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Isolation and identification of bacteria from *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae)

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Biological control studies have been increasingly performed against agricultural and forest pests. To develop a biological control agent, bacteria was isolated from harmful pests and identified using various tests. *Xylosandrus germanus* (Blandford, 1894) (Coleoptera: Curculionidae) is a harmful pest in the hazelnut orchards and other fruit-tree cultures. In this study, we identified 16 bacteria isolates from healthy *X. germanus* collected in hazelnut orchards in Turkey. Isolates were characterized based on morphological, physiological and biochemical properties using the VITEK 2 Identification System and the fatty acid methyl esters (FAME) analysis. In addition, 16S rRNA gene sequencing of bacterial isolates was performed. Associated bacteria were identified as *Acinetobacter psychrotolerans* (2 strains), *Stenotrophomonas maltophilia*, *Pseudomonas fluorescens* (two strains), *Staphylococcus sciuri, Staphylococcus warneri*, *Pantoea agglomerans* (two strains), *Staphylococcus hominis* subsp. *hominis*, *Erwinia billingiae* (two strains), *Brevibacterium linens*, *Advenella* sp., *Pantoea cedenensis* and *Brevibacterium permense*. Several species of these bacteria are used in biological control as an antifungal and insecticidal against agricultural pest. In the future, their biological control properties will be investigated. This is the first study on the bacterial community of *X. germanus*.

Key words: *Xylosandrus germanus*, hazelnut, 16S rRNA, fatty acid methyl esters (FAME), VITEK 2, bacterial symbionts, mutualism, biological control.

INTRODUCTION

The main purpose of most agricultural studies is to increase the yield of agricultural crops. Although Turkey is first among all hazelnut producing countries (Kılıç 1994), the average yield of hazelnut per unit field is very low. Approximately 150 insect species have been detected in hazelnut orchards. However, only 10-15 of these species result in economic losses (Isık et al., 1987). Ambrosia beetles are an important pest in hazelnuts (Ak et al., 2005a, b, c).

Chemicals used against pest insects have harmful effects on the environment. Intensive use of chemicals leads to resistance in insects, and is also harmful to the environment. Biological pest control is thought to be an alternative method. Biological control provides a safety approach that is less toxic to the environment, credit to its capability of causing disease in insects, it does not harm other animals or plants. Using natural enemies against pest organism has developed the new environmentally friendly methods and microbial pest control strategies have been preferred instead of chemical pesticides worldwide.

Bacteriological studies have been made with the aim of developing biological control agents, especially against other hazelnut pest insects, such as the ambrosia beetles

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Xyleborus dispar (Sezen et al., 2007, 2008; Kati et al., 2007). Another closely related beetle, the black stem borer *Xylosandrus germanus* (Blandford 1894) (Coleoptera: Curculionidae) is also an important hazelnut pest, but its bacterial community is currently unknown. These invasive beetles are native to Asia and were first detected the US in 1932 and introduced to Europe in the 1950`s (Solomon, 1995; Lawrence, 2006). It is polyphagous and attacks a wide variety of host trees (Frank and Sadof, 2011). Bacteria are abundant and diverse on the body surface and within galleries of ambrosia and bark beetles (Hulcr et al., 2012). Here, we aimed to identify the bacterial community of *X. germanus* for the first time.

MATERIALS AND METHODS

Collection of insects and isolation of bacteria

In this study, branches with galleries creating adults of *X. germanus* in the bark were collected from the hazelnut orchards in Giresun, Turkey, in June and July 2008 and taken to the laboratory. Insects were individually put into sterilized tubes to prevent possible contamination. They were identified by Dr. Kibar Ak (Black Sea Agricultural Research Institute, Samsun, Turkey). Collected adults were surface sterilized with 70% ethanol. The adults were homogenized in a Nutrient broth (NB; containing per liter: 5 g peptone from meat; 3 g meat extract) by using a glass tissue grinder. Then, samples were ten-fold diluted. 100 µl of the suspensions were plated on a Nutrient agar (NA; containing per liter: 5 g peptone from meat; 3 g meat extract; 12 g agar-agar). Plates were incubated at 30°C for 24 or 48 h. Bacteria were selected based on their colours and colony morphologies. Then, pure cultures were prepared and these cultures were identified using various assays.

Phenotypical, physiological, biochemical properties and fatty acid methyl ester analysis of the isolated bacteria

Colony morphologies of the isolates were observed on NA by direct and stereomicroscopic observations of single colonies. Bacteria morphology and motility were examined by light microscopy of native preparations. Gram staining was performed (Claus, 1992). Endospores were observed in light microscopy using negative staining (Elcin, 1995). Temperature, NaCl and pH tolerance values were determined in NB. The VITEK 2 analysis system was used to detect biochemical properties. Fatty acid methyl ester (FAME) analysis of isolates was performed as suggested by Sasser (1990) using the Microbial Identification System (Hewlett-Packard model 5898A, Palo Alto, CA) and using the Tryptic Soy Agar (TSA) database of the Microbial Identification System software package (MIDI; Microbial ID, Inc., Newark, DE).

