Identification and Biochemical Characterization of some Strains of *Clavibacter michiganensis* subsp. *Michiganensis*, in Romania

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Abstract

Clavibacter michiganensis subsp. *michiganensis* is responsible for the 'bacterial canker of tomato'. The study aimed to identify and characterize biochemically some strains of the bacteria. Five strains were used for biochemical identification and characterization and two reference strains were used as positive controls. The biochemical characterization was performed with Biolog Microbial Identification System. All the strains used many carbon sources and were positive for growth in the presence of: 1%NaCl, Nalidixic Acid, Aztreonam, Potassium Tellurite and pH 6, but negative for growth and sensitive to: Troleandomycin, Lincomycin, Vancomycin, Fusidic Acid, Eimfamycin SV, Guanidine HCl, D-Serine, Minocycline and Niaproof 4. The differences between the Biolog system database and the studied strains did not change their classification at the level of genus and species, but modified or did not allow the classification at the subspecies level. Furthermore, the analyzed strains had metabolic diversity.

Keywords: Biolog system, spectrophotometry, optic

Introduction

At the beginning of the 20th century, in 1910, Smith discovered in the state of Michigan, United States of America, a harmful bacteria that causes the wilting of tomato plants (Singh and Bharat, 2017). In Romania, it was reported by Elena Bucur, since 1955 (Severin and Iliescu, 2006).

It is a Gram positive bacteria, aerobic, non-mobile, solitary or in pairs, capsulated, slightly curved rod-shaped, sometimes with swellings or branches at some ends (Severin and Iliescu, 2006). Optimal growth and development conditions of *Clavibacter michiganensis* subsp. *michiganensis* are temperatures between 20 and 30°C and pH 7-8 (Sen *et al.*, 2015).

Clavibactermichiganensissubsp.michiganensis produces very different symptoms which depend on: the type of infection (localized or systemic), the sensitivity of the cultivar, the age of the plant, the virulence of the pathogen, the nutritional status of the host plant and the environmental conditions (humidity and temperature).

The main host plant of economic importance is *Solanum lycopersicum*, but this bacteria also attacks other sensitive species of *Solanum* spp. (<https://gd.eppo.int/taxon/CORBMI/hosts>). Other plants of the *Solanaceae* family can naturally be infected (*Capsicum sativum*, *Capsicum annuum*, *Capsicum frutescens* and *Solanum melongena*).

Solanaceous and non-solanaceous weeds can spread the bacteria being natural reservoirs of infection.

Mainly, *Clavibacter michiganensis* subsp. *michiganensis* can be transmitted with the planting material (seedlings and seeds). Secondary, it spreads with: infected debris and soil, infected host plants with or without symptoms, equipment and other contaminated materials and agricultural practices (https://wiki.bugwood.org/Clavibacter_michiganensis_subsp._michiganensis).

According to the literature there are three mechanisms that could explain the wilting of plants in case of infection with *Clavibacter michiganensis* subsp. *michiganensis* namely: mechanical obstruction of xylem due to colonization of vessels with bacteria, toxic action of exopolysaccharides and enzymatic attack on plant tissues (Jahr *et al.*, 2000).

Studies show that strains of *Clavibacter michiganensis* subsp. *michiganensis* are different morphologically, biochemically, phenotypically and genetically. The present study aims to identify and characterize biochemically some strains of the bacteria from different counties of Romania.

Materials and Methods

Five strains of *Clavibacter michiganensis* subsp. *michiganensis*, from three counties (Argeş, Suceava and Satu Mare) of our country were used for biochemical identification and characterization. The strains Cmm 19-3857, Cmm 19-3861 and Cmm 19-4326 were isolated from *Solanum lycopersicum* cv. 'Colibri', the strain Cmm 19-4327 from *S. lycopersicum* cv. 'Kyveli' and the strain Cmm 19-4819 from *S. lycopersicum* cv 'Inima de bou'.

As positive controls were used two reference strains of *Clavibacter michiganensis* subsp. *michiganensis* (NCPBP 2979 and PD 223) both originating in Hungary, one purchased from the United Kingdom (Food and Environment Research Agency Sand Hutton) and the other from the Netherlands (Plant Protection Service). All strains analyzed were cryopreserved at -80°C using the Protect™ system.

The biochemical characterization was performed with 'Biolog's Microbial Identification System' which determines a phenotypic fingerprint of the strains of *Clavibacter michiganensis* subsp. *michiganensis*. This system uses MicroPlates with

94 phenotypic tests: 71 carbon sources and 23 chemical sensitivity tests. Microorganisms use or not use different carbon sources from MicroPlate wells. Where the carbon source is used, a redox reaction which reduces tetrazole occurs and due to cellular respiration the well is colored in purple. Where the microorganism does not utilize the carbon source no reaction occurs and the wells remain colorless. The pattern of colored and non-colored wells is specific to each strain (User guide - Identification system Biolog).

