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EVALUATION OF THE BIOCONTROL EFFICACY OF *Serratia proteamaculans* AND *S. liquefaciens* ISOLATED FROM BATS GUANO PILE FROM A SUBTERRESTRIAL CAVE (GREECE)

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Abstract

Members of the genus *Serratia* are of great research interest because they are almost ubiquitous and exhibit emulsifying, surfactant, antifouling, antitumor and antimicrobial properties. Water is a natural habitat for several species of serrations. This paper reports on the first isolation of *S. proteamaculans* from bats guano. The aim of the present study is to evaluate the biocontrol activity of *Serratia* strains isolated from bats guano pile from a subterrestrial cave of Thessaly region (Aeolia), Greece. *Serratia* strains initial designated as strains Sl2, Sl4 and were able to ferment glucose (D-glucose), other carbohydrates (i.e. D-mannitol, D-mannose), and saccharose/sucrose as a source of carbon and sugars. Both strains have an optimal growth at 28 °C whereas strain Sl4 were able to grow and at 4 °C. Bacteria strains Sl2 and Sl4 were classified within the *Serratia liquefaciens* group by the VITEK® 2 system (bioMerieux SA, France) and were accurately identified at the species level by MALDI-TOF MS (bioMerieux SA, France). MALDI-TOF MS classified Sl2 strain as *S. proteamaculans* and Sl4 strain as *S. liquefaciens*. To the best of our knowledge, this paper is the first to report the detection and classification in detail of the *S. proteamaculans* in bat guano. Both *Serratia* strains produced prodigiosin at 28 °C with optimum prodigiosin production recorded 72 h after incubation. Further the antifungal activity of *S. liquefaciens* and *S. proteamaculans* strains were investigated in vitro against plant pathogenic fungi (*Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*). This is the first report that *S. liquefaciens* and *S. proteamaculans* strains isolated from bat guano were able to produce freely diffusible compounds with fungistatic activity in vitro. Studies on the interaction between pathogen and bacteria confirmed the biocontrol efficacy of both *Serratia* strains (*S. liquefaciens* and *S. proteamaculans*).

Keywords: *Serratia* spp., bat guano, subterranean aquatic environment, secondary metabolites, biocontrol.

Serratia species, which includes up to 18 species, can be found in several different environments [1]. Strains of the genus *Serratia* have been isolated from water, soil, plants, and animals [2]. In mammals, *Serratia* strains have been associated with infections such as mastitis in cattle, conjunctivitis in equine, septicaemia in foals, goats, and pigs, but have also been associated with several clinically healthy individuals [3]. *S. liquefaciens* and *S. marcescens* have been reported as opportunistic pathogens for the chiropteran species [4].

Serratia liquefaciens group consists of the species *S. liquefaciens*, *S. proteamaculans*, and *S. grimesii* [3]. Strains of the *S. liquefaciens* group predominantly cause sepsis and bloodstream infections via contaminated clinical equipment and blood components [5]. In 1980, the "Approved list of Bacterial Names" listed *S. liquefaciens* and *S. proteamaculans* as separate species [6]. Although probably rarely reported in clinical samples due to inability to easily discriminate between

group species, *S. proteamaculans* has been shown to cause human disease [7].

Water appears to be a natural environment for several species, including *S. marcescens*, *S. fonticola*, *S. grimesii*, *S. liquefaciens*, *S. plymuthica*, *S. rubidaea*, and *S. ureilytica* [8]. *S. marcescens*, *S. liquefaciens*, *S. proteamaculans*, *S. grimesii*, and *S. plymuthica* were found in river water in one study, with the predominant species being *S. marcescens* followed by *S. liquefaciens* [3]. *S. marcescens* subsp. *sakuensis* was originally isolated from the suspended water of a wastewater treatment tank in Japan [9]. *Serratia* species are also associated with animals and cause important animal diseases. *S. marcescens* was described in 1958 as a cause of illness in animals, when part of a dairy herd was diagnosed with mastitis. There are many other reports of colonization or disease caused by *Serratia* species in animals, including but not limited to reptiles, rodents, birds, chicks, goats, pigs, fish, and horses [8].

Members of the *Serratia* genus are gaining increased scientific interest as they have been shown to exhibit emulsifying, surface, antifouling, antitumour and antimicrobial activity [10, 11]. Literature shows that the ubiquitous nature of this genus is due to the synthesis of numerous extracellular products, including exoenzymes, nucleases and secondary metabolites that aid in the adaption of *Serratia* to harsh environmental conditions [12] including prodigiosin. Certain strains of *S. marcescens*, *S. rubidaea*, and *S. surfactantfaciens* produce prodigiosin and show antibacterial and antifungal activity [2, 10].

