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Whole Cell Fatty Acid Composition of *Xanthomonas axonopodis* pv. *vesicatoria* Strains, Isolated From Pepper

Mustafa MIRIK* **Yesim AYSAN**** **Fikrettin SAHIN*****

* Department of Plant Protection, Faculty of Agriculture, University of Namık Kemal, 59030 Tekirdag, TURKEY
Corresponding Author: Mustafa MIRIK

** Department of Plant Protection, Faculty of Agriculture, University of Cukurova, 01330 Adana, TURKEY

*** Department of Plant Protection, Faculty of Agriculture, University of Ataturk, 25240 Erzurum, TURKEY

ABSTRACT

Bacterial spot, caused by *Xanthomonas axonopodis* pv. *vesicatoria,* is one of the most important disease of pepper (*Capsicum* spp.) in the eastern Mediterranean region of Turkey. During 2002-2004, plant samples with bacterial spot symptoms were collected from 83 commercial fields in Adana, Osmaniye, and Mersin provinces. *X. axonopodis* pv. *vesicatoria* were isolated and identified by traditional methods. Whole cell fatty acid methyl ester (FAME) analysis in Sherlock Microbial Identification System (MIS) with database of prokaryotes was used together with traditional methods for identifying and phenotypically characterization of the strains. One hundred and seventy bacterial strains were identified as *X. axonopodis* pv. v*esicatoria* by traditional methods and pathogenicity tests. Identification of the selected 19 strains was confirmed by FAME analysis with similarity indices ranging from 37-76%. Total 24 different fatty acids were detected in the whole cell of *X. axonopodis* pv. v*esicatoria* strains selected. Fifteen fatty acids were found in all strains as major fatty acids. *X. axonopodis* pv. v*esicatoria* strains tested were divided into two different groups in terms of FAs concentration and composition. There was no correlation between geographical origin and FA groups of the strains. The results suggested that MIDI system was useful for confirming the identity of bacterial spot pathogen at the species level, but not pathovar and strain level. Therefore, FAME analysis combined with traditional methods and pathogenicity test was good enough for identification and characterization of the pathogen at the pathovar and strain level.

Keywords: Bacterial spot, pepper, microbial identification, fatty acid methyl ester analysis

INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important greenhouse and field grown vegetable in Turkey with an annual production of 1.750.000 tons in 2004 (DİE, 2005). Bacterial spot of pepper, caused, by *Xanthomonas axonopodis* (syn:*campestris*) pv.

v*esicatoria* (Doidge) Dye, has been identified as the major bacterial disease of pepper cultivars in the eastern Mediterranean region of Turkey (Aysan and Sahin, 2003). The disease occurs in 52-100% of the pepper fields inspected in the region (Mirik et al., 2005). The disease can cause significant losses, particulary in warm and humid environments (Schaad, 1976; Lelliott and Stead, 1987). The symptoms of the disease on pepper are characterized by small, angular spots with yellow halo on leaves. The lesions on fruit are blister-like, irregular, dark, raised spots, frequently surrounded by a water soaked border. When the fruit lesions enlarge, they become brown and rough and have a cracked or warty appearance. Affected fruits may not be marketable (Mirik et al., 2005).

Bacterial spot pathogen, *X. axonopodis* pv. v*esicatoria,* of tomato and pepper identify by morphological, physiological, biochemical, and pathogenicity tests. Most species of *X. axonopodis* can be differentiated by a combination of several phenotypic tests, growth on semi-selective media and pathogenicity tests on their host (Schaad et al., 2001). Most of the time, identification of the pathogen is difficult and time-consuming. Thus, many scientists have focused on rapid, accurate, reliable, and inexpensive identification methods. In recent years, molecular studies, such as PCR, are most popular for identification of plant pathogenic bacteria. In the study, we used whole cell fatty acid methyl ester analysis (FAME) in Sherlock Microbial Identification System (MIS) to identify and find out the relationship between our bacterial strains, isolated from different diseased pepper fields in Adana, Osmaniye, and Mersin provinces of the eastern Mediterranean region of Turkey. The aim of the present study was to explore the possibilities for using MIDI for identification and characterization of *X. axonopodis* pv. v*esicatoria* strains collected from pepper plants grown in commercial fields in the eastern Mediterranean region of Turkey.