Four steps were required to perform this test: isolating the culture, preparing a bacterial suspension in an inoculation fluid with a certain density, incubating the GenIII microplates at a certain temperature, reading and interpreting the results. Thus, 1-2 balls loaded with bacterial cells from each strain of *Clavibacter michiganensis* subsp. *michiganensis* were placed on culture media BUG (Biolog Universal Growth, special media for this test prepared according to the manufacturer's recommendations) or on non-selective media (Yeast extract Peptone Glucose Agar or Nutrient Agar). Isolated Petri dishes were incubated, at 27°C, between 24 and 48 hours, until the first bacterial growths appeared. Extended incubation (for more than 48 hours) was avoided, because the bacteria had to be viable and it had to have metabolic activity, in order to react with the different carbon sources on the Gen III microplates (96 wells). After the first growths appeared, a bacterial colony was taken with a loopful and introduced into an inoculation fluid (IF-A) specific to aerobic bacteria. The fluid tubes were agitated by reversing until the colonies were dispersed in the liquid media. Then, using a turbidimeter, the cell density (transmittance) of the vials with the inoculation fluid was measured. If the transmittance of the samples was between 92-98%, the fluid was pipetted onto a Gen III microplate. If the fluid transmittance was below 92%, it is diluted by adding IF-A, until it reaches the desired value. If the transmittance was over 98%, biological material (colonies) is added, until it reaches the desired value. Then the Gen III microplates (Fig. 1) were loaded with 100 µl of inoculation fluid per well and incubated at 30°C, for 22 hours, in an OmniLog incubator.

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D- Cellobiose	A6 Gentiobiose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 pH 6	A12 pH 5
B1 D-Raffinose	B2 α -D-Lactose	B3 D-Melibiose	B4 β -Methyl-D- Glucoside	B5 D-Salicin	B6 N-Acetyl-D- Glucosamine	B7 N-Acetyl- β -D- Mannosamine	B8 N-Acetyl-D- Galactosamine	B9 N-Acetyl- Neuramini c Acid	B10 1%NaCl	B11 4%NaCl	B12 8%NaCl
C1 α -D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic Acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 myo-Inositol	D5 Glycerol	D6 D-Glucose-6- PO4	D7 D-Fructose-6- PO4	D8 D-Aspartic Acid	D9 D-Serine	D10 Troleandomyc in	D11 Rimfamycin SV	D12 Minocycline
E1 Gelatin	E2 Glycyl-L- Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic Acid	E6 L-Glutamic Acid	E7 L-Histidine	E8 L-Pyrogutamic Acid	E9 L-Serine	E10 Lincomycin	E11 Guanidine HCl	E12 Niaproof 4
F1 Pectin	F2 D- Galacturonic Acid	F3 L-Galactonic Acid Lactone	F4 D-Gluconic Acid	F5 D- Glucuronic Acid	F6 Glucuronamide	F7 Mucic Acid	F8 Quinic Acid	F9 D- Saccharic Acid	F10 Vancomycin	F11 Tetrazolium Violet	F12 Tetrazolium Blue
G1 p-Hydroxy- Phenylacetic Acid	G2 Methyl Pyruvate	G3 D-Lactic Acid Methyl Ester	G4 L-Lactic Acid	G5 Citric Acid	G6 α -Keto-Glutaric Acid	G7 D-Malic Acid	G8 L-Malic Acid	G9 Bromo- Succinic Acid	G10 Nalidixic Acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween 40	H2 γ -Anino- Butyric Acid	H3 α -Hydroxy Butyric Acid	H4 β -Hydroxy- D,L-Butyric Acid	H5 α -Keto- Butyric Acid	H6 Acetoacetic Acid	H7 Propionic Acid	H8 Acetic Acid	H9 Formic Acid	H10 Aztreonam	H11 Sodium Butyrate	H12 Sodium Bromate

Figure 1. MicroPlates GEN III (User guide - Identification system Biolog)

Results and discussions

After the end of the incubation period, there were performed three types of readings: visual, optical and spectrophotometric.

Visual readings of the microplates established that in some wells the redox reaction took place, therefore the bacteria of *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) were metabolically

active, breathed and reduced tetrazole, coloring the well into lilac (Fig. 2).

Optical readings were made at the Omnilog reader (Biolog equipment), provided with a video camera that takes the images, at certain times. The readings were performed for 22 hours, from half to half an hour. During this period the bacterial strains were biochemically characterized and identified,

Figure 2. Color reactions - GEN III MicroPlates contaminated with live cells of *Cmm*

Figure 3. Phenotypic profile of *Cmm* (User guide - Identification system Biolog)

by comparing their metabolic fingerprint with a database with over 2000 profiles, for Gram-positive and Gram-negative microorganisms.

Spectrophotometric readings were performed at MicroStation™ reader (Biolog equipment) at two densities (absorbance) 590 nm and 750 nm.

In the database of the Biolog system (GEN III) there is the metabolic profile of a strain of *Clavibacter michiganensis* subsp. *michiganensis* (Fig. 3). The interpretation of the reactions of this strain is shown in Figure 4. The strains taken in the study were characterized by comparison with this metabolic profile.

Columns 1-9 were used for carbon source utilization tests. All reactions in these columns are compared to the reaction in well A1, which is the negative control well. Therefore, the reactions in the wells in columns 1-9 which give a color similar to well A1 (the absence of any color) are considered negative because they do not use the respective carbon source. The wells that have been colored in lilac (more intense or weaker) are considered positive because they metabolize the respective substrate, using the carbon source.

