Bacterial Wilt of Bananas in Libya: Isolation and Characterization of the Pathogen

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ABSTRACT

A bacterial disease of banana *Musa chinensis cv. petit naine* grown in a plastic house on a site west of Tripoli (Libya) was identified as banana wilt. The bacterium was isolated from parts of plants showing symptoms similar to that of Moko disease. Typical wilt symptoms were induced in young banana suckers following inoculation with the pathogen. Based on its cultural characteristics, biochemical and pathogenicity tests, the causal bacterium was identified as *Pseudsomonas solanacearum,* banana isolate biotype 2.

INTRODUCTION

Growing banana under cover is a new practice that was developed in Morocco and introduced into Libya in 1989 by the General Company for Agricultural Marketing. The project started with an area of 1.75 hectare on a site west of Tripoli. By the end of 1992, the total area of banana under cover managed by the Secretariat of Reclamation and land Reform reached 100 hectares. An additional area of about 10 hectares is run by farmers in the private sector in and around Tripoli.

The banana crop is subject to several economically important diseases. Bacterial wilt (Moko disease) occurs in many parts of the World where banana is grown. The disease is caused by *Pseudomonas solanacearum* (E. F. Smith) and is destructive in Trinidad, Costa Rica, Panama, Honduras, Venezuela and South India (1, 4).

Severely wilted banana plants of the short type Petit naine showing symptoms that were very similar to Moko disease were observed in one of the plastic houses owned by the General Company for Agricultural Marketing in 1991. Infected samples were collected and attempts to identify the disease and causal agent were carried out.

MATERIALS AND METHODS

I. Isolation of the pathogen:

Pieces of diseased tissue from the inner and outer parts of a pseudostem showing discoloration were removed aseptically from samples collected on 20.2.1991. The segments were placed in 10 ml of sterile distilled water, and left to stand for 15-30 min. Alliquots of diffusates were streaked with a sterile loop on tetrazolium chloride agar (TZC) (6). All plates were incubated at 30 °C ±1 for 48 hr. Characterization of isolates

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was based on colony morphology observed on TZC medium, and on King's medium (7). After incubation at 30 °C ± 1, colonies with diameter >2.5mm, with elliptical to irregular borders and containing diffuse formazan pigmentation were designated as fluidal (F) [F4b]. On the other hand, round colonies usually <1.5mm in diameter with narrow clear borders surrounding intensely concentrated formazan pigmentation were designated as afluidal (F4a).

The presence of poly-B-hydroxybutryate was determined in smears using Sudan black B (2). Oxidase (8), and Catalase (9) tests were performed. The carbohydrates tested were glucose, sucrose, mannitol and sorbitol (5).

II. Inoculation and disease evaluation:

Bacterial isolates used in this experiment were of fluidal type F4b, and afluidal type F4a. Purified isolates were obtained by subculturing on TZC medium for 48 hr at 30°C ± 1. Inoculum was prepared by suspending 48 hrs old colonies grown on TZC agar in sterile distilled water, the concentration of bacterial suspension amounted to about 10^9 C.F.U. Three sets of four potted banana plants (approximately 15 cm tall) were used for inoculation. Plants in each set were inoculated by root dipping (3) after cutting off their tips.

Isolates F4b and F4a were used to inoculate banana plants in the first and second set, respectively, while roots of plants in the third set were dipped in sterile distilled water alone to serve as controls.

All plants were held in the greenhouse at 28-35 °C. The tests were repeated 2 times with different sets of plants.

RESULTS AND DISCUSSION

I. Isolation and characterization of the bacteria:

On the basis of colony morphlogy observed on TZC media and formazan formation, two different isolates of bacteria were obtained from pseudostems of banana plants showing discoloration of the inner tissues (vascular discoloration). Colonies of F4b isolates were highly fluidal on TZC medium with diffuse formazan pigmentation (Fig.1-A), However the F4a type isolate colonies appeared nearly round, smooth with centralized formazan pigmentation (Fig. 1-B).