MATERIALS and METHODS

Collection and Identification of Bacterial Strains

Sampling was performed in 83 fields of Adana (Ceyhan, Karaisalı, Salbaş, Pirili, Kuyucu, and Kuzgun villages), Osmaniye (Sumbas, Mehmetli and Armağanlı villages) and Mersin (Kazanlı) provinces during June and August of 2002, 2003, and 2004 (Mirik et.al, 2005). Pepper plants (fruits and leaves) showing typical symptom of bacterial spot disease were placed in paper bags and transported to the laboratory for isolation and identification of the causal agent. Surface-sterilized small pieces of leaf spot were macerated in one ml of sterile distilled water. A loopful of suspension was streaked onto YDC (McGuire et al., 1986) into petri plates and incubated at 25^{o}C for 3-4 days. Single yellow colonies on YDC and were selected for further tests. Pepper strains were identified traditional methods by using gram reaction by KOH, oxidase, catalase reaction, starch and esculin hydrolysis, HR on tobacco leaves as described by Schaad et al., (2001). All strains were tested for pathogenicity plants on four weeks-old pepper plants (cv. Bursa Yağlik) by spray inoculating with 10^{8} cfu/ml suspension of bacteria as three replicates. A reference strain of *X. campestris* pv. *vesicatoria*, GSPB 224 (Göttinger Sammlung Phytopathogener Bakterien, Germany), was used as positive control in the tests. Saline buffer was used as negative control.

Fatty Acid Methyl Ester Analysis

Selected 18 bacterial strains and a reference strain (GSPB 224) were also identified based on FAME analysis in MIDI system (Sherlock Microbial Identification System (MIS), Microbial ID, Newark, DE) with TSBA databases ((De Boer and Sasser, 1986; Janse et al., 1992). The part of the study was carried out in Biotechnology Application and Research Center of Atatürk University, Erzurum. The bacterial strains were grown on Tryptic Soy Agar (TSA) (casein peptone 15.0 g l^{-1}; soya peptone 5.0 g l^{-1}; sodium chloride 5.0 g l^{-1}; agar 15 g l^{-1}) and incubated for 24 hour at 25^{o}C. Whole cell fatty acids from each bacterial strains were performed according to the method described by manufacturing Manual (Sherlock Microbial Identification System version 4.0, MIDI, inc., Newark, DE). Approximately 40 mg of living cells from each samples was harvested, and added to 1 ml 1.2M NaOH in 50% aqueous methanol with 5 glass beads (3 mm dia) in a screw cap tube, then incubated at 100^{o}C for 30 min in a water bath. After the saponified samples were cooled at room temperature for 25 min, they were acidified and methylated by adding 2 ml 54% 6 N HCL in 46% aqueous methanol and incubated at 80^{o}C for 10 min in a water bath. After rapid cooling, methylated fatty acids were extracted with 1.25 ml 50% methyl-tert butyl ether (MTBE) in hexane. Each sample was mixed for 10 min and the bottom phase was removed with a Pasteur pipette. The top phase was washed with 3 ml 0.3 M NaOH. After mixing for 5 min then the top phase was removed and then transferred into a GC sample vial for analysis.

FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA) with a fused-silica capillary column (25m by 0.2mm) with cross-linked 5% phenylmethyl silicone. The operating parameters for the study were set and controlled automatically by computer program. The chromatograms with peak retention times and areas were produced on the recording integrator and were electronically transferred to the computer for analysis, storage and report generation. Peak naming and column performance was achieved through the use of calibration standard mix (Microbial ID 1200-A) containing nC9-nC20 saturated and 2&3 hydroxy fatty acids. Fatty acids were analysis by gas chromatography (HP6890, Palo Alto, CA, USA). Peaks were identified using Sherlock Microbial Identification System (MIS Sherlock 3.6 MIDI, Inc., Newark, DE, USA). The variables in the statistical analysis were the relative quantities of named fatty acids in each single isolate. Cluster analysis techniques were used to produce unweighted pair matching based on fatty acid composition (Güven et al., 2004). Standard deviation calculations were performed using SPSS (Statistical Package for Social Sciences) version 5.5a for statistical analysis.