Molecular characterization

DNA isolation was carried out according to the procedure of Sambrook et al. (1989). The 16S rRNA gene was amplified using primers designed to anneal to conserved positions. In polymerase chain reaction (PCR), the forward primer, UNI16S-L (5'-ATTCTAGAGTTTGATCATGGCTTCA-3'), and the reverse primer, UNI16S-R (5'-ATGGTACCGTGTGACGGGCGGTGTTGTA-3') (Brosius et al., 1978) were used. The total 50 µl PCR mixture included the template DNA (10 ng), each primer (50 ng), 25 mM of each deoxyribonucleoside triphosphate (0.5 µl), 10X PCR buffer (10 µl), GoTaq polymerase (0.2 U) and distilled water.

The PCR was conducted using the following conditions: 5 min at 95°C for initial denaturation, followed by 30 amplification cycles (20 s at 95°C, 45 s at 55°C 1 min at 72°C) and 7 min at 72°C for final primer extension. All PCR products were analysed by 1.3% agarose gel electrophoresis. The resulting gene sequences (length approximately 1,400 bp) were cloned into a pGEM-T easy cloning vector. Sequencing of the cloned products was performed at Macrogen Inc. (Wageningen, Holland). These sequences comparisons were blasted against the GenBank database (Pearson, 1990; Altschul et al., 1990, 1997).

G±C analysis of Xg5 isolate

Analysis of the G±C content of the bacterial isolate Xg5 was performed using the DSMZ Identification Service. Its G±C content was determined by HPLC (Cashion et al., 1977; Tamaoka et al., 1984; Mesbah et al., 1989). The DNA was purified on hydroxyapatit according to the procedure of Cashion et al. (1977).

RESULTS

In this study, 16 bacterial isolates from *X. germanus* were identified using phenotypic, biochemical, physiological, FAME and molecular techniques. According to morphological results, five isolates were Gram-positive, the others were Gram-negative and all isolates were non-sporulating, eight isolates were motile and eight were non-motile. Moreover, the colony colours of two isolates were yellow, that of the other two isolates were orange and the others produced a creamy pigment. Four isolates had the shape of coccobacilli; five isolates were bacilli; seven isolates were cocci (Table 1).

According to pH test results, none of the isolates grow at pH 3 media; and six isolates grow at pH 5. All isolates grew at pH 7. According to heat tolerance test results, all isolates grew at 25 and 30°C, and some isolates grew at 37 and 40°C. According to NaCl tolerance test results, six isolates grow at 2% NaCl media; two isolates grow weakly; the others did not grow (Table 2). Biochemical characteristics of isolates were examined using the VITEK 2 system (Table 3 and 4). In order to identify FAME profiles of the isolates, MIS was used. In this study, according to FAME profiles, all isolates had 9-20 carbons and 46 different fatty acids were detected. Moreover, all the isolates had a C16:0 saturated fatty acid. The FAME profiles of isolates are listed in Table 5. Molecular studies of isolates were performed using 16S rRNA gene sequencing analysis. The isolates were identified as *Acinetobacter psychrotolerans* (Xg1 and Xg2), *Stenotrophomonas maltophilia* (Xg3), *Pseudomonas fluorescens* (Xg4 and Xg9), *Staphylococcus sciuri* (Xg5), *Staphylococcus warneri* (Xg6), *Pantoea agglomerans* (Xg7 and Xg15), *Staphylococcus hominis* subsp. *hominis* (Xg8), *Erwinia*

Table 1. Morphological characteristics of bacterial isolates of *Xylosandrus germanus*.

Isolate ID	Colour of colonies	Shape of colonies	Shape of bacteria	Gram stain	Motility
Xg1	Cream	Round	Coccobacili	-	-
Xg2	Cream	Round	Coccobacili	-	-
Xg3	Cream	Round	Bacili	-	+
Xg4	Cream	Wavy round	Bacili	-	+
Xg5	Cream	Round	Cocci	+	-
Xg6	Cream	Round	Cocci	+	-
Xg7	Yellow	Round	Cocci	-	+
Xg8	Cream	Round	Cocci	+	-
Xg9	Cream	Round	Bacili	-	+
Xg10	Translucent	Wavy round	Cocci	-	+
Xg11	Translucent	Round	Cocci	-	+
Xg12	Yellow-Orange	Round	Bacili	+	-
Xg13	Cream	Round	Coccobacili	-	+
Xg14	Cream	Round	Coccobacili	-	-
Xg15	Yellow	Round	Cocci	-	+
Xg16	Orange	Round	Bacili	+	-

Table 2. Physiological characteristics of bacterial isolates of *X. germanus*.