Columns 10-12 were used for chemical sensitivity tests. The reactions in these wells are com-

A1 Negative Control -	A2 Dextrin +	A3 D-Maltose +	A4 D-Trehalose +	A5 D-Cellobiose +	A6 Gentiobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control +	A11 pH 6 +	A12 pH 5 +
B1 D-Raffinose +	B2 α -D-Lactose +	B3 D-Melibiose +	B4 β -Methyl-D- Glucoside +	B5 D-Salicin +	B6 N-Acetyl-D- Glucosamine -	B7 N-Acetyl- β -D- Mannosamine -	B8 N-Acetyl-D- Galactosamine -	B9 N-Acetyl Neuraminic Acid -	B10 1%NaCl +	B11 4%NaCl +	B12 8%NaCl + (slab)
C1 α -D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose + (slab)	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose -	C9 Inosine + (slab)	C10 1% Sodium Lactate +	C11 Fusidic Acid -	C12 D-Serine -
D1 D-Sorbitol +	D2 D-Mannitol +	D3 D-Arabinol -	D4 myo-Inositol +	D5 Glycerol +	D6 D-Glucose-6- PO4 + (slab)	D7 D-Fructose-6- PO4 + (slab)	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Troleandomycin -	D11 Rimfamycin SV -	D12 Minocycline -
E1 Gelatin + (slab)	E2 Glycyl-L- Proline -	E3 L-Alanine +	E4 L-Arginine -	E5 L-Aspartic Acid + (slab)	E6 L-Glutamic Acid +	E7 L-Histidine -	E8 L- Pyroglutamic Acid -	E9 L-Serine +	E10 Lincomycin -	E11 Guanidine HCl -	E12 Niaproof 4 -
F1 Pectin +	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Gluconic Acid +	F5 D-Gluconic Acid -	F6 Glucuronamid e -	F7 Mucic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancomycin -	F11 Tetrazolium Violet -	F12 Tetrazolium Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid +	G6 α -Keto- Glutaric Acid + (slab)	G7 D-Malic Acid -	G8 L-Malic Acid +	G9 Bromo- Succinic Acid +	G10 Nalidixic Acid +	G11 Lithium Chloride +	G12 Potassium Tellurite +
H1 Tween 40 +	H2 γ -Amino- Butyric Acid -	H3 α -Hydroxy Butyric Acid -	H4 β -Hydroxy- D,L-Butyric Acid -	H5 α -Keto-Butyric Acid + (slab)	H6 Acetoacetic Acid +	H7 Propionic Acid +	H8 Acetic Acid +	H9 Formic Acid -	H10 Aztreonam +	H11 Sodium Butyrate + (slab)	H12 Sodium Bromate +

Figure 4. Interpretation of the phenotypic profile of strain of *Cmm*
+ = positive reaction; - = negative reaction

A1 Negative Control -	A2 Dextrin +	A3 D-Maltose -	A4 D-Trehalose -	A5 D-Cellobiose -	A6 Gentiobiose -	A7 Sucrose +	A8 D-Turanose -	A9 Stachyose -	A10 Positive Control +	A11 pH 6 +	A12 pH 5 -
B1 D-Raffinose -	B2 α -D-Lactose -	B3 D-Melibiose -	B4 β -Methyl-D- Glucoside -	B5 D-Salicin -	B6 N-Acetyl-D- Glucosamine -	B7 N-Acetyl- β -D- Mannosamine -	B8 N-Acetyl-D- Galactosamine -	B9 N-Acetyl Neuraminic Acid -	B10 1%NaCl +	B11 4%NaCl +	B12 8%NaCl -
C1 α -D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose /	C5 3-Methyl Glucose /	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose -	C9 Inosine -	C10 1% Sodium Lactate -	C11 Fusidic Acid -	C12 D-Serine -
D1 D-Sorbitol /	D2 D-Mannitol +	D3 D-Arabinol -	D4 myo-Inositol -	D5 Glycerol /	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Troleandomycin -	D11 Rimfamycin SV -	D12 Minocycline -
E1 Gelatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L- Pyroglutamic Acid -	E9 L-Serine -	E10 Lincomycin -	E11 Guanidine HCl -	E12 Niaproof 4 -
F1 Pectin /	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Gluconic Acid -	F5 D-Gluconic Acid -	F6 Glucuronamid e -	F7 Mucic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancomycin -	F11 Tetrazolium Violet +	F12 Tetrazolium Blue /
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α -Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Bromo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride /	G12 Potassium Tellurite /
H1 Tween 40 +	H2 γ -Amino- Butyric Acid -	H3 α -Hydroxy Butyric Acid -	H4 β -Hydroxy- D,L-Butyric Acid -	H5 α -Keto- Butyric Acid +	H6 Acetoacetic Acid +	H7 Propionic Acid -	H8 Acetic Acid +	H9 Formic Acid -	H10 Aztreonam -	H11 Sodium Butyrate -	H12 Sodium Bromate /

Figure 5. Interpretation of metabolic profile *Cmm* Ncpcb 2979
optical reading (left), spectrophotometric reading (right)

