas* diseases of aroids are characterized by marginal chlorosis and necrosis, interveinal necrosis, and in some case a systemic infection resulting in plant death. Although a large number of *Pseudomonas* strains cause plant disease in most agricultural and commercial crops such as tomato, peanut, potato, tobacco (*Pseudomonas solanacearum*) among others, a review of literature has indicated no reported incidences of pathogenic disease implicating *Pseudomonas* Sp. as phytopathogens of dasheen. *Pseudomonas cichorii* is responsible for the bacterial leaf spot in some susceptible horticultural aroid foliage plants inclusive of *Epipremnum aureum* (Pothos), *Philodendron panuduraeforme* (Fiddleleaf Philodendron), *Agglaonema spp.* (Chinese Evergreen) and Monstera spp. (Pfleger and Gould, 1998). However, there has been no reported case of diseases of the edible aroids caused by *Pseudomonas cichorii.* This study focuses on the effect that *Pseudomonas* sp has on dasheen plantlets in vitro with a view to better understanding this plant-microbe interaction that occurs in the field.

Materials And Methods

Materials

In vitro derived dasheen plantlets.

In vitro derived dasheen plantlets, cultured from the apical meristem of young dasheen shoots, were initiated and multiplied on Murashige and Skoog basal salt mixture, MS (Murashige and Skoog, 1962), pH 5.8 containing 30g/L sucrose, 0.5mg/L (for initiation) and 0.1 mg/L (for multiplication) benzene amino purine (BAP). At both the initiation and multiplication stages *in vitro* dasheen cultures were transferred to the tissue culture growth room of the Biotechnology Centre, University of the West Indies, which has a 16-hour photoperiod at 24-27^{0}C (Mitchell *et al*, 1994). Mature plantlets, approximately six (6) months old were used in the pathogenicity study.

Isolation of *Pseudomonas* sp.

The pathogenic bacteria were isolated from samples of infected dasheen leaves collected from a dasheen cultivation located in Sligoville, St. Catherine, Jamaica, W.I. These bacteria were cultured microbiologically on Nutrient Yeast Glycerol Agar [NYGA), 5g of Bacto Peptone (Difco Laboratories, Detroit, Michigan) per litre, 3g of yeast extract per litre, 20g of glycerol per litre and 18g of Bacto Agar per litre] plates as indicated by Bradbury (1970). The predominant bacterial colonies within the mixed culture that grew on the NYGA plates were found to be yellow and mucoid in appearance. These microorganisms were selected and re-streaked to obtain pure cultures.

Identification of Pseudomonas sp

Initial preliminary identification of the bacterial isolates obtained from infected dasheen (*Colocasia esculenta* var. *esculenta)* from a farm in Sligoville, St. Catherine, was carried out using phenotypic characteristics. Subsequent identification of one of the yellow – pigmented phytopathogenic bacterial isolate, UWIOSKGI, was based on the 16S ribonucleic acid (rRNA) gene sequence similarity, performed by MIDI Labs, Incorporated, 125 Sandy Drive, Newark, Delaware 19713, USA. The 16S rRNA gene was PCR amplified from genomic DNA isolated from pure bacterial colonies. Primers used for the amplification correspond to *E. coli* positions 005 and 1540 (full length packages) and 005 and 531 (500 bp packages). Amplification products were purified from excess primers and dNTPs using Microcon 100 (Millipore) molecular weight cut off membranes and checked for quality and quantity by running a portion of the products on agarose gel. The samples were electrophoresed on an ABI Prism 377 DNA Sequencer. The data was analyzed using Applied Biosystems DNA editing and assembly software and sequence comparisons were obtained using the MicroSeq software.

BLAST Search of UWIOSKGI 500bp 16S rRNA sequence.

The five hundred (500) base pair nucleotide sequence of 16S rRNa sequence obtained from MIDI Labs Incorporated was analyzed using BLASTIN located at http://www.ncbi.nih.gov/blast

Infection of Dasheen with *Pseudomonas* sp

Overnight broth cultures of the bacteria isolates were used to inoculate the leaves of these tissue culture plantlets. For the inoculation, a sterile needle was used to create wounds at least 1 cm apart on the leaf's top surface at the same point where sterile cotton previously soaked in broth culture was placed on the underside. The development of infection in the plant was monitored for 18 days and the disease symptoms recorded.

