Pak.J.Bot., 29(2): 235-241, 1997.

USE OF CELLULAR FATTY ACIDS IN THE CHARACTERIZATION AND DIFFERENTIATION OF BACTERIAL LEAF STREAK PATHOGENS ON MILLET

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Abstract

Analyses of cellular fatty acids (FAs) were used to identify bacterial leaf streaking pathogen(s) infecting foxtail and pearl millet in Colorado, USA. Isolates from both hosts were identified as unknown pathovar(s) of *Xanthomonas campestris*. To determine any similarity the most discriminatory FA profiles of the US isolates were compared with those of *X. campestris*. pv. *pennamericanum* infecting pearl millet in Africa and 11 other *X. campestris* pathovars infecting members of the Poaceae family. The US isolates were different from *X. campestris*. pv. *pennamericanum* and the other *X. campestris* pathovars but similar among themselves. When a dendogram of the millet isolates was constructed with 35 other *X. campestris* pathovars, the different nature of the US isolates was further clarified. However, this evidence was not enough to give the American isolates a separate pathovar status.

Introduction

Traditional characterization of bacteria is a complex, cumbersome and time-consuming procedure (Stead, 1988). Previously, most researchers relied on the conventional physiological and biochemical tests to identify bacterial isolates. However, these tests are not very authentic (Dye, 1962). Scientists have been continuously striving for rapid and accurate identification techniques. Therefore, at present more accurate and fast methods of bacterial identification are used. Some of these techniques are: monoclonal antibodies (Alvarez *et al.*, 1985), restriction fragment-length polymorphism (RFLP) (Lazo *et al.*, 1987), Biolog (Jones *et al.*, 1993; Ayub *et al.*, 1995), DNA hybridization (Vauterin *et al.*, 1992) and fatty acid analysis (Roy, 1988). Among these techniques analysis of whole-cell fatty acid (FA) profiles has become one of the important tools in the accurate identification of bacteria (Roy, 1988; Stead, 1988, 1989; Sasser, 1990). Since FA profiling is computer assisted therefore, it is less expensive, rapid and most importantly accurate (Stead, 1988). In plant pathogenic bacteria, FAs between 9 and 20 carbons in length are in appreciable amount and they are found in the membrane and in some bacteria in the lipopolysaccharides (Sasser, 1990). It is also interesting to note that FA profilings agree with DNA and RNA homology study in some bacterial strains (Sasser & Smith, 1987).

A bacterial leaf streaking pathogen, provisionally identified as *Xanthomonas* of unknown species and pathovar affiliations, was reported on pearl millet (*Pennisetum americanum*) in the state of Colorado, USA (Swift *et al.*, 1991). Later on these and other isolates infecting foxtail millet (*Setaria italica*) were identified with Biolog as

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Xanthomonas campestris of unknown pathovar specification (Ayub *et al.*, 1995). At the same time a bacterial leaf streaking pathogen of pearl millet, identified to be *X. campestris* pv. *pennamericanum*, was reported as a new pathovar from Nigeria, Africa (Qhobela & Claflin, 1988). Since Biolog did not differentiate the US isolates from *X. campestris* pv. *pennamericanum* (Ayub *et al*, 1995) therefore, it was believed that African isolate and the US isolates, both from pearl and foxtail millets, were similar. As FA analyses have been shown to differentiate *X. campestris* strains from Poaceae (Stead, 1989) therefore, this method was used as identification and differentiation technique.

The present report describes the use of fatty acids in the characterization and differentation of bacterial leaf streak pathogens on millet.

Materials and Methods

Isolates: Foxtail and pearl millet leaves having water-soaked leaf streaks were collected from the fields in Colorado during 1992 and 1993 growing seasons. Isolation procedure from these leaves has been discussed elsewhere (Ayub *et al.*, 1995). Isolates from foxtail and pearl millet were designated as isolate X and Y, respectively. For comparison isolate B6, identified to be *X. campestris* pv. *pennamericanum* (Qhobela & Claflin, 1988), and provided by Dr. Larry Claflin of Kansas State University, USA was also included.

Identification and comparison of fatty acids: Lyophilized cultures of isolate X, Y and B6 were grown over night on separate Petri dishes containing tripticase soy agar (TSA) medium. These Petri dishes were sent through expedited mail (Federal Express) to "The Microbe Inotech Laboratories, Inc. St. Louis, Missouri, USA" for FA analyses. These isolates were examined against both versions of aerobes (TSBA, Rev. 3.70) and the clinical aerobes (Clin, Rev. 3.70) databases. FA results of these three isolates were compared with FA profiles of *X. campestris* pvs. *graminis, translucens* and *vasculorum* provided by Dr. Ralph Paisley of "The Microbial Identification Inc. (MIDI) Newark, Delaware, USA". For comparison with additional *X. campestris* pathovars like *hordei, cerealis, secalis, undulosa, holcicola, coracana, oryzicola*, and *oryzae* FA data was adopted from Stead (1989) and Vauterin *et al.*, (1992). All these pathovars are pathogenic on various members of Poaceae.