Parameter	Isolate ID															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Growth at pH 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at pH 5	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-
Growth at pH 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at pH 9	-	+	+	-	+	W	+	-	-	-	-	+	-	-	-	W
Growth at pH 10	-	-	+	-	+	-	W	-	-	-	-	+	-	-	-	W
Control (NB)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in NB +2% NaCl	W	-	+	-	W	+	-	-	+	+	-	+	-	+	+	+
Growth in NB +3% NaCl	-	-	-	-	W	+	-	-	+	-	-	+	-	+	-	+
Growth in NB +4% NaCl	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	+
Growth in NB +5% NaCl	-	-	-	-	-	W	-	-	+	-	-	+	-	-	-	+
Growth in NB +7% NaCl	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+
Growth in NB +10% NaCl	-	-	-	-	-	-	-	-	+	-	-	W	-	-	-	+
Growth in NB +12% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 25˚C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 30˚C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	-	-	-	-	+	+	W	+	-	-	-	+	+	-	-	+
Growth at 40˚C	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-	+

+: Growth, -: no growth, W: weak growth.

billingiae (Xg10 and Xg11), *Brevibacterium linens* (Xg12), *Advenella* sp. (Xg13), *Pantoea cedenensis* (Xg14) and *Brevibacterium permense* (Xg16) (Table 6).

DISCUSSION

In order to develop effective biological control agents, it is necessary to identify the bacterial community of insect pests. For this purpose, we aimed to identify the bacterial community of the hazelnut pest *X. germanus*. In this study, 16 bacteria isolated from *X. germanus* were identified.

According to FAME analysis and VITEK 2 results, Xg1 and Xg2 isolates were determined as *Acinetobacter*

Table 3. Biochemical characteristics of Gram negative bacterial isolates (tested with VITEK 2).

Parameter	Isolate ID										
	1	**2**	**3**	**4**	**7**	**9**	**10**	**11**	**13**	**14**	**15**
Ala-Phe-Pro-arilamidaz	-	-	+	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-
L-Pyrrlydonyl- arilamidaz	-	-	-	+	+	+	+	+	-	+	+
L-Arabitol	-	-	-	-	-	-	-	-	-	-	-
D-Cellobiose	-	-	-	-	-	-	-	-	+	-	-
Beta-galactosidase	-	-	-	-	+	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-	-
Beta-N-acetyl-glucosaminidase	-	-	(+)	-	-	-	-	-	-	-	-
Glutamyl arilamidaz pNA	-	-	-	-	-	-	-	-	-	-	-
D-Glucose	-	-	-	-	+	+	+	+	+	+	+
Gamma-glutamyl-transferase	-	-	+	-	-	-	-	-	+	-	-
Fermentation/glucose	-	-	-	-	+	-	-	-	-	-	+
Beta-glucosidase	-	-	+	-	+	-	+	+	-	+	+
D-Maltose	-	-	-	-	-	-	-	-	-	-	-
D-Mannitol	-	-	-	-	+	-	+	+	-	+	+
D-Mannose	-	-	-	-	+	-	(-)	-	+	+	+
Beta-Xylosidase	-	-	-	-	-	-	-	-	-	-	-
Beta-Alanine arilamidaz pNA	-	-	-	-	-	-	-	-	-	-	-
L-proline arilamidaz	-	-	+	+	-	+	-	-	+	-	-
Lipase	+	-	+	-	-	-	-	-	-	-	-
Palatinose	-	-	-	-	-	-	-	-	-	-	-
Tyrosine Arilamidaz	(-)	-	-	+	-	+	+	+	-	+	-
Urease	-	-	-	-	-	-	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	-	-	-	-	-	-
Saccharose/sucrose	-	-	-	-	+	-	-	-	-	-	+
D-Tagatose	-	-	-	-	-	-	-	-	-	-	-
D-Trehalose	-	-	-	-	+	-	+	+	-	+	+
Citrate (sodium)	+	+	+	+	-	+	-	-	+	-	-
Malonate	-	-	-	-	+	+	-	-	-	+	+
5-Keto-D-gluconate	-	-	-	-	-	-	-	-	-	-	-
L-Lactate alkalinisation	-	-	-	+	+	+	+	+	+	+	+
Alpha-Glucosidase	-	-	-	-	-	-	-	-	-	-	-
Succinate alkalinisation	-	-	+	+	+	+	+	+	+	+	-
Beta-N-Acetyl-galactosaminidase	-	-	-	-	-	-	-	-	-	-	-
Alpha-galactosidase	-	-	-	-	-	-	-	-	-	-	-
Phosphatase	-	-	+	-	+	-	+	+	-	+	(+)
Glycine arilamidaz	-	-	-	+	-	+	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	-	-	-	-	-	-	-	-	-	-	-
L-Histidine assimilation	+	+	-	-	-	-	-	-	-	-	-
Courmarate	-	-	-	+	-	+	-	-	-	+	-
Beta-glucoronidase	-	-	-	-	-	-	-	-	-	-	-
O/129Resistance (comp.vibrio.)	-	-	-	-	+	-	-	-	-	+	-
Glu-Gly-Arg-Arilamidaz	-	-	+	-	-	-	-	-	-	-	-
L-Malate assimilation	-	-	-	-	-	-	-	-	-	-	-
Ellman	+	-	-	-	-	-	-	-	+	-	-
L-Lactate assimilation	-	-	-	-	-	-	-	-	+	-	-