Results And Discussion

The phenotypic characteristics of the pathogenic bacteria isolates were found to be rod-shaped, motile, and Gram negative. On Nutrient Yeast Glycerol Agar (NYGA), the colonies appeared yellow, circular, entire, glistening and raised. The bacterial isolate, UWIOSKGI, was identified as *Pseudomonas fulva* based on the 16S rRNA gene sequence similarity of five hundred (500) base pairs. Results obtained from the 16S ribosomal ribonucleic acid (rRNA) gene sequence identified the partially sequenced isolate, UWIOSKGI, as *Pseudomonas fulva*. The results in Table 1 showed that there was a 0.19% genetic difference between the *Pseudomonas fulva* 16S rRNA sequence contained in MIcroSeq^{TM} database, used by MIDI labs and the five (500) hundred base pair sequence of the 16S rRNA from UWIOSKGI. *Pseudomonas fulva* was the closest matching bacteria to the test strain followed by nine (9) others in increasing percentage of genetic difference. (Table1). It can therefore be determined from this alignment of the genetic differences that the UWIOSKG1 isolated is a P*seudomonas sp*, and more definitely, P*seudomonas fulva*.

The BLAST further confirmed the identity of the bacterial isolate as the search analysis indicated that the 500 base pair sequence for UWIOSKGI bacterial isolate, identified as *Pseudomonas fulva* by MIDI Labs, was 99% similar to *Pseudomonas fulva* 16S ribosomal RNA gene over 509 of 511 nucleotides. In addition, the queried sequence had 99% similarity to seven (7) other 16S rRNA gene sequences of *Pseudomonas* sp. namely *Pseudomonas parafulva*, *Pseudomonas* sp. Fa27, *Pseudomonas* sp. Fa24, *Pseudomonas* sp. AEBL3, *Pseudomonas* sp. NZ096, *Pseudomonas* sp. NZ017 and *Pseudomonas* sp. NZWT2.

Table1 The alignment report for UWIOSKGI obtained from the 16S rRNA analysis.

Percent Genetic Differences (% GD)	Size of DNA Sequence (Base Pairs)	*Pseudomonas* sp.
0.19	522	*Pseudomonas fulva*
1.44	522	*Pseudomonas straminae*
2.97	522	*Pseudomonas fluorescens* A (bt)
2.97	522	*Pseudomonas fluorescens* G (bt)
3.16	522	*Pseudomonas mucidolens*
3.16	522	*Pseudomonas fuscovaginae*
3.16	522	*Pseudomonas synxantha*
3.26	522	*Pseudomonas alcaligenes*
3.35	522	*Pseudomonas agarici*
3.35	522	*Pseudomonas asplenii*

Prepared by MIDI Labs Incorporated, 125 Sandy Drive, Newark, DE19713, USA

Pseudomonas fulva was first isolated during the taxonomic study of the genus *Pseudomonas*, from rice and oil fields and oil brine in Japan (Iizuka and Komagata, 1963 a, b, c, d, e). Other closely related species include *P.straminea* and *P. azotoformans.* The phylogenetic relationships deduced from 16S rRNA gene sequences along with the production of a water-insoluble yellow pigment facilitated the inclusion of these species in group 1 of Palleroni (1984). It has been proposed that *Pseudomonas fulva* may be related to *P. straminea*, *P. oryzihabitans*, *P. flavescens* and *P. graminis* (isolated from grass) considering the ecological similarity that these species are plant inhabiting and produce a water-insoluble yellow pigment (Uchino, et al, 2001).

During the pathogenicity study of *Pseudomonas* sp. on dasheen, a brown coloration was detected around the wounds created on the leaves of infected dasheen plantlets 4 to 6 days following the inoculation of the leaves with *Pseudomonas* sp. These areas became rapidly necrotic and coalesced to produce larger areas of dead tissues. During days 6 to 12 the entire leaf became a soaked brown mass due to severe necrosis (Figure1). In the last 6 days of the infection, that is, days 12 to 18, the severe necrosis seen in the leaf had spread to the petioles causing loss of structural coherence and the subsequent death of this organ (Figure 2). It was difficult to visually detect any physiological changes in the roots of the infected plantlets. This infection of dasheen by *Pseudomonas* sp *in vitro* is the first report of a disease affecting dasheen with *Pseudomonas* sp being the causative agent.