Plant pathogenic fungi are responsible for severe losses of agriculture worldwide. An effective approach is to use chemical fungicides, to control the spread of fungi plant diseases, which have been applied to several fruits and other crop species. The rising threat of fungicide resistance in plant e.g. the grey mold fungus *Botrytis cinerea* resistance development is well-known. *B. cinerea* is notorious as a 'high risk' organism for rapid resistance development and the introduction of new fungicide classes for grey mold control was always followed by the appearance of resistance in field populations [13]. Under these circumstances, there is a growing need for identifying alternatives to fungicides in the prevention and treatment of microbial infections.

Base to above, the primary aim of this study was to examine bat guano from a subterrestrial aquatic ecosystem, for *Serratia* isolates capable of presenting biocontrol activity against plant pathogenic fungi.

Materials and methods. The Malaki cave is located in Thessaly (Aeolia), Greece (lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.).

Gram-negative bacteria were isolated from bats guano. NA (Nutrient Agar), Potato Dextrose Agar (PDA), and MacConkey agar (MCA, Oxoid Limited, Great Britain) were used for routine isolation of bacteria. We used quadrant streak method, and agar plates were incubated at 22 °C for 2 days [1].

VITEK® 2 and MALTI-TOF MS (bioMerieux SA, France) were used to identify bacterial isolates at the species level [14, 15]. Single colonies on the nutrient agar slant were selected and suspended in 2.5 ml of 0.45% sterile saline to adjust the bacterial suspension to a 0.5 McFarland turbidity standard using a densitometer (bioMerieux SA, France) according to the manufacturer's instructions. Each bacterial suspension was prepared within 30 minutes of inoculation into the gram-negative (GN) cassette for identification by VITEK® 2. The biochemical test array of the GN cassette is presented in Table 1. The analysis was performed using VITEK® 2 software version 07.01 (bioMerieux SA, France) [16]. Higher probability than 80 % with a sufficient profile was considered a satisfactory identification within the possible identification spectrum of species or genera (taxa) by the VITEK® 2 [15, 16].

Prior to MALDI-TOF MS measurement, bacterial isolates were freshly inoculated on PCA (Oxoid Limited, UK) and cultivated for 24 h at 28 °C. The common direct transfer protocol (commonly referred to as whole-cell or intact-cell measurement) was followed to obtain mass spectra. Briefly, ~ 0.1 mg of cell material was directly transferred from a bacterial colony or smear of colonies to a MALDI target spot. After drying at laboratory temperature, sample spots were overlaid with 1 μl of matrix solution (10 mg/ml α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid). To determine mass spectra generation reproducibility, all cultures were cultivated independently four times (biological replicates); each measurement was carried out in triplicate. MS analysis was performed on an Autoflex MALDITOF mass spectrometer (Bruker Daltonics, Germany) using Flex Frontiers [16] with dereplication (Recurrent Bacterial Isolates Control 3.4 software, Bruker Daltonics, Germany): calibration was carried out with the use of the Bacterial Test Standard (Bruker Daltonics, Germany) (http://www.bruker.com/jp/products/mass-spectrometry-andseparations/literature/literatureroom.html?eID=dam_frontend_ push&stream=1&docID=58883). All MS spectra were measured automatically using Flex Control software (Bruker Corporation», USA, Germany) according to the standard measurement method for microbial identification. Specifically, our set-up values in linear positive mode were as follows: ion source 1 voltage, 20 kV; ion source 2 voltage, 19 kV; lens voltage, 6.5 kV; mass range, 2-20 kDa; the final spectrum was the sum of 10 single spectra, each obtained by 200 laser shots on random target spot positions. With regard to the functioning of MALDI-TOF MS, by which +1 ions are predominantly generated and detected, Da is used as a unit of m/z throughout the study. For bacterial classification using BioTyper 3.1 software (Bruker Daltonics, Germany) equipped with MBT 6903 MPS Library (released in April 2016), the MALDI Biotyper Preprocessing Standard Method and the MALDI Biotyper MSP Identification Standard Method adjusted by the manufacturer (Bruker Daltonics, Germany) were used [15, 16].

A VITEK® 2 GN identification card was used (the VITEK® 2 system, bioMerieux SA, France) [17] was used to assign the *Serratia proteamaculans* (Sl2) and *Serratia liquefaciens* (Sl4) strains to the *Serratia liquefaciens* group.

The *Serratia* strains (Sl2, Sl4) isolated from bat guano identified as *S. proteamaculans* (Sl2 strain) and *S. liquefaciens* (Sl4 strain) using a MALDI-TOF instrument, were screened for prodigiosin production on MacConkey's medium. Plates were incubated at 28 °C for 24, 48, and 72 h and screened for *Serratia* colonies with hyperpigmentation. The pigment producing strains (hyperpigmentation) were detected by appearance of pink-red growth.