+: Growth, -: no growth, (**+**):weak growth, (**-**):almost no growth.

Table 4. Biochemical characteristics of Gram positive bacterial isolates (tested with VITEK 2).

Parameter	Isolate ID				
	5	6	8	12	16
D-Amygdalin	+	-	-	-	-
Phosphatidylinositol phospholipase C	-	-	-	-	-
D-Xylose	+	-	-	-	-
Arginine dihydrolase 1	+	+	+	+	-
Beta-galactosidase	-	-	+	-	-
Alpha-glucosidase	-	-	+	-	-
Ala-Phe-Pro arilamidaz	-	-	-	-	-
Cyclodextrin	-	-	-	-	-
L-Aspartate arilamidaz	-	-	-	-	-
Beta galactopyranosidase	-	-	-	-	-
Alpha-mannosidase	-	-	-	-	-
Phosphatase	-	-	-	-	-
Leucine arilamidaz	-	-	-	-	-
L-Proline arilamidaz	-	-	-	+	+
Beta glucuronidase	+	-	-	-	-
Alpha-galactosidase	-	-	-	-	-
L-Pyrrolydonyl-arilamidaz	-	+	-	-	-
Beta-glucuronidase	+	+	-	-	-
Alanine arilamidaz	-	-	-	+	+
Tyrosine arilamidaz	-	-	-	-	-
D-Sorbitol	-	-	-	-	-
Urease	-	+	+	-	-
Polymixin B resistance	-	-	-	-	-
D-Galactose	-	-	+	-	-
D-Ribose	+	+	-	-	-
L-Lactate alkalinization	+	-	-	+	+
Lactose	+	-	-	-	-
N-Acetyl-D-glucosamine	-	-	-	-	-
D-Maltose	+	+	+	-	-
Bacitracin resistance	+	-	-	-	-
Novobiocin resistance	+	-	-	-	-
Growth in 6.5% NaCl	+	+	+	-	-
D-Mannitol	+	-	-	-	-
D-Mannose	+	-	+	-	-
Methyl-B-D-glucopyranoside	+	-	-	-	-
Pullulan	-	-	-	-	-
D-Raffinose	-	-	-	-	-
O/129 Resistance (comp. Vibrio.)	-	+	+	-	-
Salicin	+	-	-	-	-
Saccharose/sucrose	+	+	+	-	-
D-Trehalose	+	+	+	-	-
Arginine dihydrolase 2	-	-	-	+	-
Optochin resistance	+	+	+	-	-

+: Growth, - : no growth.

haemolyticus. Jung-Sook et al. (2009) reported the presence of the following major fatty acid components in *Acinetobacter* species: 16:0, 18:1ω9c and summed feature 3. These results were consistent with ours. According to 16S rRNA gene sequencing, isolates resembled *Acinetobacter psychrotolerans* by 99%. *Acinetobacter*

Table 5. FAME profiles of bacterial isolates.

