

# Genetic Diversity Within *Acidovorax avenae* subsp. *citrulli* in Cucurbits Production Areas of Northeast, Thailand

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## Introduction

*Acidovorax avenae* subsp. *citrulli* (Aac) causes bacterial fruit blotch disease, the most important bacterial disease in commercially grown watermelon and melon. Aac infects all cucurbit plants. The objective of this study was to evaluate the genetic variability of Aac in cucurbit production areas of Northeast, Thailand through determination of their biological properties and DNA fingerprint of 16S rDNA.

## Material and Methods

**Aac isolation:** In 2000 and 2001, samples from infected fruits and leaves of watermelon, melon, white gourd, squash, pickle melon and cucumber were collected from production field in Khon Kaen, Audornthani, Kalasin, Nakornratchasima, Yasothorn provinces and unknown growing place but delivered to Khon Kaen fresh markets. The Aac was isolated on nutrient agar (NA) and confirmed by ELISA using antibody against Aac-KK9, reference strain of Aac.

**Pathogenicity and severity test:** The selected 183 Aac isolates were used. Bacterial cells grown for 48 h on NA were suspended in sterile distilled water and diluted to OD 0.1 at 600nm by spectrophotometer. Bacterial suspensions were injected underneath of cotyledon of 15 days old watermelon, melon and cucumber seedlings. The plants were maintained under favor conditions for bacterial fruit blotch disease and rated disease severity at 12 days after inoculation on a scale of 0-8 range from no symptom (0) to dead plant (8).

**Formation of bacterial colony:** All selected 183 Aac isolates were grown on NA at 28°C. Different of colony formation was evaluated at 2 days and after 6 days of growing on culture.

**Amplified Ribosomal DNA Restriction Analysis (ARDRA):** Total genomic DNA was extracted from 16 h shaken culture cells by boiling method. The 16s rRNA gene was amplified using primers A1 and B6 according to Manceau and Horvias (1997). The amplified fragment of each Aac isolate was digested with *Hae* III, *Msp* I, *Taq* I, *Rsa* I and *Hind* III. Digested DNA samples were electrophoresed through 3 % agarose gel and stained with ethidium bromide. DNA fingerprint profiles were recorded. Dendrograms were constructed by UPGMA with NTSYS pc. Version 2.

## Results and Discussion

The selected 183 isolates of Aac were divided into 4 pathogenically groups consisted of group I: non-pathogenically on all test plants, group II: pathogenically on all test plant, group III: pathogenically only on watermelon and melon seedlings and group IV: pathogenically only on melon and cucumber seedlings. Based on disease severity scale, pathogenic isolates of Aac were sub-divided into 9 severity groups. Aac isolates from watermelon were the most diverse, belong to 7 severity groups, followed by Aac from squash and melon was placing into 4 severity groups. Aac isolates from white gourd, pickle melon and cucumber could not determine their diversity in this work. The different in colony formation was observed at 6 days old culture and categorized to 3 types as follows, type A: typical "fried egg" appearance, type B: circular form and undulate margin and type C: circular form and diffuse colony. Colony typing is related to Aac pathogenicity, type A is non-pathogenically grouped whereas type B and C are pathogenically grouped. DNA fingerprints of 16s rDNA after digested with restriction *Rsa* I and *Hind* III showed distinct pattern between non-pathogenically and pathogenically groups of Aac. Digestion with *Hae* III, *Msp* I and *Taq* I did not show polymorphism within this gene of all selected Aac isolates. These results conclude that genetic diversity of Aac occurrence in Northeast area of Thailand. However, ARDRA of 16S rDNA could not use to evaluate genetic relatedness inside pathogenically groups.

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## Reference

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