Relationship with other *X. campestris* pathovars: Data from the two laboratories (Delaware and Missouri) and previous reports (Stead, 1989 and Vauterin *et al.*, 1992) were compared with millet isolates of the US and African origins. Only those FAs that are diagnostic for *X. campestris* pathovars were considered. Cluster analysis was performed with 35 different *X. campestris* pathovars to determine relationship of millet isolates with them. Dendogram was constructed to determine similarities or differences in these pathovars by measuring euclidian distances.

Results and Discussion

Isolates identification: All the millet isolates were identified as unknown *X. campestris* pathovars using gas chromatography of fatty acid methyl esters (GC-FAMEs). Similari-

Table 1. Isolate identification with GC-FAMEs profile.

Isolate Designation	Identification by FAME	Similarity Coefficient	Distance Coefficient
X	*Xanthomonas campestris*	0.719	2.625
Y	*Xanthomonas campestris*	0.804	2.134
B6	*Xanthomonas campestris*	0.625	3.132

X: Foxtail Millet Isolate
Y: Pearl Millet Isolate
B6: X.c.pv. *pennamericanum*

ty indices (SIs) were 0.72 for the foxtail isolate, and 0.80 and 0.63 for the pearl millet isolates from the US and Africa, respectively (Table 1). SIs demonstrate closeness of the unknown isolates with those already stored in the database (Roy, 1988). SIs range from 0 to 1.0 and SI values between 0.6-1.0 are considered excellent for positive identification (Mihail *et al.*, 1993). Since SIs of all isolates were above 0.6 therefore, they were identified as *X. campestris* (Table 1). Currently the available database contains FA profiles of more than 9000 strains of bacteria and can identify most bacteria to the pathovar or subspecies level (Sasser, 1990). FA analyses identified isolates from millet to the species level but did not identify them to the pathovar level. The reason why the

Table 2. Ratios of FAs that constituted >5% of the total profile in millet isolates and their comparison with 11 other *X. campestris* pathovars from Poaceae.

Pathovar	Percent fatty acid of total profile					
	15:0 Iso	15:0 Anteiso	16:1 Cis 9	16:0	Iso 17:1 w9C	17:0 Iso
Graminis	31.2	4.4	20.2	4.2	9.5	7.6
Translucens	24.1	6.1	24.3	6.8	6.8	4.1
Vasculorum	21.3	1.9	20.2	4.5	19.3	17.2
Hordei	21.5	5.4	21.0	4.6	7.0	3.2
Cerealis	26.3	5.5	21.1	4.3	7.4	3.2
Secalis	27.9	7.0	21.1	3.5	6.8	2.9
Undulosa	24.5	5.9	23.5	4.3	7.1	3.4
Holcicola	16.0	2.7	23.1	14.6	11.4	6.7
Coracana	19.9	7.8	17.2	5.0	4.3	5.5
Oryzicola	8.6	1.5	23.5	13.8	12.8	17.5
Oryzae	3.0	---	24.8	22.5	7.6	14.8
Pennamericanum	28.4	10.8	19.5	7.3	6.9	8.0
X	22.7	11.9	21.5	8.0	4.7	7.0
Y	26.2	10.8	22.3	7.3	4.4	6.0

isolates were not accurately identified at the pathovar level is that the isolates included in our study were new and identified for the first time therefore, it is most probable that the existing database did not have their suitable match.

FAME profile comparison in various *X. campestris* pathovars and differentiation of millet isolates: Even though *X. campestris* from cereals have upto 40 FAs (Stead, 1988) the most discriminatory FAs for them are 11:0 ISO, 11:0 ISO 3OH, 13:0 ISO 3OH, 15:0 ISO, and 15:0 ANTEISO (Roy, 1988). The percent profiles of 11:0 ISO were 3.4, 4.1 and 4.4% for isolate B6, X and Y, respectively. Combined profiles of 15:0 ISO and 15:0 ANTEISO accounted for 39.2% of the total profile for isolate B6, 34.6% and 37.0% for the US isolate X and Y, respectively (Table 2). This is in line with the findings of Chase *et al.* (1992) who stated that they constitute 32-55% of the total profile in *X. campestris* from aroids. Profiles of these three FAs show clear distinction among the millet isolates among themselves as well as other *X. campestris* pathovars. Percent profile of 11:0 ISO 3OH were 1.4% for the isolates X and Y, and 1.2% for *X. campestris* pv. *pennamericanum*. For 13:0 ISO 3OH these profiles were 3.2% for isolates X and Y, and 2.8% for *X. campestris* pv. *pennamericanum*. Analyses of these two FAs suggest that the US isolates of both hosts are similar while isolate B6 is different from them. Ratios of these five FAs among the various millet isolates are shown in Fig.1. which further clarifies the difference between the US and African isolates. Different nature of the US and African isolates is further supported by the ratios of 15:0 ISO to 15:0 ANTEISO (Fig.2) and 15:0 ISO to 16:0 ISO (Fig.3) because these ratios are also used to differentiate some *X. campestris* strains (Stead, 1989).