RESULTS and DISCUSSIONS

Bacteria strains, which were consistently isolated from leaf and fruits spots formed mucoid and yellow colored colonies on YDC medium. One hundred and seventy bacterial strains were purified and used for further studies. All the strains were gram- and oxidase-negative, catalase-, starch-, esculin- and HR-positive on tobacco leaves. Leaf spots similar to those observed in the fields developed on inoculated pepper plants in 7-14 days. No symptoms developed on control plants inoculated with saline

buffer. The bacterium was re-isolated from the inoculated plants and characterized as identical to the reference strain, GSPB 224. All strains were identified as *X. axonopodis* pv. v*esicatoria* by traditional methods and pathogenicity tests. Among the strains isolated, 19 different strains were selected for FAME analysis according to their sources and reactions to some tests (Table 1).

Table 1. List of *X. axonopodis* pv *vesicatoria* strains, place, pathogenicity tests and biochemical tests

Designation No.	Location	KOH	O	Starch	C	Esculin	HR	Patojenicity
GSPB 224	Germany	-	-	++	+	+	+	+
XCVA1-2	Karaisalı	-	-	+	+	+	+	+
XCVA1-6	Karaisalı	-	-	+	+	+	+	+
XCVA2-2	Karaisalı	-	-	+	+	+	+	+
XCVA3-4	Ceyhan	-	-	+	+	+	+	+
XCVA4-2	Pirili	-	-	+	+	+	+	+
XCVA4-4	Pirili	-	-	+	+	+	+	+
XCVA5-3	Salbaş	-	-	+	+	+	+	+
XCVA5-5	Salbaş	-	-	+	+	+	+	+
XCVA5-9	Salbaş	-	-	+	+	+	+	+
XCVA6-3	Kuzgun	-	-	+	+	+	+	+
XCVA8-2	Karaisalı	-	-	+	+	+	+	+
Kuyucu	Karaisalı	-	-	+	+	+	+	+
XCVME1-2	Mersin	-	-	+	+	+	+	+
XCVME1-3	Kazanlı	-	-	+	+	+	+	+
XCV01-3	Osmaniye	-	-	+	+	+	+	+
XCVO2-3	Armağanlı	-	-	+	+	+	+	+
XCVO4-1	Mehmetli	-	-	+	+	+	+	+
XCVO4-2	Sunbas	-	-	+	+	+	+	+

KOH: Potassium hydroxide, O: Oxidase, Starch: Strach hydrolise, C: Catalase, Esculin: Esculin hydrolysis, HR: Hypersensitve Reaction on Tobacco, ++: Positive, +: Weakly positive

The identity of the selected 19 strains was confirmed to be *X. axonopodis* pv. v*esicatoria* on the basis of FAME analysis with similarity indices ranging from 37-76%. When microbial identification system was used for FAME analysis, all strains were identified correctly to the species level. But, ability of the system to identify *X. axonopodis* pv. v*esicatoria* strains to pathovar level was limited. Many pathovars of *Xanthomonas axonopodis* such as v*esicatoria*, *malvacearum, phaseoli, begoniae* were named by FAME analysis. When microbial identification system was used for FAME analysis, all strains were identified correctly to the species level. But, ability of the system to identify *X. axonopodis* pv. v*esicatoria* strains to pathovar level was limited. The pepper strains were identified as *X. axonopodis* pv. v*esicatoria* based on traditional tests and pathogenicity on pepper plants. The limitation of microbial identification system in identification of *Xanthomonas* previously reported by Weller et. al., (2000) and Massoma et al., (2003). Similar results reported by Janse (1997) on *Pseudomonas syringae* pathovars.