Five different fungal strains causing plant disease were used in this study to assess antifungal properties of the *Serratia* strains. The fungal strains *Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani* were isolated from affected tomato plants in Central Greece during earlier study [18, 19], with techniques described in [20, 21].

The isolated *Serratia* strains (*S. liquefaciens* and *S. proteamaculans*) were screened in vitro by dual culture techniques for the presence of antagonistic activity against the five different fungal species, *F. oxysporum* (BFI 2550), *A. alternata* (BFI 2596), *B. cinerea* (BFI 1952), *S. sclerotiorum* (BFI 2529), and *R. solani* (BFI 2531) obtained from Benaki Phytopathological Institute (BFI) as described by [22]. Specifically, in PDA plate, a 40 mm streak was made from 24h culture of bacteria 30 mm away from the centre of a petri dish. A 5 mm agar plug from a 5-day old fungal culture was placed at the centre of the petri dish with the test bacterial strain. Plates were incubated at 25 °C for 5 days and monitored for zone

of inhibition daily. The mycelial radial growth (diameter) of the plant pathogens was measured. The features of the manifestation of antifungal activity were examined. All treatments were performed in eight replications.

Data were analysed using the Minitab statistical package (https://www.minitab.com/en-us/support/downloads/). Analysis of variance was used to assess antagonistic effect, the results are shown in an excel graph plotted the mean/treatment (*M*) and the standard error of differences between means (±SEM).

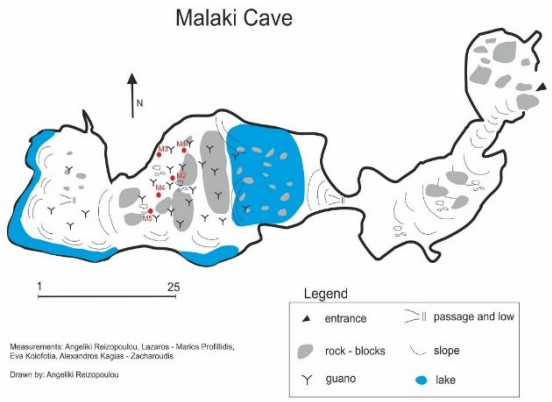

Fig. 1. Cave mapping (the Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.). Red dots indicate sampling sites close to indigenous bat nests. Drawn by Angeliki Reizopoulou.

Results.. Malaki cave is a small semi light limestones subterrestrial cave (Fig. 1). Malaki cave environment is being separated into three zones I) a twilight zone close to the entrance, II) a middle zone with scarce light and varying temperatures, and III) the deep zone of total darkness and a steady temperature (17 °C) throughout the year. In Cave's deep zone, there is a small subterrestrial lake, and several large bat guano piles. In cave's deep zone the main species fauna found are the genus *Miniopterus*, *Myotis*, and *Rhinolophus* [23].

Serratia strains initially designated as Sl2 and Sl4 were able to ferment glucose (D-glucose), utilize maltose, trehalose, other carbohydrates (i.e., D-mannitol, D-mannose), and saccharose/sucrose as a source of carbon and sugar (Table 1). Both strains have an optimal growth at 28 °C whereas strain Sl4 can also grow at 4 °C.

All bacterial strains with negative straight rod morphology, capable of growth on MacConkey Agar were correctly identified to the species level (99.9% probability) by the VITEK® 2 system using VITEK 2 GN ID card (see Table 1). VITEK 2 GN ID card classified both strains (Sl2 and Sl4) as *Serratia liquefaciens* group. The direct identification reporting time of VITEK® 2 ranged from 4.17 h to 4.65 h for all isolates. Furthermore, all strains identified using the VITEK® 2 were classified to the species level (99.9% confidence level) with MALDI-TOF MS system as *S. proteamaculans* (Sl2) and *S. liquefaciens* (Sl4). It is clear from the results of MALDI-TOF MS analysis that the confidence value of 99.9% as presented is beyond doubt evident of the clear distinction and presence of *S. proteamaculans* and *S. liquefaciens* strains.

1. Evaluation of VITEK® 2 GN ID Card for rapid identification of gram negative bacteria isolated from bats guano (Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.)