+ = positive reaction, lilac color; - = negative reaction, no color; / = doubtful reaction, yellow color

A1 Negative Control	A2 Dextrin +	A3 D-Mannose +	A4 D-Trehalose +	A5 D-Glucose +	A6 Gentobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control	A11 pH 6 +	A12 pH 5 +
B1 D-Raffinose -	B2 α-D-Lactose +	B3 D-Melibiose -	B4 β-Methyl-D- Glucoside +	B5 D-Salicin +	B6 N-Acetyl-β-D- Glucosamine -	B7 N-Acetyl-β-D- Mannosamine -	B8 N-Acetyl-D- Galactosamine -	B9 N-Acetyl Neuraminic Acid -	B10 1%NaCl /	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose -	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose -	C9 Inosine -	C10 1% Sodium Lactate -	C11 Fusidic Acid -	C12 D-Serine -
D1 D-Sorbitol /	D2 D-Mannitol +	D3 D-Arabinol -	D4 myo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Tetrasodium in -	D11 Rifampicin SV -	D12 Miconazole -
E1 Gutatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L- Pyroglutamic Acid -	E9 L-Serine -	E10 L-tyrosine -	E11 Guandine HCl -	E12 Niprop 4 -
F1 Pectin +	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Gluconic Acid -	F5 D-Gluconic Acid -	F6 Glucuronamide -	F7 Malic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancomycin -	F11 Tetrazolium Violet -	F12 Tetrazolium Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Benzo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride +	G12 Potassium Tellurite +
H1 Tween 80 +	H2 γ-Anino- Butyric Acid -	H3 β-Hydroxy- Butyric Acid -	H4 β-Hydroxy- D,L-Butyric Acid -	H5 α-Keto- Butyric Acid -	H6 Acetoacetic Acid -	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Acetamin -	H11 Sodium Butyrate -	H12 Sodium Bromate +

A1 Negative Control	A2 Dextrin +	A3 D-Mannose +	A4 D-Trehalose +	A5 D-Glucose +	A6 Gentobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control	A11 pH 6 +	A12 pH 5 +
B1 D-Raffinose /	B2 α-D-Lactose +	B3 D-Melibiose -	B4 β-Methyl-D- Glucoside +	B5 D-Salicin /	B6 N-Acetyl-β-D- Glucosamine -	B7 N-Acetyl-β-D- Mannosamine -	B8 N-Acetyl-D- Galactosamine -	B9 N-Acetyl Neuraminic Acid -	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose -	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose -	C9 Inosine -	C10 1% Sodium Lactate -	C11 Fusidic Acid -	C12 D-Serine -
D1 D-Sorbitol +	D2 D-Mannitol +	D3 D-Arabinol -	D4 myo-Inositol /	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Tetrasodium in -	D11 Rifampicin SV -	D12 Miconazole -
E1 Gutatin -	E2 Glycyl-L- Proline -	E3 L-Alanine /	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L- Pyroglutamic Acid -	E9 L-Serine -	E10 L-tyrosine -	E11 Guandine HCl -	E12 Niprop 4 -
F1 Pectin -	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Gluconic Acid +	F5 D-Gluconic Acid /	F6 Glucuronamide -	F7 Malic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancomycin -	F11 Tetrazolium Violet /	F12 Tetrazolium Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Benzo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride /	G12 Potassium Tellurite +
H1 Tween 80 +	H2 γ-Anino- Butyric Acid -	H3 β-Hydroxy- Butyric Acid -	H4 β-Hydroxy- D,L-Butyric Acid -	H5 α-Keto- Butyric Acid -	H6 Acetoacetic Acid +	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Acetamin +	H11 Sodium Butyrate -	H12 Sodium Bromate /

Figure 6. Interpretation of metabolic profile *Cmm* PD223
optical reading (left), spectrophotometric reading (right)

+ = positive reaction, lilac color; - = negative reaction, no color; / = doubtful reaction, yellow color

A1 Negative Control	A2 Dextrin +	A3 D-Mannose +	A4 D-Trehalose /	A5 D-Glucose +	A6 Gentobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control	A11 pH 6 +	A12 pH 5 +
B1 D-Raffinose -	B2 α-D-Lactose /	B3 D-Melibiose -	B4 β-Methyl-D- Glucoside -	B5 D-Salicin +	B6 N-Acetyl-β-D- Glucosamine -	B7 N-Acetyl-β-D- Mannosamine -	B8 N-Acetyl-D- Galactosamine -	B9 N-Acetyl Neuraminic Acid -	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose -	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose -	C9 Inosine -	C10 1% Sodium Lactate /	C11 Fusidic Acid -	C12 D-Serine -
D1 D-Sorbitol -	D2 D-Mannitol -	D3 D-Arabinol -	D4 myo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Tetrasodium in -	D11 Rifampicin SV -	D12 Miconazole -
E1 Gutatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid /	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L- Pyroglutamic Acid -	E9 L-Serine -	E10 L-tyrosine -	E11 Guandine HCl -	E12 Niprop 4 -
F1 Pectin +	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Gluconic Acid -	F5 D-Gluconic Acid -	F6 Glucuronamide -	F7 Malic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancomycin -	F11 Tetrazolium Violet -	F12 Tetrazolium Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Benzo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride +	G12 Potassium Tellurite +
H1 Tween 80 /	H2 γ-Anino- Butyric Acid -	H3 β-Hydroxy- Butyric Acid -	H4 β-Hydroxy- D,L-Butyric Acid -	H5 α-Keto- Butyric Acid -	H6 Acetoacetic Acid -	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Acetamin +	H11 Sodium Butyrate -	H12 Sodium Bromate /