According to result of whole cell fatty acid composition, 24 different fatty acids were found as 9.531 (%0.5), 10:0 (%0.71), 11:0 ISO (%3.93), 11:0 ANTEISO (%0.23), 10:0-3OH (%0.38), unknown 11.799 (%1.33), 11:0 ISO-3OH (%1.88), 12:0-3OH (%2.34), 14:0 (%1.60), 13:0 ISO-3OH (%3.78), 13:0-2OH (%0.48), 15:0 ISO (%31.01), 15:0 ANTEISO (%12.66), 15:0 (%0.95), 16:0 ISO (%1.62), 16:1 w9c (%1.95), Sum in feature 3 (%22.27), 16:0 (%3.61), ISO 17:1 w9c (%6.52), 17:0 ISO (%5.26), 17:0 ANTEISO (%1.17), 17:1 w8c (%0.48), Sum in feature 5 (%1.88), and 18:1 w9c (%0.80) (Table 2). These fatty acids were saturated (10:0, 14:0, 15:0, and 16:0), unsaturated (16:1 w9c, ISO17:1 w8c, and 18:1 w9c), hydroxyl (10:0 3OH, 12:0-3OH, and 13:0-2OH), methyl branched (11:0 ISO, 11:0 ANTEISO, 11:0 ISO 3OH, 13:0 ISO-3OH, 15:0 ISO, 15:0 ANTEISO, 16:0 ISO, 17:0 ISO, 17:0 ANTEISO), two Sum in features (3 and 5) and unknown fatty acid (unknown 9.531, and unknown 11.799) (Table 2).

Table 2. Fatty acid compositions of *Xanthomonas axonopodis* pv. v*esicatoria* strains

Fatty Acids	No. of Strains	Range	Mean	Standard Derivation
unknown9.531	2	0.25-0.74	0.50	0.35
10:0	14	0.44-0.87	0.63	0.09
11:0 ISO	19	3.38-4.29	3.92	0.24
11:0 ANTEISO	1	0.23	0.23	-
10:0 3OH	3	0.31-0.51	0.38	0.11
unknown11:799	17	0.83-2.35	1.31	0.40
11:0 ISO 3OH	19	1.50-2.53	1.86	0.27
12:0 3OH	19	1.78-3.31	2.35	0.39
14:0	19	1.16-1.90	1.57	0.20
13:0 ISO 3OH	19	3.52-4.52	3.79	0.24
13:0 2OH	1	0.48	0.48	-
15:0 ISO	19	26.10-34.76	30.86	2.59
15:0 ANTEISO	19	9.43-15.34	12.84	1.75
15:0	10	0.59-1.27	0.95	0.16
16:0 ISO	16	1.20-2.12	1.64	0.33
16:1 w9c	9	1.66-215	1.95	0.16
Sum In Feature 3	19	20.13-23.78	22.24	1.20
16:0	19	2.95-4.68	3.64	0.49
ISO 17:1 w9c	19	5.40-7.68	6.47	0.52
17:0 ISO	19	3.02-7.32	5.23	1.08
17:0 ANTEISO	5	0.43-2.65	1.17	0.53
17:1 w8c	2	0.44-0.52	0.44	0.06
Sum In Feature 5	1	1.88	1.88	-
18:1 w9c	2	0.31-1.28	0.80	0.69
Summed Feature 3	16:1 w7c /15 ISO 2OH			
Summed Feature 5	18:2w6,9c/18:0ANTE			

There were no great differences between the bacterial spot strains isolated from different regions, based on their fatty acids amounts and frequency. Fifteen fatty acids (11:0 ISO, 10:0-3OH, unknown 11.799, 11:0 ISO-3OH, 12:0-3OH, 14:0, 13:0 ISO-3OH, 15:0 ISO, 15:0 ANTEISO, 16:O ISO, Sum in feature 3, 16:0, ISO 17:1 w9c, 17:0 ISO, and Summed feature 3) were found all strains of *X. axonopodis* pv. v*esicatoria* as major fatty acids. Overall evaluation showed that fatty acid composition of the strains was qualitatively similar.