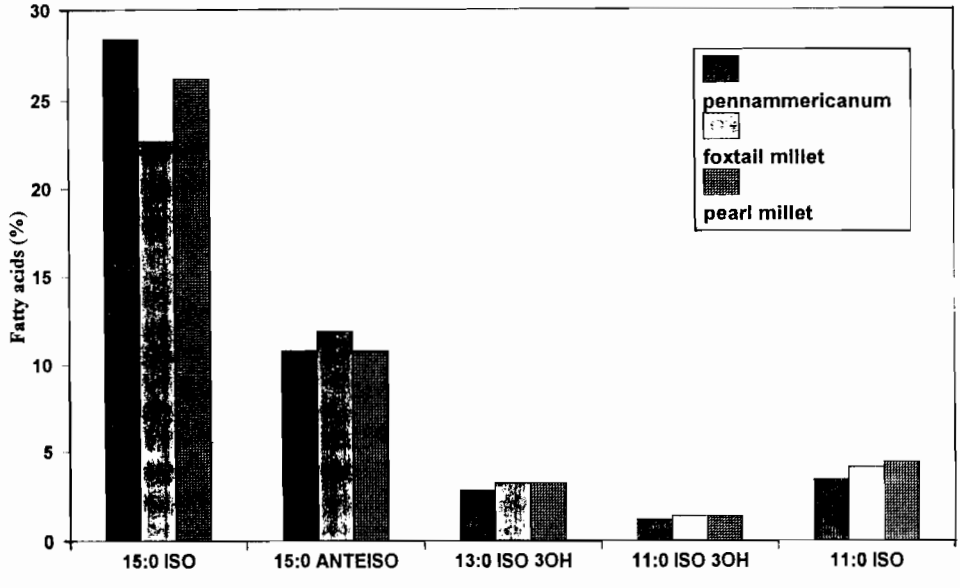

Fig.1. Ratio of different fatty acids in various isolates from millet

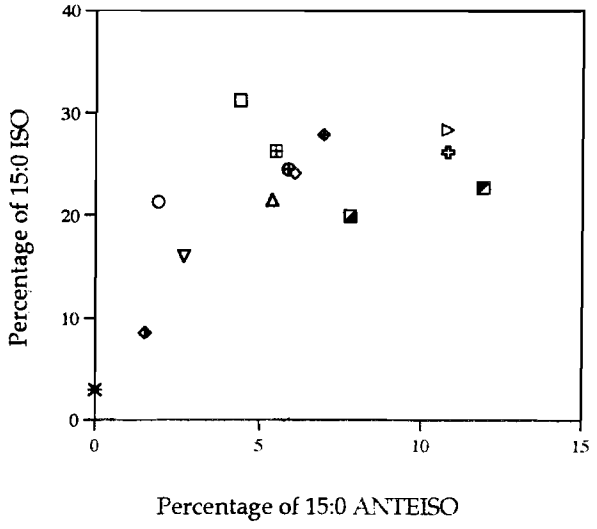

Fig.2. *Xanthmonas compestris* pathovars differentitation according to 15:0 ISO/15:0 ANTEISO ratio.

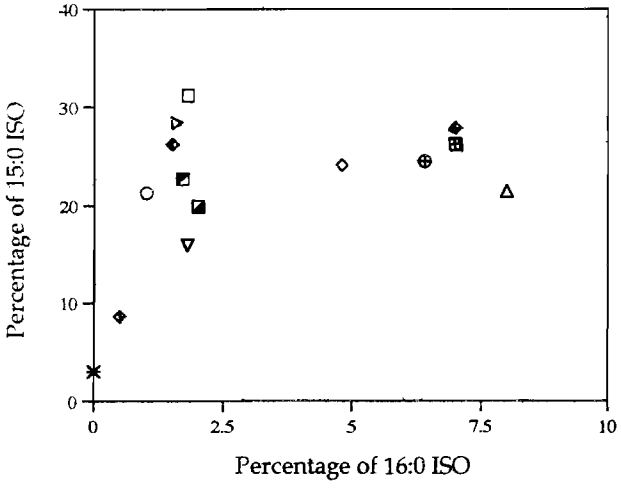

Fig.3. *Xanthomonas campestris* pathovars differentiation according to 15:0 ISO/16:0 ISO ratio.