A1 Negative Control	A2 Dextrin +	A3 D-Mannose +	A4 D-Trehalose +	A5 D-Glucose +	A6 Gentobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control	A11 pH 6 +	A12 pH 5 +
B1 D-Raffinose -	B2 α-D-Lactose -	B3 D-Melibiose -	B4 β-Methyl-D- Glucoside +	B5 D-Salicin -	B6 N-Acetyl-β-D- Glucosamine -	B7 N-Acetyl-β-D- Mannosamine -	B8 N-Acetyl-D- Galactosamine -	B9 N-Acetyl Neuraminic Acid -	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose -	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose -	C9 Inosine -	C10 1% Sodium Lactate -	C11 Fusidic Acid -	C12 D-Serine -
D1 D-Sorbitol /	D2 D-Mannitol -	D3 D-Arabinol -	D4 myo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Tetrasodium in -	D11 Rifampicin SV -	D12 Miconazole -
E1 Gutatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L- Pyroglutamic Acid -	E9 L-Serine -	E10 L-tyrosine -	E11 Guandine HCl -	E12 Niprop 4 -
F1 Pectin -	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Gluconic Acid -	F5 D-Gluconic Acid -	F6 Glucuronamide -	F7 Malic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancomycin -	F11 Tetrazolium Violet -	F12 Tetrazolium Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Benzo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride +	G12 Potassium Tellurite +
H1 Tween 80 /	H2 γ-Anino- Butyric Acid -	H3 β-Hydroxy- Butyric Acid -	H4 β-Hydroxy- D,L-Butyric Acid -	H5 α-Keto- Butyric Acid -	H6 Acetoacetic Acid /	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Acetamin +	H11 Sodium Butyrate -	H12 Sodium Bromate /

Figure 7. Interpretation of metabolic profile *Cmm* 19-3857
optical reading (left), spectrophotometric reading (right)

+ = positive reaction, lilac color; - = negative reaction, no color; / = doubtful reaction, yellow color

pared with the reaction in well A10 which is the positive Control reaction. The positive control color is lilac. Therefore, the reactions in the wells in columns 10-12 which give a lilac color are considered positive for growth and indicate a lack of sensitivity to the respective chemical inhibitor. The wells that were not stained are considered negative for growth and indicate a significant sensitivity to the chemical inhibitor.

The metabolic profiles of the reference controls used (*Cmm* Ncpcb2979 and *Cmm* PD223) and the interpretation of the color reactions are shown in Figures 5-6 and for the used strains (*Cmm* 19-

3857, *Cmm* 19-3861, *Cmm* 19-4326, *Cmm* 19-4327, *Cmm* 19-4819) are noted in Figures 7-11.

Comparing the strains of *Clavibacter michiganensis* subsp. *michiganensis* taken in the study we can conclude that all strains use the carbon sources of the following compounds: α-D-Glucose, Dextrin, D-Mannose, D-Mannitol, D-Fructose, D-Galactose and Sucrose. They does not use carbon sources from: p-Hydroxy-Phenylacetic Acid, Glycyl-L-Proline, D-Galacturonic, Methyl Pyruvate, γ-Anino-Butyric Acid, D-Arabinol, L-Galactonic Acid Lactone, D-Lactic Acid Methyl Ester, α-Hydroxy-Butyric Acid, L-Arginine, L-Lactic Acid, β-Hydroxy-

A1 Negative Control -	A2 Dextrin +	A3 D-Maltose +	A4 D-Trehalose +	A5 D-Cellobiose +	A6 Gentiobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control +	A11 pH 6 +	A12 pH 5 -
B1 D-Raffinose -	B2 α-D-Lactose +	B3 D-Melibiose +	B4 β-Methyl-D- Glucoside +	B5 D-Salicin +	B6 N-Acetyl-D- Glucosamine +	B7 N-Acetyl-β-D- Mannosamine +	B8 N-Acetyl-D- Galactosamine +	B9 N-Acetyl Neuraminic Acid +	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose +	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose +	C9 Inosine -	C10 1% Sodium Lactate +	C11 Fusidic Acid +	C12 D-Serine -
D1 D-Sorbitol +	D2 D-Mannitol +	D3 D-Arabitol -	D4 mpo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Troleandomy- cin -	D11 Rimfamycin SV -	D12 Minocycline -
E1 Gelatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L-Aspartic Pyroglutamic Acid -	E9 L-Serine -	E10 Lincomycin -	E11 Guanidine HCl -	E12 Niaproof 4 -
F1 Pectin -	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Glucuronic Acid -	F5 D-Gluconic Acid -	F6 Glucuronamide +	F7 Mucic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancocycin -	F11 Tetrastatin Violet -	F12 Tetrastatin Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Bromo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride -	G12 Potassium Tellurite -
H1 Tween 40 -	H2 γ-Amino- Butyric Acid -	H3 α-Hydroxy- Butyric Acid -	H4 β-Hydroxy- DL-Butyric Acid -	H5 α-Keto- Butyric Acid -	H6 Acetoacetic Acid -	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Aztreonam +	H11 Sodium Butyrate -	H12 Sodium Bromate -