Fatty acid*	Isolate ID															
	Xg1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Saturated																
09:00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.16	-
10:00	1.57	1.67	0.33	0.13	-	-	-	-	-	-	-	-	-	-	-	-
12:00	3.27	3.77	-	2.09	-	-	3.77	-	2.52	3.89	4.14	-	3	4.18	4.08	-
14:00	0.31	0.38	1.99	0.57	0.65	-	5.37	-	0.59	5.45	6.26	-	0.81	5.75	5.52	-
15:00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16:00	19.25	20.09	6.95	36.83	2.17	1.59	30.96	0.67	36.37	33.55	35.76	0.62	29.24	37.03	30.45	0.53
17:00	1.81	1.95	0.15	0.14	-	-	0.49	-	-	-	-	-	0.75	1.08	-	-
18:00	1.95	1.77	-	1.32	0.75	8.57	0.53	5.48	1.52	-	0.55	-	0.97	0.6	-	-
19:00	-	-	-	-	-	-	-	0.99	-	-	-	-	-	-	-	-
20:00	-	-	-	-	3.98	9.61	-	9.37	-	-	-	-	-	-	-	-
Unsaturated																
15:1 iso F	-	-	1.22	-	-	-	-	-	-	-	-	-	-	-	-	-
16:1 ω9c	-	-	2.05	-	-	-	-	-	-	-	-	-	-	-	-	-
16:1 ω11c	-	-	-	-	0.36	-	-	-	-	-	-	-	-	-	-	-
17:1 ω 8c	1.46	1.9	0.44	-	-	-	-	-	-	-	-	-	-	-	-	-
17:1 iso ω10c	-	-	-	-	0.37	-	-	-	-	-	-	-	-	-	-	-
18:1 ω 9c	41.76	39.4	0.31	-	-	-	-	-	-	-	-	-	-	-	-	-
Branched																
11:0 iso	-	-	3.27	-	-	-	-	-	-	-	-	-	-	-	-	-
11:0 anteiso	-	-	0.22	-	-	-	-	-	-	-	-	-	-	-	-	-
13:0 iso	-	-	0.22	-	1.02	-	-	-	-	-	-	-	-	-	-	-
13:0 anteiso	-	-	0.21	-	-	-	-	-	-	-	-	-	-	-	-	0.32
14:0 iso	-	-	2.26	-	0.84	0.26	-	0.91	-	-	-	-	-	-	-	0.27
15:0 iso	-	-	26.58	-	42.14	2.05	-	6.39	-	-	-	4.71	-	-	-	3.63
15:0 antesio	-	-	26.24	-	22.57	50.27	-	39.56	-	-	-	54.24	-	-	-	63.97
16:0 iso	-	-	4.59	-	1.14	-	-	0.53	-	-	-	4.44	-	-	-	3.37
17:0 iso	0.35	-	2.36	-	14.31	2.71	-	4.36	-	-	-	0.84	-	-	-	0.5
17:0 antesio	-	-	0.37	-	6.96	18.06	-	7.99	-	-	-	35.14	-	-	-	27.41
18:0 iso	-	-	-	-	-	-	-	1.25	-	-	-	-	-	-	-	-
19:0 iso	-	-	-	0.35	1.49	1.53	-	10.5	0.37	-	-	-	-	0.35	-	-
19:0 antesio	-	-	-	-	1.25	5.35	-	11.35	-	-	-	-	-	-	-	-
20:0 iso	-	-	-	-	-	-	-	0.65	-	-	-	-	-	-	-	-
Hydroxy																
10:0 3OH	-	-	0.23	3.03	-	-	-	-	2.7	-	-	-	-	-	-	-
11:0 iso 3OH	-	-	1.57	-	-	-	-	-	-	-	-	-	-	-	-	-
11:0 3OH	-	-	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-
12:0 2OH	4.02	3.74	-	4.7	-	-	-	-	4.17	-	-	-	-	-	-	-
12:0 iso 3OH	7.06	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-
12:0 3OH	-	7.14	2.05	4.35	-	-	-	-	4.41	-	-	-	-	-	-	-
13:0 2OH	-	-	1.46	-	-	-	-	-	-	-	-	-	-	-	-	-
13:0 iso 3OH	-	-	2.11	-	-	-	-	-	-	-	-	-	-	-	-	-
14:0 2OH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16:0 3OH	-	-	-	-	-	-	-	-	-	-	-	-	1.9	-	-	-
Cyc CYCLO																
17:0 cyclo	-	-	0.99	22.55	-	-	11.94	-	16.84	8.67	12.25	-	8.84	21.89	6.98	-

Table 5. Contd.

19:0 cyclo ω8c	-	-	-	2.38	-	-	0.43	-	1.49	-	-	-	1.43	4.95	-	-
Summed Feature 2 12:0 ALDE?																
16.1: iso I 14:0 3 OH	-	-	-	-	-	-	9.43	-	-	10.48	8.73	-	9.56	9.43	9.83	-
Unknown 10.928																
Summed Feature 3 16:1ω7c/16:1 ω7/6c	16.27	17.59	6.45	12.96	-	-	21.22	-	18.41	26.25	22.89	-	22.92	6.48	25.73	-
Summed Feature 8 18:1 ω7/6c	0.58	0.6	0.41	8.6	-	-	15.86	-	10.59	11.71	9.42	-	20.58	8.25	16.59	-
Summed Feature 9 17:1 iso ω9c	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-

*:9:0 pelargonic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 15:0 pentadecylic acid, 16:0 palmitic acid, 17:0 margaric acid, 18:0 stearic acid, 20:0 arachidic acid, 15:1pentadecenoic acid, 16:1 palmitoleic acid, 17:1 heptadecenoic acid, 18:1cis oleic acid.

