

ISOLATION OF NON PATHOGENIC BACTERIAL CONTAMINANTS OF MICROPROPAGATED DATE PALM (*Phoenix dactilyfera* L.) and banana (*Musa* sp.) in Morocco

BENJAMA* Ah.

ملخص

لوحظت العدوى البكتيرية على بعض النخيلات الناتجة عن زراعة الأنسجة ولقد تبث أن هذه العدوى تأتي مع الفسائل القادمة من الواحات. إن الجراثيم المسؤولة قد عزلت وعينت كـ *Bacillus* بنوعين : نوع Gram - بدون سوط flagella ممثل بصنفي *Bacillus pumillus* و *B. sphaericus* المعزولة من شجر النخيل، ونوع Gram + دو سوط ممثل بـ 5 أصناف *B. brevis* , *B. laterosporus* , *B. circulans* المعزولة من شجر النخيل و *B. subtilis* المعزولة من شجر الموز و *B. cereus* المعزولة من شجر النخيل والموز.

الجراثيم تكون حجابا خفيفا أو كثيفا سفافا داخل الأوساط (Milieu) المستعملة في زراعة الأنسجة أو تكون طبقة صفراء أو بيضاء على السطح.

* INRA, Phytobacteriology, Marrakech, Morocco.

RESUME

Des contaminations bactériennes ont été observées sur des vitroplants de palmier dattier. Il a été confirmé que ces contaminations viennent avec le rejet issu de la palmeraie. Les bactéries ont été isolées et identifiées comme *Bacillus* avec 2 types : Un type gram négatif, sans flagelle représenté par *Bacillus pumilus* et *B. sphaericus* isolées de palmier dattier, et type gram positif, flagellé représenté par 5 espèces; *Bacillus brevis*, *B. laterosporus*, *B. circulans* isolés du palmier dattier, *B. subtilis* isolé du bananier et *B. cereus* isolé du palmier dattier et du bananier.

Les bactéries forment un voile léger ou dense, transparent à l'intérieur des milieux de culture ou un film jaune ou blanc à sa surface.

SUMMARY

Bacterial contaminants were observed contaminating date palm and banana explants in culture test tubes. It was shown that these contaminants are brought with date palm hearts selected in the field for seedling multiplication. Identification has revealed the presence of two types of *Bacillus* : a Gram negative, a flagellate type represented by *B. pumilus* and *B. sphaericus* isolated from date palm; and a Gram positive, flagellate type represented by five species *B. brevis*, *B. laterosporus*, *B. circulans* isolated from date palm, *B. subtilis* isolated from banana, and *B. cereus* isolated from both date palm and banana. These bacteria form a more or less dense and translucent, white, yellow or creamy layer in the medium used to grow of date palm or banana tissues.

Key words : date palm, banana, vitro-plant, bacterial contamination, *Bacillus* spp

INTRODUCTION

In-vitro culture of date palm has developed rapidly in Morocco during the past few years. This technique allows a rapid multiplication of healthy date palm seedlings used to replace the trees killed by "bayoud", a most devastating fungus disease (6). The first in-vitro produced date palm seedlings were produced in Morocco in 1977 (23). They were obtained from the heart meristem of offshoots selected in date palm groves. Despite surface sterilisation of plant material used to start seedling production, accidental bacterial contaminations can occur (27). There are reports about the association of bacteria and healthy plant fruits (8), seeds (25), seedlings (16) and ovules (22). Various bacterial contaminants have been reported. Leary et al. (15) and Leary & Chun (14) isolated a pathogenic Gram negative *Bacillus circulans* from date palm culture of tissue in United State. Cornu & Michel (5) isolated a non-pathogenic *Pseudomonas* from *Prunus avium* in France while Boxus & Terzi (3) in Belgium and Fisse et al. (7) in Spain identified other bacterial genera like *Torulopsis*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium* and *Corynebacterium* on strawberry; and *Bacillus* on ornamental *Syngonium* sp. and *Ficus* sp. Leifert et al. (17, 18, 19), working with nine plant species, found that 90% of bacterial isolates were *Bacillus* and 30% constituted by *Enterobacter*, *Pseudomonas*, *Staphylococcus* or *Lactobacillus*.

Here in Morocco, a bacterial veil was often observed to develop in the medium around the mass propagation tissue. We were contacted in 1987 to solve the problem.

If the tissue culturists don't pay a permanent attention, they can select and spread very quickly some resistant lines of bacteria.

MATERIALS AND METHODS

Isolation

Bacterial contaminants were isolated from explants using YPGA medium (Yeast 5g, Peptone 5g, Glucose 10g and Agar 20g/L). Direct isolations were also made from date palm and banana hearts. The hearts were surface desinfected one minute with chlorox and their meristematic part was cut into pieces in sterile distilled water. After 30mn of maceration, a drop of the macerating liquid was spread on two media, YPGA (20) and King's B (11) and incubated at 27°C.

Identification by biochemical characters

The tests used were : the standard Gram staining as well as the KOH Gram method (26), the Glucose in soft and deep agar (9), Oxidase (12), flagella staining (21), sporulation, motility (4,25).

The biochemical tests were Glucose, L (+) tartrate, mannitol, casein, gelatin, saccharose, citrate, malonate, erythritol, starch, nitrate, vp32°C and vp37°C, NaCl 7%, xylose, arabinose and growth under 45° - 55° - 65°C (1, 2 10, 13; 24).