A1 Negative Control -	A2 Dextrin +	A3 D-Maltose +	A4 D-Trehalose +	A5 D-Cellobiose +	A6 Gentiobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control +	A11 pH 6 +	A12 pH 5 -
B1 D-Raffinose -	B2 α-D-Lactose +	B3 D-Melibiose +	B4 β-Methyl-D- Glucoside +	B5 D-Salicin +	B6 N-Acetyl-D- Glucosamine +	B7 N-Acetyl-β-D- Mannosamine +	B8 N-Acetyl-D- Galactosamine +	B9 N-Acetyl Neuraminic Acid +	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose +	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose +	C9 Inosine -	C10 1% Sodium Lactate +	C11 Fusidic Acid +	C12 D-Serine -
D1 D-Sorbitol +	D2 D-Mannitol +	D3 D-Arabitol -	D4 mpo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Troleandomy- cin -	D11 Rimfamycin SV -	D12 Minocycline -
E1 Gelatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L-Aspartic Pyroglutamic Acid -	E9 L-Serine -	E10 Lincomycin -	E11 Guanidine HCl -	E12 Niaproof 4 -
F1 Pectin -	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Glucuronic Acid -	F5 D-Gluconic Acid -	F6 Glucuronamide +	F7 Mucic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancocycin -	F11 Tetrastatin Violet -	F12 Tetrastatin Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Bromo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride -	G12 Potassium Tellurite -
H1 Tween 40 -	H2 γ-Amino- Butyric Acid -	H3 α-Hydroxy- Butyric Acid -	H4 β-Hydroxy- DL-Butyric Acid -	H5 α-Keto- Butyric Acid -	H6 Acetoacetic Acid -	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Aztreonam +	H11 Sodium Butyrate -	H12 Sodium Bromate -

Figure 8. Interpretation of metabolic profile *Cmm* 19-3861 - optical reading
optical reading (left), spectrophotometric reading (right)

+ = positive reaction, lilac color; - = negative reaction, no color; / = doubtful reaction, yellow color

A1 Negative Control -	A2 Dextrin +	A3 D-Maltose +	A4 D-Trehalose +	A5 D-Cellobiose +	A6 Gentiobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control +	A11 pH 6 +	A12 pH 5 -
B1 D-Raffinose -	B2 α-D-Lactose +	B3 D-Melibiose +	B4 β-Methyl-D- Glucoside +	B5 D-Salicin +	B6 N-Acetyl-D- Glucosamine +	B7 N-Acetyl-β-D- Mannosamine +	B8 N-Acetyl-D- Galactosamine +	B9 N-Acetyl Neuraminic Acid +	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose +	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose +	C9 Inosine -	C10 1% Sodium Lactate +	C11 Fusidic Acid +	C12 D-Serine -
D1 D-Sorbitol +	D2 D-Mannitol +	D3 D-Arabitol -	D4 mpo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Troleandomy- cin -	D11 Rimfamycin SV -	D12 Minocycline -
E1 Gelatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L-Aspartic Pyroglutamic Acid -	E9 L-Serine -	E10 Lincomycin -	E11 Guanidine HCl -	E12 Niaproof 4 -
F1 Pectin -	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Glucuronic Acid -	F5 D-Gluconic Acid -	F6 Glucuronamide +	F7 Mucic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancocycin -	F11 Tetrastatin Violet -	F12 Tetrastatin Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Bromo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride -	G12 Potassium Tellurite -
H1 Tween 40 -	H2 γ-Amino- Butyric Acid -	H3 α-Hydroxy- Butyric Acid -	H4 β-Hydroxy- DL-Butyric Acid -	H5 α-Keto- Butyric Acid -	H6 Acetoacetic Acid -	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Aztreonam +	H11 Sodium Butyrate -	H12 Sodium Bromate -

