ISOLATION AND IDENTIFICATION OF BACTERIA FROM SOYBEAN LEAVES

Koushi Nishiyama[1]; Maria José D'avila Charchar[2]; Norio Iizuka[1]

Introduction

The objective of this study is to isolate and identify some bacteries that cause disease on soybean (*Glycine max* (L.) Merrill).

Although it was initially planned to study bacterial diseases of common bean (*Phaseolus vulgaris* L.), only two locations could be surveyed, ie. a small field in Brasília D.F. and an experimental field of EPABA at Barreiras in Bahia State (Figure 1). However, bean plants in those fields were affected severely with rust and other fungal diseases, and no typical bacterial diseases were detected. Therefore in this study, bacterial isolates from soybean plants were examined.

Materials and Methods

Diseased plants were collected from fields located in Brasília D.F., Londrina in Paraná State, and Barreiras in Bahia State (Table 1). A fragment of a specimen obtained from a leaf where bacterial exudation was observed was treated with a 0.3% hypochlorite solution for surface sterilization. Then the fragment was homogenized with 3 ml of sterilized water with a glass-homogenizer. A loop of the homogenate was smeared onto a nutrient agar plate. Some prominent colonies grown on the medium were transferred to King's B agar slants after 4 to 6 days of culture. These isolates were used for tests of the bacteriological characteristics and pathogenicity. No specimen was obtained where two or more kinds of pathogenic bacteria were isolated together in a segment. Therefore, one isolate was selected from the isolates obtained form one and the same plant and used for further tests.

[1] Phytopathology Specialist, Consultant from EMBRAPA/JICA.
[2] Eng.-Agr., Ph.D., EMBRAPA/Centro de Pesquisa Agropecuária dos Cerrados (CPAC), Caixa Postal 08223, CEP 73301-970 Planaltina,DF.

FIG. 1 - Bacterial blight of common bean (*Phaseolus vulgaris* L.).

TABLE 1 - Source of tested isolates.

Isolate number	Host plant	Date of collection	Locality	Field
BR 1	Soybean cv. Cristalina	Feb. 21, 1989	Taquara, DF	Farmer's field
BR 2	Soybean cv. Cristalina	Feb. 21, 1989	Taquara, DF	Farmer's field
BR 3	Soybean cv. Cristalina	Feb. 21, 1989	Taquara, DF	Farmer's field
BR 4	Soybean cv. Cristalina	Feb. 21, 1989	Taquara, DF	Farmer's field
BR 5	Soybean cv. Cristalina	Feb. 21, 1989	Taquara, DF	Farmer's field
BR 6	Soybean cv. Cristalina	Feb. 21, 1989	Taquara, DF	Farmer's field
BR 7	Soybean cv. Cristalina	Feb. 21, 1989	Taquara, DF	Farmer's field
BR 8	Soybean cv. Cristalina	Feb. 21, 1989	Taquara, DF	Farmer's field
BR 9	Soybean cv. Doko	Feb. 21, 1989	Rio Preto, DF	Farmer's field
BR 10	Soybean cv. Doko	Feb. 21, 1989	Rio Preto, DF	Farmer's field
BR 11	Soybean cv. Doko	Feb. 21, 1989	Rio Preto, DF	Farmer's field
BR 12	Soybean cv. Doko	Feb. 21, 1989	Rio Preto, DF	Farmer's field
BR 13	Soybean cv. Doko	Feb. 21, 1989	Rio Preto, DF	Farmer's field
LN 2	Soybean	Feb. 28, 1989	Londrina, PR	Experimental field
LN 3	Soybean	Feb. 28, 1989	Londrina, PR	Experimental field
LN 6	Soybean	Feb. 28, 1989	Londrina, PR	Experimental field
LN 10	Soybean	Feb. 28, 1989	Londrina, PR	Experimental field
LN 17	Soybean	Feb. 28, 1989	Londrina, PR	Experimental field
LN 18	Soybean	Feb. 28, 1989	Londrina, PR	Experimental field
LN 19	Soybean	Feb. 28, 1989	Londrina, PR	Experimental field
BA 24	Soybean	March 16, 1989	Barreiras, BA	Experimental field
BR 25	Soybean	March 17, 1989	Roda Velha, BA	Farmer's field
BA 27	Soybean	March 17, 1989	BR 020, Km 320, BA	Farmer's field
BA 28	Soybean	March 17, 1989	BR 020, Km 320, BA	Farmer's field

Bacteriological characteristics were tested by the methods listed in the references cited below (Nishiyama, 1978). Some modifications were made to prepare the media if the recommended reagents were not available. Leaves of potted soybean were smeared with a bacterial suspension, ca 109 cfu/ml, using a painting brush and covered with vinyl film over-night to maintain moist conditions. Then the inoculated plants were kept in the green house to observe the pathogenicity.