It was found that all strains were phenotypically similar at a rate of 96.4% in cluster analysis of fatty acids. The strains were divided into two different groups as according to cluster analysis (Figure 1). In group I was included the strains; GSPB 224, XCVA1-2, XCVA1-6, XCVA2-2, XCVA3-4, XCVA4-2, XCVA4-4, XCVA5-3, XCVA5-5, XCVA5-9, XCVA6-3, XCVA8-2, XCVME1-3, XCVO1-3, XCVO2-3, XCVO4-1 and KUYUCU. In group II was included the strains; XCVME1-2 and XCVO4-2. There was no correlation between strain and location. Fatty acid composition of the strains in the two main groups was shown in Table 3. The group I was characterized by content of 10:0, 11:0 ISO, unknown 11:799, 11:0 ISO 3OH, 11:0 ISO 3OH, 12:0 3OH, 14:0, 13:0 ISO 3OH, 15:0 ISO, 15:0 ANTEISO, 16:0 ISO, Sum In Feature 3, 16:0, ISO 17:1 w9c and 17:0 ISO. The group II was characterized by content of 11:0 ISO, 11:0 ISO 3OH, 12:0 3OH, 14:0, 13:0 ISO 3OH, 15:0 ISO, 15:0 ANTEISO, Sum In Feature 3, 16:0, ISO 17:1 w9c, 17:0 ISO and 17:0 ANTEISO.

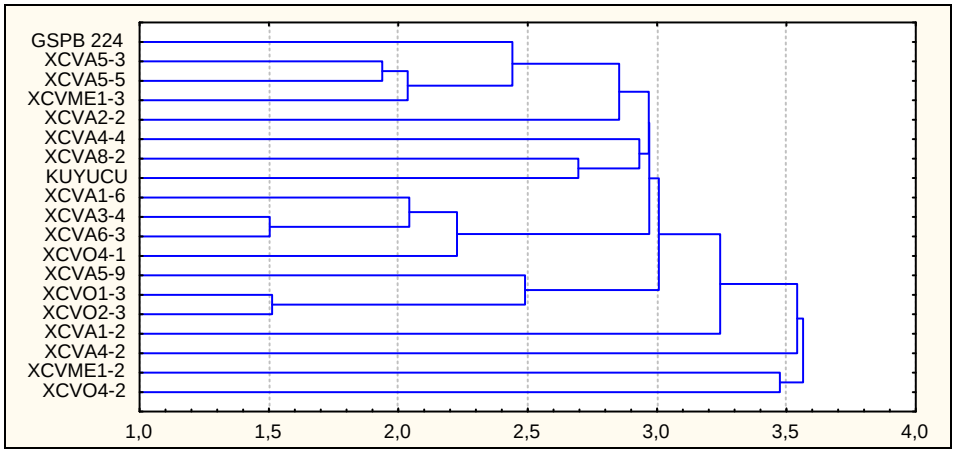

Figure 1. Cluster analysis of fatty acids *Xanthomonas axonopodis* pv. v*esicatoria* strains

FAME analysis has also been used together with traditional methods (morphological, physiological, and biochemical tests) for identifying and phenotypically characterization of bacteria (Sasser, 1990). In some studies, characterization of plant pathogenic bacteria has been described by composition of whole cell fatty acids in previous studies (Kloepper et al., 1992; Lilley et al., 1996). The FAME analysis system proved to be useful for confirming phytopathogenic bacteria as species level and often at the pathovar level. Identification should be confirmed by using pathogenicity tests for

pathovar level (Sasser, 1990; Stead et al., 1992; Weller et al., 2000). The system offers a valuable, cheap, rapid and accurate method for identification of many bacteria. The method can be used for describing individual bacterial isolates as well as mixed bacterial populations (Haack et al., 1994). The fatty acid contents could be extracted from soil or from planted populations (Cavigelli et al., 1995; Zelles et al., 1995).