A1 Negative Control -	A2 Dextrin +	A3 D-Maltose +	A4 D-Trehalose +	A5 D-Cellobiose +	A6 Gentiobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control +	A11 pH 6 +	A12 pH 5 -
B1 D-Raffinose -	B2 α-D-Lactose +	B3 D-Melibiose +	B4 β-Methyl-D- Glucoside +	B5 D-Salicin +	B6 N-Acetyl-D- Glucosamine +	B7 N-Acetyl-β-D- Mannosamine +	B8 N-Acetyl-D- Galactosamine +	B9 N-Acetyl Neuraminic Acid +	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose +	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose +	C9 Inosine -	C10 1% Sodium Lactate +	C11 Fusidic Acid +	C12 D-Serine -
D1 D-Sorbitol +	D2 D-Mannitol +	D3 D-Arabitol -	D4 mpo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Troleandomy- cin -	D11 Rimfamycin SV -	D12 Minocycline -
E1 Gelatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L-Aspartic Pyroglutamic Acid -	E9 L-Serine -	E10 Lincomycin -	E11 Guanidine HCl -	E12 Niaproof 4 -
F1 Pectin -	F2 D-Galacturonic Acid -	F3 L-Galactonic Acid Lactone -	F4 D-Glucuronic Acid -	F5 D-Gluconic Acid -	F6 Glucuronamide +	F7 Mucic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancocycin -	F11 Tetrastatin Violet -	F12 Tetrastatin Blue -
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Bromo- Succinic Acid -	G10 Nalidixic Acid -	G11 Lithium Chloride -	G12 Potassium Tellurite -
H1 Tween 40 -	H2 γ-Amino- Butyric Acid -	H3 α-Hydroxy- Butyric Acid -	H4 β-Hydroxy- DL-Butyric Acid -	H5 α-Keto- Butyric Acid -	H6 Acetoacetic Acid -	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Aztreonam +	H11 Sodium Butyrate -	H12 Sodium Bromate -

Figure 9. Interpretation of metabolic profile *Cmm* 19-4326
optical reading (left), spectrophotometric reading (right)

+ = positive reaction, lilac color; - = negative reaction, no color; / = doubtful reaction, yellow color

D,L-Butyric Acid, D-Glucuronic Acid, N-Acetyl-D-Glucosamine, D-Fucose, Glucuronamide, L-Fucose, L-Histidine, Mucic Acid, D-Malic Acid, D-Aspartic Acid, L-Pyrogutamic Acid, Quinic Acid, N-Acetyl Neuraminic Acid, D-Serine, D-Saccharic Acid and Formic Acid.

With regard to chemical sensitivity tests, it was found that these strains were positive for growth in the presence of: 1% NaCl, Nalidixic Acid, Aztreonam, Potassium Tellurite and pH 6. Instead, they were negative for growth and showed sensitivity to: Troleandomycin, Lincomycin, Vanco-

mycin, Fusidic Acid, Rimfamycin SV, Guanidine HCl, D-Serine, Minocycline and Niaproof 4.

Although the strain in the database metabolizes compounds: Gelatin, L-Aspartic Acid, Citric Acid, α-Keto-Butyric Acid, D-Glucose-6-PO4, L-Glutamic Acid, α-Keto-Glutaric Acid, D-Fructose-6-PO4, Propionic Acid, L-Malic Acid, Inosine, L-Serine and Bromo-Succinic Acid, none of the characterized strains utilize the carbon sources of these compounds. Also, although the strain of *Clavibacter michiganensis* subsp. *michiganensis* from the database growth in the presence of

A1 Negative Control	A2 Dextrin +	A3 D-Maltose +	A4 D-Trehalose +	A5 D-Cellobiose +	A6 Gentobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control	A11 pH 6 +	A12 pH 5 -
B1 D-Raffinose /	B2 α-D-Lactose +	B3 D-Melibiose -	B4 β-Methyl-D- Glucoside -	B5 D-Salicin -	B6 N-Acetyl-D- Glucosamine -	B7 N-Acetyl-β-D- Mannosamine -	B8 N-Acetyl-D- Galactosamine -	B9 N-Acetyl- Neuraminic Acid -	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose -	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose -	C9 Inositol -	C10 1%Sodium Lactate /	C11 Fosfatic Acid -	C12 D-Serine -
D1 D-Sorbitol /	D2 D-Mannitol /	D3 D-Arabinol -	D4 myo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Tetrasomye in -	D11 Rimifamycin SV -	D12 Mincycline /
E1 Gelatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L- Pyroglutamic Acid -	E9 L-Serine -	E10 Linoscin -	E11 Guandine HCl -	E12 Nigrocol 4 -
F1 Pectin +	F2 D-Galacturonic Acid -	F3 D-Galactonic Acid Lactone -	F4 D-Glucuronic Acid -	F5 D-Chronic Acid -	F6 Glucuronid -	F7 Mucic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancomycin -	F11 Tetracycline Violet /	F12 Tetracycline Blue /
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Bromo- Succinic Acid -	G10 Nalidixic Acid +	G11 Lithium Chloride /	G12 Potassium Tellurite /
H1 Tween 40 +	H2 γ-Amino- Butyric Acid -	H3 α-Hydroxy- Butyric Acid -	H4 β-Hydroxy- D,L-Butyric Acid -	H5 α-Keto- Butyric Acid +	H6 Acetoacetic Acid +	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Azotium -	H11 Sodium Butyrate -	H12 Sodium Bromate /

Figure 10. Interpretation of metabolic profile *Cmm 19-4327*
optical reading (left), spectrophotometric reading (right)

+ = positive reaction, lilac color; - = negative reaction, no color; / = doubtful reaction, yellow color