Results

The twenty four bacterial isolates listed in Table 1 were divided into two groups, 18 isolates with white colonies and 6 isolates with yellow colonies. The bacteriological characteristics tested are shown in Table 2. The bacteria with white colonies showed similar characteristics. The pathogenicity test to soybean cv. EMGOPA 302 gave inconclusive results. The isolates with yellow colonies hardly grew on the slants and did not produce acid, which was the test for the utilization of the sole carbon source. They induced typical symptoms accompanied with pustules on soybean cv. EMGOPA 302.

TABLE 2 - Bacteriological characteristics of tested isolates.

Character	Isolate									
	BR 1-4	BR 5,6	BR 7,8	BR 9-13	LN 2,3,6	LN 10	LN 17-19	BA 24	BA 25	BA 27,28
Colony color	Wa)	W	W	W	Yb)	W	Y	W	W	W
Shape of cell	SRc)	SR	SR	SR	SR	SR	SR	NTd)	NT	NT
Relation to oxygen	Ae)	A	A	A	A	A	A	NT	NT	NT
Gram reaction	-	-	-	-	-	-	-	-	-	-
Green fluorescent Pigment	+	+	+	+	-	+	-	+	+	+
Water insoluble Pigment	-	-	-	-	Y	-	Y	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+
Growth at 33°C	+	+	+	+	+	+	+	NT	NT	NT
35°C	-	-	-	-	+	-	+	NT	NT	NT
40°C	-	-	-	-	-	-	-	NT	NT	NT
Potato soft rot	-	-	-	-	-	-	-	NT	NT	NT
H2S production	-	-	-	-	+	-	+	NT	NT	NT
Levan formation	+	+	+	+	N	+	NT	+	+	+
Slimy growth on 5% Sucrose	NT	NT	NT	NT	d) +	NT	+	NT	NT	NT
Hydrolysis of esculin	-	-	-	-	+	-	+	-	NT	-
Utilization of Asparagine	+	+	+	+	-	+	-	NT	NT	NT
Reduction of nitrate	-	-	-	-	-	-	-	NT	NT	NT
Liquefaction of gelatin	-	-	-	-	+	-	+	-	NT	-
Acid production from										
Glucose	+	+	+	+	-	+	-	+	NT	+
Sucrose	+	+	+	+	-	+	-	+	NT	+
Trehalose	-	-	-	-	-	-	-	-	NT	-
Mannitol	-	-	-	-	-	-	-	-	NT	-
Sorbitol	-	-	-	-	-	-	-	-	NT	-
Inositol	-	-	-	-	-	-	-	-	NT	-
Galactitol	-	-	-	-	-	-	-	-	NT	-
Erythrytol	-	-	-	-	-	-	-	-	NT	-
Alkali production from L-Tartrate	-	-	-	-	-	-	-	-	NT	-
Path-ogenicity to Soybean cv. EMGOPA 302	Xf)	X	X	X	+	X	+	NT	NT	NT

+: positive, -: negative, a) W: white, b) Y: yellow, c) SR: short rod, d) NT: not tested, e) A: aerobic, f) X: inconclusive result

Discussion

The eighteen isolates with white colonies consisted of short-rods, that were aerobic and Gram-negative. They produced green fluorescent pigment, and utilized L-tartrate and sucrose. They were obtained from soybean leaves which showed symptoms of bacterial blight (Figure 2). Though their pathogenicity was not fully confirmed, they were tentatively identified as *Pseudomonas syringae* pv. *glycinea* (Coerper, 1919) Young, Dye and Wilkie 1978.

FIG. 2 - Bacterial pustule of soybean (*Glycine max* (L.) Merrill).

The six isolates of with yellow colonies which consisted of short-rods and were Gram-negative. They appeared slimy on nutrient agar supplemented with 5% sucrose and produced a water-insoluble yellow pigment and hydrogen sulfide. They did not grow in asparagine medium where asparagine was the sole source of carbon and nitrogen. They were pathogenic to soybean cv. EMGOPA 302 and induced the formation of pustules. They were therefore identified as *Xanthomonas* campestris pv. *glycines* (Nakano, 1919) Dye 1978.

Bacterial diseases of soybean like *Pseudomonas syringae* pv. *glycinea* (Bacterial Blight), that is more common in Brazil, *Xanthomonas campestris* pv. *glycines* (Bacterial Pustule) and *Pseudomonas syringae* pv. tabaci (Wildfire) have bean studied in this country (Yorinori, 1986).

Soybean fields with the cultivar "Doko" were severely attacked by bacterial blight in Brasília D.F. and no other cultivar, was affected to such an extent. In the inoculation test, the soybean cultivar "EMGOPA 302" did not show typical symptoms after infection with the isolates of *P. syringae* pv. *glycina*, presumably due to differences in varietal resistance to the pathogen.

In the experimental station, EPABA, in Barreiras, one soybean cultivar out of hundreds showed symptoms of bacterial blight white the others were not affected. Those cultivars were supplied by EPABA with seeds from EMBRAPA/CNPSoja in Londrina and were grown to observe their genetic characteristics under field conditions. The cultivar affected may indicate that the seeds were infected by the pathogen before sowing.

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During this period, we conducted some experiments on bacterial diseases of legumes. The results obtained are described in the present paper.

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