Table 3. Fatty acid composition of the strains in the two main groups

Fatty Acids (FA)	Group I		Group II	
	Amount of FAs	No. of Strains	Amount of FAs	No. of Strains
unknown9.531	0.25-0.74	2	-	-
10:0	0.44-0.86	14	-	-
11:0 ISO	3.49-4.29	17	3.76-3.38	2
11:0 ANTEISO	0.23	1	-	-
10:0 3OH	0.31-0.51	3	-	-
unknown 11:799	0.83-2.35	17	-	-
11:0 ISO 3OH	1.50-209	17	2.38-2.53	2
12:0 3OH	1.78-3.31	17	2.23-2.76	2
14:0	1.27-1.72	17	1.25-1.90	2
13:0 ISO 3OH	3.45-4.05	17	3.95-4.52	2
13:0 2OH	0.48	1	-	-
15:0 ISO	26.10-34.76	17	28.48-28.58	2
15:0 ANTEISO	9.43-15.34	17	14.57-14.93	2
15:0	0.59-1.27	10	-	-
16:0 ISO	1.12-2.12	16	-	-
16:1 w9c	1.66-2.13	9	-	-
Sum In Feature 3	20.13-23.78	17	20.39-23.25	2
16:0	2.95-4.68	17	3.88-4.36	2
ISO 17:1 w9c	5.40-7.10	17	6.66-7.68	2
17:0 ISO	3.02-6.60	17	7.08-7.32	2
17:0 ANTEISO	0.43-0.67	3	1.54-2.65	2
17:1 w8c	0.44-0.52	2	-	-
Sum In Feature 5	1.88	1	-	-
18:1 w9c	0.31-1.28	2	-	-

ÖZET

BIBERDEN İZOLE EDILEN *XANTHOMONAS AXONOPODIS* PV. *VESICATORIA* İZOLATLARININ TÜM HÜCRE YAG ASIT KOMPOZISYONU

Bakteriyel hastalık etmeni *Xanthomonas axonopodis* pv. *vesicatoria* tarafından neden olunan bakteriyel leke hastalığı Türkiye'nin Doğu Akdeniz Bölgesin'de yetiştiriciliği yapılan biber (*Capsicum* spp.) bitkilerinin önemli bir bakteriyel hastalığıdır. 2002 ve 2004 yılları arasında Adana, Osmaniye ve Mersin illerinde sörveyi yapılan 83 tarladan hastalık belirtileri gösteren biber bitkileri toplanmıştır. *X. axonopodis* pv. *vesicatoria* izole edilmiş ve geleneksel yöntemlerle tanılanmıştır. Tüm hücre yağ asit metil ester (FAME) analizleriyle prokaryotların mikrobiyal tanı sistemi (MIS) bu

izolatların tanısı ve fenotipik karakterizasyonu için geleneksel yöntemlerle birlikte kullanılmıştır. 170 bakteriyel izolat geleneksel yöntemler ve patojenite testine göre *X. axonopodis* pv. v*esicatoria* olarak tanılanmıştır. Seçilen 19 bakteri izolatı, FAME analizleriyle %37-76 benzerlik oranında bu etmen olduğu desteklenmiştir. Seçilen *X. axonopodis* pv. v*esicatoria* izolatlarının tüm hücrelerinden 24 farklı yağ asidi belirlenmiştir. 15 yağ asidi izolatların tamamında belirlenen temel yağ asitleridir. Testlenen *X. axonopodis* pv. v*esicatoria* izolatları yağ asitlerin kompozisyonları ve konsantrasyonlarına göre iki farklı gruba ayrılmıştır. İzolatların yağ asit grupları ve coğrafik yerleri arasında bir korelasyon bulunmamaktadır. Sonuçlara göre bakteriyel leke patojeninin tür düzeyinde tanısını desteklemek için uygundur fakat pathovar ve ırk düzeyinde uygun değildir. Bundan dolayı FAME analizi geleneksel yöntemler ve konukçu testleriyle birlikte kombine edildiğinde patojenin pathovar ve ırk düzeyinde karakterizasyonu ve tanılanması için yeterli olmaktadır.

Anahtar kelimeler: bakteriyel leke, biber, mikrobiyal tanı, yağ asit metil ester analizi

LITERATURE CITED

AYSAN, Y., and F. SAHIN, 2003. Occurrence of bacterial spot disease, caused by *Xanthomonas axonopodis* pv. *vesicatoria*, on pepper in the eastern Mediterranean region of Turkey. **Plant Pathology, 52**:781.

CAVIGELLI, M. A., G. P. ROBERTSON, and M. J. KLUG, 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. **Plant Soil, 170:**99-113.

DE BOER, S. H., and M. SASSER, 1986. Differantiation of *Erwinia carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroceptica* on the basis of cellular fatty acid composition. **Canadian Journal of Microbiology**, **32:**796-800.

DİE, 2002. Tarımsal Yapı ve Üretim. Başbakanlık D.İ.E. Yayınları.