□	*Translucence*	▽	*Holeicola*
◇	*Translucence*	◪	*Coracana*
○	*Vasculorum*	◆	*Oryzicola*
△	*Hordei*	✱	*Oryzae*
⊞	*Cerealis*	▷	*Pennamericanum*
◆	*Secalis*	◪	*Foxtail Isolate*
⊕	*Undulosa*	✣	*Pearl Isolate*

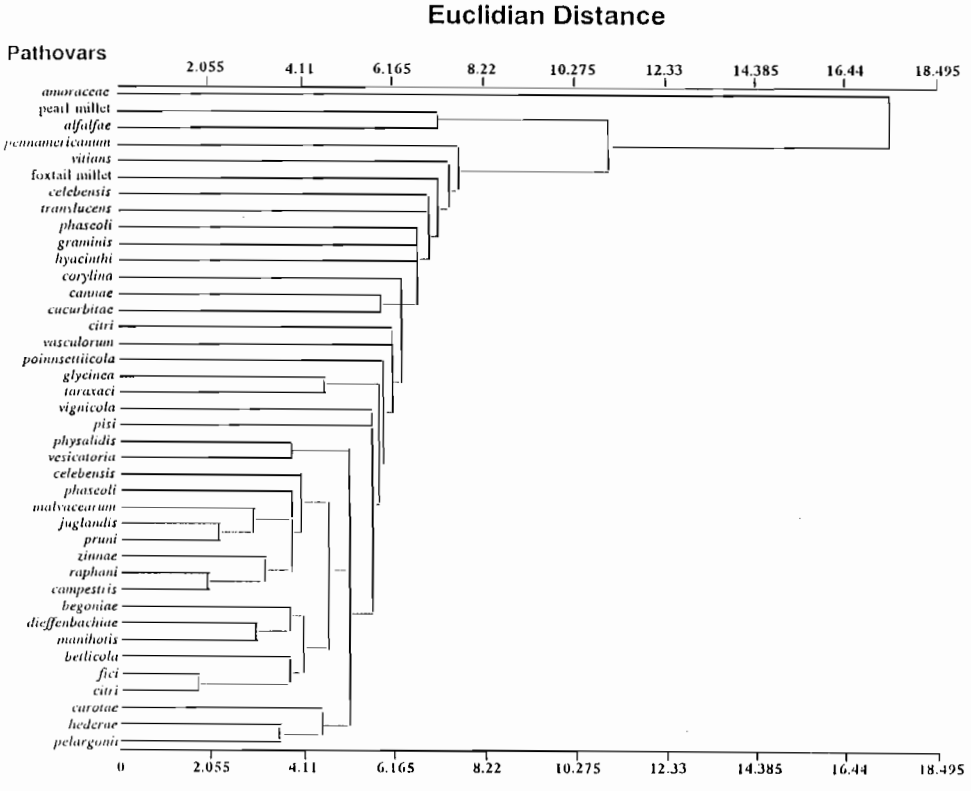

Fig.4. Dendogram obtained by average linkage of euclidian distance values among FAME profiles of various *Xanthomonas campestris* pathovars.

Atleast 20-23 FAs were identified in the millet isolates. FAs that constituted more than 5% of the total profile are given in Table 2. Nearly same FAs have been reported as major ones (more than 5%) for 14 *X. campestris* from Poaceae by Stead (1989). These were compared with 11 other *X. campestris* pathovars of Poaceae (Table 2). Based on the analyses of the distinctive FA profiles it is concluded that the US isolates are different from *X. campestris* pv. *pennamericanum* as well as other *X. campestris* pathovars. Instead of the different nature of the US isolates they can not be given a different pathovar status since FA analysis is a single criterion and bacteria can not be classified on a single character (Stead, 1988). Further, the US isolates of both hosts are similar.

Relationship with other *X. campestris* pathovars: Dendogram based on FA analyses, of 35 different *X. campestris* pathovars alongwith three millet isolates was constructed. It shows that the US isolates are not related to African and other pathovars. At an Euclidian distance of 7.20 the US isolates from both hosts are similar but different from the African isolate. The African isolate occurs at an Euclidian distance of 7.715 but at this

distance all the three isolates are not distinguishable (Fig.4). This further suggests that the US isolates are similar whereas isolate B6 is different from them.

Acknowledgement

The senior author is grateful to Dr. Carol Ishimaru, Assistant Professor of Plant Pathology, Colorado State University, Fort Collins Colorado, USA, for letting him use her laboratory facilities.

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(Received for publication 19 February, 1997)