Table 6. GenBank accession numbers of 16S rRNA genes of bacteria from *X.germanus.*

Isolate ID	Most likely identical taxonomic species	Accesion number
Xg1	*Acinetobacter psychrotolerans*	KF740570
Xg2	*Acinetobacter psychrotolerans*	KF740571
Xg3	*Stenotrophomonas maltophilia*	KF740572
Xg4	*Pseudomonas fluorescens*	KF740573
Xg5	*Staphylococcus sciuri*	KF740574
Xg6	*Staphylococcus warneri*	KF740575
Xg7	*Pantoea agglomerans*	KF740576
Xg8	*Staphylococcus hominis* subsp. *hominis*	KF740577
Xg9	*Pseudomonas fluorescens*	KF740578
Xg10	*Erwinia billingiae*	KF740579
Xg11	*Erwinia billingiae*	KF740580
Xg12	*Brevibacterium linens*	KF740581
Xg13	*Advenella* sp.	KF740582
Xg14	*Pantoea cedenensis*	KF740583
Xg15	*Pantoea agglomerans*	KF740584
Xg16	*Brevibacterium permense*	KF740585

described by Yamahira et al. (2008) had similar morphological characteristics with our Xg1 and Xg2 isolates. The genus *Acinetobacter* is widely distributed in nature; they were isolated from environmental sources such as soil, cotton, water, food and insect. In addition, *Acinetobacter* sp. were isolated from clinical specimens such as blood, feces (Brisou and Prévot, 1954; Nishimura et al., 1988; Carr et al., 2003; Baumann, 1968; Bifulco et al., 1989; Geiger et al., 2011).

Xg3 isolate was identified as *Stenotrophomonas maltophilia* according to FAME analysis, VITEK 2 and 16S rRNA sequencing. The FAME profiles are characterized by the occurrence of iso15:0, anteiso15:0, 16:1, and 16:0 as dominant components. These profiles were previously reported for *Stenotrophomonas* species (Wolf et al., 2002; Romanenko et al., 2008). *S. maltophilia* strains have been isolated from a variety of natural sources (Berg et al., 1996, 1999) and insects (Indiragandhi et al., 2007). Some members of these species are known as human pathogens (Drancourt et al., 1997; Denton and Kerr, 1998; Coenye et al., 2004). In addition, *S. maltophilia* strains are used in biological control as an antifungal agent for crops diseases (Berg et al., 1996; Jakobi et al., 1996; Minkwitz and Berg, 2001).

Xg4 and Xg9 isolates showed a low similarity with *Pseudomonas agarici* (12.9 and 35.7%, respectively) in

the FAME analyses, but closely resembled *Pseudomonas fluorescens* (99 and 95%, respectively) in the VITEK 2 analyses. Consistent with our results, Veys et al. (1989) reported the presence of three hydroxy acids (3-OH C10: 2-OH C12:0 and 3-OH C12) is characteristic of the fluorescent *Pseudomonas* species (*P. aeruginosa, P. putida and P. fluorescens*) and Camara et al. (2007) demonstrated *P. fluorescens* fatty-acid profiles contain 16:0 and 17:0 cyclo fatt acids. Xg4 and Xg9 isolates resembled *P. fluorescens* by 99%, according to 16S rRNA sequencing. Ribotyping, a method for classifying pseudomonads was used (Behrendt et al., 2003; Behrendt et al., 2007).

Based on FAME analyses, Xg5 and Xg6 isolates were identified as *Staphylococcus* sp. The Xg5 isolate was identified as *S. sciuri*, according to FAME analysis and VITEK 2 results. In previous studies, members of the genus *Staphylococcus* displayed large amounts of the fatty acids anteiso C15:0, C18:0, C20:0 and smaller but significant amounts of the fatty acids iso C15:0, C16:0, iso C17:0 ve anteiso C17:0 fatty acids (Kotilainen et al., 1990; Wieser and Busse, 2000). Our results of the 16S rRNA sequencing identified Xg5 as one of the *S. sciuri* subspecies: either *S. sciuri* subsp. *carnaticus*, *S. sciuri* subsp. *rodentium* or *S. sciuri* subsp. *sciuri* (Table 7). Thus, G±C analysis of this isolate was performed by DSMZ. We found a G±C content of 32.5% that suggested a new *S. sciuri* subspecies.