GÜVEN, K., J. B. JONES, M. T. MOMOL, and E. R. DICKSTEIN, 2004. Phenotypic and genetic diversity among *Pseudomonas syringae* pv. *phaseolicola*. **J. Phytopathology, 152:**658-666.

HAACK, S. K., H. GARCHOW, D. A. ODELSON, L. J. FORNEY, and M. J. KLUG, 1994. Accuracy, reproducibility and interpretation of fatty acid methyl ester profiles of model bacterial communities. **Appl. Environ. Microbiol**. **60:** 2483-2493.

JANSE, J. D., A. R. CHASE, N. C. H ODGE, and R. E. STALL, 1992. Xanthomonad diversity on Geranium leaves as determined by monoclonal antibodies MABS, carbon sources utilization patterns, fatty acid analysis FAME and pathogenicity. **Phytopathology, 82:**1066 (Abstract)

JANSE, J. D., 1997. Fatty acid analysismin the identification, taxonomy and ecology of (Plant pathogenic) bacteria. Diagnosis and Identification of Plant Pathogens, H. W. Dehne et al. (eds.) 63-70, 1997 Kluwert Academic Publishers, Netherlands.

KLOEPPER, J. W., J. A. McINROY, and K. L. BOWEN, 1992. Compaarative identification by fatty acid analysis of soil, rhizosphere, and geocarposphere of peanut (Arachis hypogaea L.). **Plant Soil, 1139:**8590.

LELLIOT, R. A., and D. E. STEAD, 1987. Diagnostic procedures for bacterial plant diseases. In Methods for the Diagnosis of Bacterial Diseases of Plants 58-59. Blackwell Scientific Publications, 216p.

LILLEY, A. K., J. C. FRY, M. J. BAILEY, M. J. DAY, 1996. Comperison of aerobic heterotrophic bacteria from rhizosphere of young sugar beet plants. **Appl. Environ. Microbiol. 56**: 3375-3381.

MASSOMO, S. M. S., H. NIELSEN, R. B. MABAGALA, K. MANSFELD-GIESSE, J. HOCKENHULL, and C. N. MORTENSEN, 2003. Identification and characterization of *Xanthomonas campestris* pv. *campestris* strains from Tanzania by pathogenicity tests, Biolog, rep-PCR and fatty acid methyl ester analysis. **European journal of Plant Pathology 109:**775-789.

McGUIRE, R. G., J.B. JONES, and M. SASSER, 1986. Tween media for semiselective isolation of Xanthomonas campestris pv. vesicatoria from soil and plant material. **Plant Disease 70:** 887-891.

MIRIK, M., Y. AYSAN, and O. ÇINAR, 2005. Prevalence and incidence of bacterial spot disease caused by *Xanthomonas campestris* pv. *vesicatoria* on pepper in the eastern Mediterranean region of Turkey. **Pakistan Journal of Biological Sciences**, **8**:1656-1658.

SASSER, M., 1990. Identification of bacteria through fatty acid analysis. In: Klement Z, Rudolph K and Sands DC (eds) Methods in Phytobacteriology (pp 199-204) Akademiai Kiado, Budapest.

SCHAAD, N. W., 1976. Control of black rot of cabbage. **Georgia Agriculture Experiment Station Res Bull, 187:**13

SCHAAD, N. W., J.B. JONES, and G. H. LACY, 2001. Gram negative bacteria, Xanthomonas. In: Laboratory Guide for Identification of Plant Pathogenic Bacteria (eds N.W. Schaad, J.B. Jones, and W. Chun), pp: 175-193. APS Press, St. Paul Minnesota.

STEAD, D. E., J. E. SELLWOOD, J. WILSON and I. VINEY, 1992. Evaluatğion of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. **Journal of Applied Bacteriology 72:**315-287.

WELLER S. A., A. ASPIN and STEAD, D. E., 2000. Classification and identification of plant-associated bacteria by fatty acid profiling. **EPPO Bulletin 30:**375-380.

ZELLES, L., R. RACKWİTZ, Q. Y. BAI, T. BECK T., F. BEESE, F., 1995. discrimintion of microbial diversity by fatty acid profiles of phospholipids and lipopolisaccharides in differently cultivated soils. **Plant and Soil, 170:**115-122.