RESULTS AND DISCUSSION

Different types of bacteria grew out of the explants and of the date palm and banana hearts (Table II).

- The first type is spherical and catenulate, aflagellate, and Gram negative (strains VP1 and 318).

- The second type is rod-shaped with 2 to 4 polar flagella, and Gram positive.

Both types of bacteria produced spores. According to the previous tests, they both belong to the genus *Bacillus*.

In the first type, isolated from date palm hearts, we found two species of *Bacillus* : *B. pumilus* (strain VP1) and *B. sphaericus* (strain 318) (Table II).

In the second type, we found 5 species of *Bacillus* : *B. brevis* (strain 161, VP7, 324), *B. laterosporus* (strain 325) and *B. circulans* (strain 326) from date palm; *B. subtilis* from banana (strain 327 . 2) and *B. cereus* (strain 314, 315, 316, 317) from both date palm and banana. It is noteworthy that *B. circulans* was isolated by Leary & Chun (14), from date palm, but was Gram negative and pathogenic.

These bacteria particularly *B. pumilus* (strain VP1) and *B. cereus* (strain VP7) were often both isolated from the bacterial veil in the test tube and associated with the hearts of the date palm shoots. This suggests that they belong to the natural microflora associated with the date palm shoots. A similar result has been reported by Hollis (8), Leben (16), Schathorst (25) and Mundt & Hinkle (22).

It must be emphasised, however, that isolations do not always yield bacteria, suggesting that these may or may not be associated with shoots. It is also important to note that these Bacilli have been isolated from shoots not showing any pathological symptoms. This suggests that the plant is a healthy carrier. Contamination of plants may have come from the soil or from irrigation water. Consequently disinfection of date palm hearts is necessary before starting in-vitro cultures.

CONCLUSION

The bacteria contaminating in-vitro plants of date palm and banana belong to species of the genus *Bacillus*. These bacteria seem to be naturally associated with date palm shoots sampled in the field. They do not produce any symptoms and are hence non-pathogenic.

Bacteria could not be isolated from all shoots. Successful isolations depend on the origin of the shoots.

Control of bacterial contaminants may prove to be difficult because of the high number of species involved and because the genus *Bacillus* is known to be difficult to control. Furthermore, bacteria may be hidden deeply between the scales, or in plant tissues.

Table I : origin of isolates

N° of isolates	origin	date of isolation	host plant	type of infection
VP1	* INRA	1987	Date palm	White veil
VP7	INRA	1987	"	White veil
161	INRA	1988	"	White veil
314	INRA	1992	"	White bacterial layer
315	** DROY	1992	"	White bacterial layer
316	DROY	1992	Banana	White bacterial layer
317	"	1992	Date palm	+ white veil
318	INRA	1992	"	yellow bacterial layer + veil
324	"	1992	"	white veil
325	"	1992	"	white bacterial layer
326	DROY	1992	Banana	cream bacterial layer
327.1	"	1992	"	cream bacterial layer
327.2	"	1992	"	cream bacterial layer

* Institut National de la Recherche Agronomique of Marrakech

** Domaine Royal de Meknes

Table II : Biochemical characterization of bacterial isolates from the date palm and banana cultured in vitro.

bacterial isolates Characters	vp1	318	161	VP7	324	314	315	316	317	3272	325	326	3271
Sporul.	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram	-	-	+	+	+	+	+	+	+	+	+	+	+
gelat.	-	-	+	+	+	+	+	+	+	+	+	+	+
casein	-	-	+	+	+	+	+	+	+	+	+	+	+
Growth 45°C	-	-	+	+	+	+	+	+	+	+	+	+	+
motility	i	i	+	+	+	+	+	+	+	+	+	+	+
vp32°	i	i	+	+	+	+	+	+	+	+	+	+	+
vp37°	i	i	+	+	+	+	+	+	+	+	+	+	+
7% NaCl	i	i	+	+	+	+	+	+	+	+	+	+	+
starch	-	-	+	+	+	+	+	+	+	+	+	+	+
growth 55°C	-	-	+	+	+	+	+	+	+	+	+	+	+
xylose	+	+	-	-	-	V	-	-	-	-	-	-	-
mannitol	+	+	-	-	-	V	-	-	-	-	-	-	-
glucose	+	+	-	-	-	V	-	-	-	-	-	-	-
nitrate	+	+	-	-	-	V	-	-	-	-	-	-	-
arabinose	+	+	-	-	-	V	-	-	-	-	-	-	-
citrate	+	+	-	-	-	V	-	-	-	-	-	-	-
growth 65° C	+	+	-	-	-	V	-	-	-	-	-	-	-
anaerob. grow.	i	i	in	in	in	-	-	-	-	-	-	-	-
L+ tartrate	+	+	-	-	-	-	-	-	-	-	-	-	-
Malonate	+	+	-	-	-	-	-	-	-	-	-	-	-
Erythritol	+	+	-	-	-	-	-	-	-	-	-	-	-
Saccharose	-	+	-	-	-	+	+	+	+	+	+	+	+
Flagella	AF	AF	PF	PF	PF	PF	PF	PF	PF	PF	PF	PF	PF

anaerob. grow. : anaerobiose growth (- : oxidative, + : fermentative)
 + : positive reaction
 - : negative reaction
 i : indet. in stab. f
 in : inerte
 PR : Peritrichous flagellation (2 or 4 flagella)
 AF : Aflagellate bacteria

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