The Xg6 isolate is similar to *S. cohnii* subsp. *cohnii* based on FAME analyses. Nevertheless, according to VITEK 2 and 16S rRNA gene sequence analysis results, this isolate resembles *Staphylococcus warneri* (Table 7). Strains of *S. warneri* have been shown to grow at 40°C and are susceptiple to novobiocin (Kloos and Schleifer, 1975). These results are consistent with ours. RNA gene restriction polymorphism has been used to differentiate *S. pasteuri* from *S. wameri* (Chesneau et al., 1993). *Staphylococcus pasteuri* should be yellow in VITEK 2 tests, whereas Xg6 appeared to be creamy in our analysis. Therefore, the Xg6 isolate was identified as *S. warneri*.

Xg7 and Xg15 isolates were identified as *Pantoea agglomerans* according to VITEK 2. According to FAME analyses results, the Xg7 isolate is similar to *P. agglomerans* and the Xg15 isolate is similar to *Serratia odorifera*. 16S rRNA gene sequencing identified the Xg15 isolate as *Serratia* sp. and Xg7 as *P. agglomerans* (99%). These results were also supported by VITEK 2 analyses.

Xg8 isolate was identified as *Staphylococcus hominis* subsp. *hominis* according to FAME analysis and VITEK 2. However, 16S rRNA sequencing indicated that isolate is similar to *S. hominis* subsp. *novobiosepticus*. Kloos et al. (1998) reported *S. hominis* subsp. *novobiosepticus* is resistant to novobiocin. We found that Xg8 is susceptible to novobiocin in VITEK 2 results and therefore we concluded that Xg8 is *S. hominis* subsp. *hominis* (Table 4).

The Xg10 and Xg11 isolates were identified as *Erwinia billingiae*. The Xg10 isolate resembled *E. rhapontici* and *Sphingomonas paucimobilis,* respectively, according to FAME and VITEK 2 analyses. Geider et al. (2006) showed that C16:0 and C16:1ω7c fatty acids profiles dominated in *Erwinia* species. 16S rRNA gene sequencing has showed that this isolate is either *Erwinia billingiae* (99%) or *E. rhapontici (*98%). Mergaert et al. (1999) reported that *E. rhapontici* produces pink pigment but our Xg10 isolate produced creamy pigment. 16S rRNA gene sequencing showed that the Xg11 isolate is *E. billingiae*.

Brevibacterium sp. has higher anteiso and iso fatty acid content than other fatty acid content (Collins et al., 1983; Collins, 1992). According to FAME analysis, Xg12 and Xg16 isolates were identified as *Brevibacterim casei* and *Brevibacterium epidermidis*/*iodinum*, respectively. The major fatty acids of Brevibacterium genus have been described to be anteiso C:17 and anteiso C:I5 (Collins et al., 1980).

These isolates resemble *Dermacoccus nishinomiyaensis*. However, Stackebrandt et al. (1995) reported that anteiso-C15:0 was not found in *Dermacoccus nishinomiyaensis*. In previous studies, colony coloures of *Brevibacterium linens*, *Brevibacterium permense*, *Brevibacterium epidermidis*, *Brevibacterium iodinum* and *B. casei* were yellow-orange, orange, pale yellow, greyish and whitish grey, respectively (Bhadra et al., 2008; Gavrish et al., 2004; Collins et al., 1983). In our study, Xg12 and Xg16 isolates were yellow-orange to orange, respectively.

16S rRNA sequencing showed that the isolates belong to the Brevibacteria. Morhopological studies showed that Xg12 and Xg16 isolates are *B. linens*, *B. permense*, respectively. *Brevibacterium* species have been isolated from insect (Katı et al., 2010).

The Xg13 isolate was highly similar to *Advenella kashmirensis* and *Advenella incenata* (98%) using 16S rRNA sequencing. 16:0 and 18:1ω7c fatty acids dominate in *Advenalla* sp. (Coenye et al., 2005). This is in accordance with our study.

The Xg14 isolate resembles *Pseudomonas luteola* (95%) according to FAME analysis and VITEK 2 results. It resembles *Pantoae cedenensis* (99%) according 16S rRNA sequencing. Fatty acids contents of this isolate were very similar to Mergaert et al. (1993). *Pseudomonas luteola* is yellow pigment (Holmes et al., 1987), but *Pantoae cedenensis* is creamy (Sezen et al., 2008), like Xg4 in our study.