Colonies of both isolates were non-fluorescent on King B medium, and accumulated poly-B-hydroxybutyrate. Oxidase and catalase tests were positive, and acid was produced in 5&7 days at 30 °C ± 1 from glucose, mannitol, and sucrose, but not from sorbitol (5).

II. Pathogenicity and symptom development:

Banana plants inoculated with isolate F4b showed wilt symptoms 15-25 days after inoculation, and became severely stunted (Fig. 2). However, plants inoculated with isolate F4a, showed slight wilting symptoms after 30 days (Fig. 2). The pathogen was readily reisolated from previously inoculated plants using TZC medium. The

Fig 1A

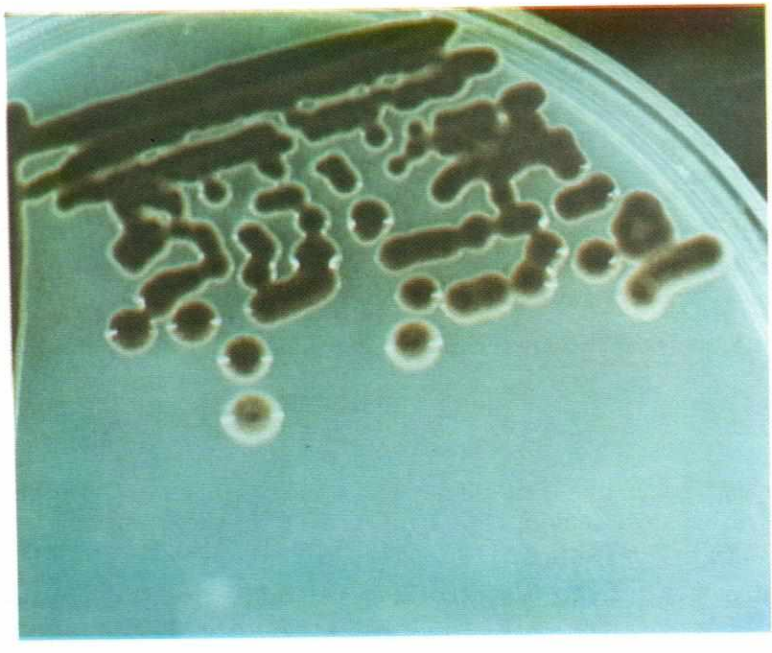

Fig 1B

Fig 2.

cultural, biochemical and pathological characteristics of the isolates under study suggested that these isolates could be designated as biotypes of *Pseudomonas solanacearum* E. F. Smith. (5, 12, 4) and the symptoms of wilting and vascular discoloration produced after inoculation with isolate F4b, strongly indicate that the disease is very similar to Moko disease described by many investigators (4, 5, 11, 12) as being caused by *Pseudomonas solanacearum* biotype 2. The characteristics of our banana isolate match closely but not exactly the banana biotype described by French and Sequeira (13). However, the difference in pathogenicity between isolates F4b, F4a under study, could be explained on the basis of virulence since the fluidal appearance of F4b colony represents the wild type that caused inoculated plants to wilt rapidly 15-25 days after inoculation in comparison with isolate F4a which represents the variant type of the fluidal type, and it produced mild wilting symptoms after 30 days.

These results are in agreement with those obtained by Kelman (6), and others (4, 5, 11).

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الذبول البكتيري على الموز: - عزل وخواص الكائن الممرض

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المستخلص

تم التعرف على أحد الأمراض البكتيرية على نبات الموز بإحدى الصوبات البلاستيكية الكائنة بغربي مدينة طرابلس على أنه مرض ذبول الموز. عزلت البكتيريا المسببة للمرض من أجزاء النباتات التي تكشفت عليها أعراض شبيهة بأعراض مرض «موكو».

إستحدثت أعراض نموذجية للذبول على نباتات موز صغيرة عن طريق الإلقاح بواسطة مسبب المرض، وبناء على الخواص المزرعية والبيوكيميائية واختبارات الأمراضية، فقد تم تعريف البكتيريا الممرضة بأنها سودوموناس سولانسيريم *Pseudomonas solanacearum* عزلة الموز طراز حيوي 2.