A1 Negative Control	A2 Dextrin +	A3 D-Maltose +	A4 D-Trehalose +	A5 D-Cellobiose +	A6 Gentobiose +	A7 Sucrose +	A8 D-Turanose +	A9 Stachyose +	A10 Positive Control	A11 pH 6 +	A12 pH 5 -
B1 D-Raffinose /	B2 α-D-Lactose +	B3 D-Melibiose -	B4 β-Methyl-D- Glucoside -	B5 D-Salicin -	B6 N-Acetyl-D- Glucosamine -	B7 N-Acetyl-β-D- Mannosamine -	B8 N-Acetyl-D- Galactosamine /	B9 N-Acetyl- Neuraminic Acid -	B10 1%NaCl +	B11 4%NaCl -	B12 8%NaCl -
C1 α-D-Glucose +	C2 D-Mannose +	C3 D-Fructose +	C4 D-Galactose +	C5 3-Methyl Glucose -	C6 D-Fucose -	C7 L-Fucose -	C8 L-Rhamnose -	C9 Inositol -	C10 1%Sodium Lactate /	C11 Fosfatic Acid -	C12 D-Serine -
D1 D-Sorbitol /	D2 D-Mannitol /	D3 D-Arabinol -	D4 myo-Inositol -	D5 Glycerol +	D6 D-Glucose-6- PO4 -	D7 D-Fructose-6- PO4 -	D8 D-Aspartic Acid -	D9 D-Serine -	D10 Tetrasomye in -	D11 Rimifamycin SV -	D12 Mincycline /
E1 Gelatin -	E2 Glycyl-L- Proline -	E3 L-Alanine -	E4 L-Arginine -	E5 L-Aspartic Acid -	E6 L-Glutamic Acid -	E7 L-Histidine -	E8 L- Pyroglutamic Acid -	E9 L-Serine -	E10 Linoscin -	E11 Guandine HCl -	E12 Nigrocol 4 -
F1 Pectin +	F2 D-Galacturonic Acid -	F3 D-Galactonic Acid Lactone -	F4 D-Glucuronic Acid +	F5 D-Chronic Acid -	F6 Glucuronid -	F7 Mucic Acid -	F8 Quinic Acid -	F9 D-Saccharic Acid -	F10 Vancomycin -	F11 Tetracycline Violet /	F12 Tetracycline Blue /
G1 p-Hydroxy- Phenylacetic Acid -	G2 Methyl Pyruvate -	G3 D-Lactic Acid Methyl Ester -	G4 L-Lactic Acid -	G5 Citric Acid -	G6 α-Keto- Glutaric Acid -	G7 D-Malic Acid -	G8 L-Malic Acid -	G9 Bromo- Succinic Acid -	G10 Nalidixic Acid +	G11 Lithium Chloride /	G12 Potassium Tellurite /
H1 Tween 40 +	H2 γ-Amino- Butyric Acid -	H3 α-Hydroxy- Butyric Acid -	H4 β-Hydroxy- D,L-Butyric Acid -	H5 α-Keto- Butyric Acid /	H6 Acetoacetic Acid /	H7 Propionic Acid -	H8 Acetic Acid -	H9 Formic Acid -	H10 Azotium -	H11 Sodium Butyrate -	H12 Sodium Bromate /

Figure 11. Interpretation of metabolic profile of *Cmm 19-4819*
optical reading (left), spectrophotometric reading (right)

+ = positive reaction, lilac color; - = negative reaction, no color; / = doubtful reaction, yellow color

Sodium Butyrate and 8% NaCl, all strains studied did not develop in the presence of these compounds.

The rest of the reactions were varied, depending on the strain, having either a positive or a negative result, without a uniformity in all the strains, regarding the response to a certain type of reaction. There were also wells on the microplates which had an uncertain result, which was interpreted as a limit reaction, which could not be classified as positive or negative, or which showed a reaction mismatch with the

reaction in the database for *Clavibacter michiganensis* subsp. *michiganensis*. The strains studied had more intense or weaker reactions compared to the strain in the database. Also, between the optical and spectrophotometric readings there were slight differences, without fundamentally altering the metabolic profile of the strain analyzed.

By summing the number of positive reactions, the metabolic diversity of the strains studied was calculated. Thus, in this case the number of positive reactions varied between 12-28 positive reac-

Figure 12. Metabolic diversity of *Clavibacter michiganensis* subsp. *michiganensis*

tions for optical readings and between 18-39 positive reactions for spectrophotometric readings (Fig. 12).

Conclusion

In this study there were identified strains of *Clavibacter michiganensis* subsp. *michiganensis* only up to the level of the genus and species (*Clavibacter michiganensis*) not to the level of the subspecies.

The existence of the differences between the database of the Biolog microbial identification system and the strains taken in the study did not essentially change the classification of the strains at the level of genus and species but did modify or did not allow their exact classification at the subspecies level.

Only one strain was erroneously classified at the subspecies level, due to the similarity of the metabolic profile with another organism of the *Clavibacter* genus. It was framed only for optical readings, not spectrophotometric ones, in the subspecies *sepedonicus*.

The visual analysis allowed only to establish that the cells existing in the microplates were alive

and had an active metabolism without allowing their biochemical characterization. Optical and spectrophotometric analysis (performed at two wavelengths) allowed us to characterize biochemically the strains of *Clavibacter michiganensis* subsp. *michiganensis* taken in the study. Following this characterization it was found that the analyzed strains had metabolic diversity, because they metabolized different substrates, using different carbon sources.

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