As a result, bacteria isolated from *X. germanus* were identified in this study. In future, biological control properties of these bacteria will be investigated. In previous studies, several species of *Acinetobacter*, *Stenotrophomonas*, *Pantoea*, *Brevibacterium* and *Pseudomonas* bacteria identified in this study exhibited antifungal or insecticidal activities (Selvakumara et al., 2011; Trotel-Aziz et al., 2008; Jankiewicz et al., 2012).

Table 7. Identity of isolates according to VITEK 2, FAME profiles and 16S rRNA sequencing.

Isolate ID	FAME profile	Similarity (%)	VITEK 2 analysis	Similarity (%)	16S rRNA results	Closest match GenBank accession no.	Similarity (%)
Xg1	*Acinetobacter haemolyticus*	84.6	*Acinetobacter haemolyticus*	91	*Acinetobacter psychrotolerans*	AB207814	99
Xg2	*Acinetobacter haemolyticus*	76.5	*Acinetobacter haemolyticus*	91	*Acinetobacter psychrotolerans*	AB207814	99
Xg3	*Stenotrophomonas maltophilia*	51.1	*Stenotrophomonas maltophilia*	99	*Stenotrophomonas maltophilia* strain ISSDS-429	EF620448	99
Xg4	*Pseudomonas agarici*	12.9	*Pseudomonas fluorescens*	99	*Pseudomonas fluorescens* strain ESR94	EF602564	99
	Staphylococcus schleiferi	54.3	*Staphylococcus sciuri*	97	*Staphylococcus sciuri* subsp. *carnaticus*	AB233331	99
Xg5	*Staphylococcus sciuri*	43.3			*Staphylococcus sciuri* subsp. *rodentium*	AB233332	99
					Staphylococcus sciuri subsp. *sciuri* strain DSM 20345	NR_025520	99
Xg6	*Staphylococcus cohnii* subsp. *cohnii*	23.8	*Staphylococcus warneri*	99	*Staphylococcus warneri* strain E21	GU397393	99
Xg7	*Raoultella terrigena*	76.2	*Pantoea agglomerans*	98	*Staphylococcus pasteuri* strain SSL11	EU373323	99
					Pantoea agglomerans strain PGHL1	EF050808	99
	Pantoea agglomerans GC subgroup B (Enterobacter)	75.7			*Pantoea ananatis* strain SAD2-6	HQ236020	99
Xg8	*Staphylococcus hominis* subsp. *hominis*	66.6	*Staphylococcus hominis* subsp. *hominis*	94	*Staphylococcus hominis* subsp. *novobiosepticus* strain: GTC 1228	AB233326	99
Xg9	*Pseudomonas agarici*	35.7	*Pseudomonas fluorescens*	95	*Pseudomonas fluorescens* strain CN078	EU364534	99
Xg10	*Erwinia rhapontici*	71.2	*Sphingomonas paucimobilis*	89	*Erwinia billingiae* strain Eb661	AM055711	99
					Erwinia rhapontici strain M52	HM008951	98
					Erwinia persicinus strain 52	AM184098	98
Xg11	*Erwinia amylovora*	57.7	*Sphingomonas paucimobilis*	89	*Erwinia billingiae* strain Eb661	FP236843	99

Table 7. Contd.

Xg12	*Brevibacterium casei*	80.5	*Dermacoccus nishinomiyaensis*/*Kytococcus sedentarius*	93	*Brevibacterium aureum* strain Enb17	AY299093	99
					Brevibacterium linens strain VKM Ac-2119	AY243345	99
					Brevibacterium iodinum strain ATCC 15728	FJ652620	98
					Brevibacterium epidermidis strain ZJB-07021	EU046495	98
					Brevibacterium permense strain VKM Ac-2280	NR_025732	98
Xg13	*Pantoea agglomerans* GC subgroup C (Enterobacter)	61.7	*Acinetobacter lwoffi*	93	*Advenella kashmirensis* strain 445A	AJ864471	98
					Advenella incenata	AM944735	98
Xg14	*Ewingella americana*	76.5	*Pseudomonas luteola*	95	*Pantoea cedenensis* strain 16-CDF	FJ811867	99
Xg15	*Serratia odorifera*	75.9	*Pantoea agglomerans*	95	*Pantoea agglomerans* strain EQH21	FJ999950	99
					Pantoea ananatis strain SAD2-6	HQ236020	98
Xg16	*Brevibacterium epidermidis*/*iodinum*	81.6	*Dermacoccus nishinomiyaensis*/ *Kytococcus sedentarius*	97	*Brevibacterium epidermidis* strain SW34	GU576981	99
					Brevibacterium linens	AB211980	98
					Brevibacterium aureum strain Enb15	AY299092	99
					Brevibacterium iodinum strain DSM 2062	NR_026241	98
					Brevibacterium permense strain VKM Ac 2280	NR_025732	98

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