Studies and reports have indicated that the major diseases of *C. esculenta* (L.) Schott, include corn rots [mainly caused by *Pythium* and *Phytophthora* species (Centre for Overseas Pest Research, 1982; Jackson, 1980; Holiday, 1980], viral diseases [such as those caused by Dasheen Mosaic Virus, (Jackson, 1980; Jackson , 1982; Shaw *et al*, 1979; Zettler and Hartman, 1986; Zettler and Hartman, 1987; Zettler and Jackson, 1979; Zettler *et al*, 1978) Alomae (Gollifer *et al*, 1977; Jackson, 1980) and Bobone viruses (Gollifer *et al*, 1977; Jackson, 1980)] and leaf blight due to *Phytophthora colocasiae* infection (Anonymous, 1970; Gollifer *et al*, 1980).

Information concerning bacterial diseases of the Araceae family (inclusive of the horticultural species and cultivars of *Aglaonema, Anthurium, Dieffenbachia, Epipremnum, Philodendron,* Spathiphyllum, Syngonium, and those of the edible aroids *Colocasia* and *Xanthosoma*) has implicated *Xanthomonas campestris* as one of the phytopathogenes for these host plants (Chase *at el*, 1992). The pathogenicity of the *Pseudomonas* sp. used in this study, indicated that these microorganisms were able to firstly penetrate the incisions created on the leaves of the *in vitro* dasheen plantlets subsequent to the application of the bacterial inoculums, and then develop an infection by establishing contact with susceptible cells or tissues of these plantlets. The development of the symptomatic brown colouration around the wounds created on the leaves, 4 to 6 days following the inoculation of the leaves with *Pseudomonas sp*, suggested that there was an incubationperiod during this plant-microbe association, which is

necessary for disease development, as against a hypersensitive response, in which a limited number of plant cells in direct contact with the invading phytopathogen dies rapidly (a programmed cell death) to prevent the spread of the infection (Dixon *et al*, 1994). However, it was observed that the *Pseudomonas sp.* later extensively invaded the leaf tissue of these plantlets by deriving the required nutrients from the breakdown of the plant cell wall and other cell components to facilitate its proliferation and further invasion of the intercellular spaces of the plant organs. This was evident, as the leaf became a soaked brown mass during days 6 to 12. During the final 6 days of the pathogenicity study the severe necrosis seen in the leaf had spread to the petiole causing the loss of structural integrity and the total deterioration of this plant organ. It can therefore be suggested that *Pseudomonas sp* is a phytopathogen of the susceptible in vitro derived dasheen (*C. esculenta* var. *esculenta)* plantlets, facilitated by favourable growth conditions.

Figure 1. Dasheen (*C. esculenta* var. *esculenta*) plantlet at day 12 after inoculation with *Pseudomonas* sp. Two of the three leaves of the plantlet showed symptoms of chlorosis

Figure 2. Dasheen (*C. esculenta* var. *esculenta*) plantlet at day 18 after inoculation with *Pseudomonas* sp. Loss of structural integrity in the petioles.

References

ANONYMOUS. 1970. *Phytophtora colocasiae* Racib. Commonwealth Mycological Institute Distribution Maps of Plant Diseases. Map No. 466. Commonwealth Agricultural Bureaux, Slough.

BRADBURY, J.F. 1970. Isolation and preliminary study of bacteria from plants. *Rev Pl* Path 49, 213-218.

CENTRE FOR OVERSEAS PEST RESEARCH, 1982. Pest control in Tropical root crops. PANS Manual, No. 4, London.

CHASE, A.R., Stall, R.E., Hodge, N.C. and Jones, J.B. 1992. Characterization of Xanthomonas campestris strains from aroids using physiological, pathological, and fatty acid analyses. Phytopathology 82, 745-759.

DIXON, R.A. Harrison, M.J. and Lamb, C.J., 1994. Early events in the activation of plant defense responses. Annu Rev Phytopathol 32, 479-501.

GOLLIFER, D.E., Jackson, G.V.H., Dabek, A.J., Plumb, R.T. and MAY, Y.Y. 1977. The occurrence and transmission of viruses of edible aroids in the Solomon Islands and the Southwest Pacific. Pesticide Articles and News Summaries 23, 171-177.

HOLLIDAY, P. 1980. Fungus Diseases of Tropical Crops. Cambridge University Press, Cambridge.

LIZUKA, H. and Komagata, K. 1963a. An attempt at grouping of the genus Pseudomonas. *J Gen Appl Microbiol 9, 73-82.*

LIZUKA, H. and Komagata, K. 1963b. Taxonomy of genus Pseudomonas with special reference to their modes of metabolism of carbon compounds. *J Gen Appl Microbiol* 9, 83-95.

LIZUKA, H and Komagata, K. 1963c. On the studies of microorganisms of cereal grains. III. Pseudomonas isolated from rice, with special reference to the taxonomic studies of chromogenic group of genus *Pseudomonas. Nippon Nogeikagaku Kaishi* 37, 71-76 (in Japanese).

LIZUKA, H. and Komagata, K. 1963d. On the studies of microorganisms of cereal grains. IV. *Pseudomonas* isolated from rice, with special reference to the taxonomic studies of fluorescent group of genus *Pseudomonas. Nippon Nogeikagaku Kaishi* 37, 77-80 (in Japanese).

LIZUKA, H. and Komagata, K. 1963e. Studies on the microorganisms of cereal grains. V. Nee species of Pseudomonas belonging to fluorescent group. *Nippon Nogeikagaku Kaishi* 37, 137-141 (in Japanese).

JACKSON, G.V. H. 1977. Taro leaf blight. Advisory Leaflet 3. South Pacific Commission, Noumea.

JACKSON, G.V.H. 1980. Diseases and Pests of Taro. South Pacific Commission, Noumea.

JACKSON G.V.H. 1982. A virus disease of Taro on French Polynesia. Report to the South Pacific Commission, Noumea.

KAY, D.E. (revised by Gooding, E. G.B). 1987. Crop and Product Digest No. 2 Root Crops, Second Edition. London: Tropical Development and Research Institute, XV & 380pp.

MITCHELL, S.A., Asemota, H.N. and Ahmad, M.H. 1994. Factors affecting the *in vitro* establishment of Jamaican yam (*Dioscorea spp*) from nodal pieces. *J Sci Agi* 67, 541-550.

MURASHIGE, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant* 15, 473-497.

PALLERONI, N.J. 1984. Genus I. *Pseudomonas*. In *Bergey's Manual of Systematic Bacteriology*, Vol. 1, pp. 141-199. Edited by N. R. Krieg & J.G. Holt. Baltimore: Williams & Wikins.

PFLEGER, F.L. and Gould, S.L., 1998. Bacterial leaf diseases of foliage plants. 20 Jul. 2002. University of Minnesota Extension Service. (URL: <http/:www.extension.umn.edu/distribution/horticulture/DG1170.html>).

SHAW, D.E., Plumb, R.T. and Jackson, G.V.H. 1979. Virus diseases of Taro (*Colocasia esculenta*) and *Xanthosoma* sp. In Papua New Guinea. Papua New Guinea Agric J. 30, 71-97.

UCHINO, M., Kosako, Y., Uchimura, T., and Komagata, K. 2000. Emendation of *Pseudomonas straminae* lizuka and Komagata 1963. Int J. Sys Evol Microbiol 50, 1513-1519.

ZETTLER, F. W., Abo El-Nil, M.M. and Hartman, R.D. 1978. Dasheen mosaic virus.

ZETTLER, F.W. and Hartman, R.D. 1986. Dasheen mosaic virus and its control in cultivated aroids. Pp. 91-102. In: *Virus Diseases of Horticultural Crops in the Tropics and Subtropics*. FFTC Book Ser. 33. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taipei.

ZETTLER, F.W. and Hartman, R.D. 1987. Dasheen mosaic virus as a pathogen of cultivated aroids and control of the virus by tissue culture. *Plant Dis* 71, 958-963.

ZETTLER, F. W. and Jackson, G.V.H. 1979. Dasheen mosaic virus. Advisory Leaflet 10. South Pacific Commission, Noumea.