Test	Mnemonic	Amount	Strain *Serratia*	
			Sl2 (*S. proteamaculans*)	Sl4 (*S. liquefaciens*)
Ala-Phe-Pro-arylamidase	APPA	0,0384 mg	–	–
Adonitol	ADO	0,1875 mg	–	–
L-pyrrolydonyl arylamidase	PyrA	0,018 mg	+	+
L-Arabitol	lARL	0,3 mg	–	–
D-Cellobiose	dCEL	0,3 mg	–	–
Beta-galactosidase	BGAL	0,036 mg	+	+
H_2S	H_2S	0,0024 mg	–	–
Beta-N-acetyl-glucosaminidase	BNAG	0,0408 mg	+	+
Glutamyl arylamidase pNA	AG LTp	0,0324 mg	–	–
D-glucose	dGLU	0,3 mg	+	+

Continued Table 1

Gamma-glutamyl-transferase	GGT	0,0228 mg	+	–
Fermentation/glucose	OFF	0,45 mg	+	+
Beta-glucosidase	BGLU	0,036 mg	+	+
D-maltose	dMAL	0,3 mg	–	–
D-mannitol	dMAN	0,1875 mg	+	+
D-mannose	dMNE	0,3 mg	+	+
Beta-xylosidase	BXYL	0,0324 mg	–	–
Beta-alanine arylamidase pNA	BAlap	0,0174 mg	–	–
L-proline arylamidase	ProA	0,0234 mg	+	+
Lipase	LIP	0,0192 mg	–	–
Palatinose	PLE	0,3 mg	+	–
Tyrosine arylamidase	TyrA	0,0276 mg	+	+
Urease	URE	0,15 mg	–	–
D-sorbitol	dSOR	0,1875 mg	+	+
Saccharose/sucrose	SAC	0,3 mg	+	+
D-tagatose	dTAG	0,3 mg	–	–
D-trehalose	dTRE	0,3 mg	+	+
Citrate (sodium)	CIT	0,054 mg	+	+
Malonate	MNT	0,15 mg	–	–
5-keto-D-gluconate	5KG	0,3 mg	+	+
[-Lactate alkalinisation	1LATk	0,15 mg	+	+
Alpha-glucosidase	AGLU	0,036 mg	+	–
Succinate alkalinisation	SUCT	0,15 mg	+	+
Beta-N-acetyl-galactosaminidase	NAGA	0,0306 mg	+	+
Alpha-galactosidase	AGAL	0,036 mg	–	+
Phosphatase	PHOS	0,0504 mg	+	–
Glycine arylamidase	GIyA	0,012 mg	–	N/A
Ornithine decarboxylase	ODC	0,3 mg	+	+
Lysine decarboxylase	LDC	0,15 mg	+	+
Decarboxylase Base	ODEC	N/A		
L-histidine assimilation	1H1Sa	0,087 mg	–	–
Coumarate	CMT	0,126 mg	+	+
Beta-glucoronidase	BGUR	0,0378 mg	–	–
O/129 resistance (comp. *vibrio*)	0129R	0,0105 mg	+	+
Glu-Gly-Arg-arylamidase	GGAA	0,0576 mg	+	+
L-malate assimilation	1MLTa	0,042 mg	–	–
ELLMAN	ELLM	0,03 mg	–	–
[-Lactate assimilation	1LATa	0,186 mg	–	–

N o t e. «–» or «+» mark gives the absence or presence of the corresponding trait, N/A — not applicable. The data for Sl2 has been reported earlier (Michail G., Reizopoulou A., Vagelas I. First report of *Serratia* species isolated from subterranean cave aquatic environment. *International Research Journal of Engineering and Technology*, 2020, 07(12): 1776-1780).

The microorganism identification by MADLI-TOF is based on four commercial systems and their databases: I) the MALDI Biotyper (Bruker Daltonics, Bremen, Germany); II) the Spectral ARchive and Microbial Identification System (SARAMIS™) (AnagnosTec, Potsdam, Germany); III) the Andromas (Andromas, Paris, France) and IV) the Vitek MS (bioMйrieux SA, Marcy l'Etoile, France). Each of the systems includes a MALDI-TOF instrument from either Bruker Daltonics or Shimadzu Corporation (Japan), and the most installed in routine laboratories systems are the MALDI Biotyper and the Vitek MS, which are the FDA-cleared platforms [24]. The systems differ in databases, identification algorithms, and instrumentation [25]. In daily laboratory practice, MALDI-TOF is used for bacterial or fungal identification from colonies grown on solid media. Up to now the procedure has been used for the identification of Gram-negative and positive rods, Gram-positive cocci, fastidious organisms, like mycobacteria, Nocardia and other actinomycetes, anaerobic bacteria, yeasts and filamentous fungi [26-29]. According to the data the identification rates of genus are extremely high (97-99%) and varies from 85% to 97% at the level of species [30-35].

Both microorganisms (*S. liquefaciens* and *S. proteamaculans*) appeared as lactose fermenters on MacConkey's medium (Fig. 2, A). *Serratia liquefaciens* can ferment lactose rapidly compared to *S. proteamaculans. S. liquefaciens* colonies are circular, entire, convex, medium, shiny red and opaque whereas *S. proteamaculans* colonies are circular, filamentous, convex, small, orange-red and opaque (Fig. 2,

B). *S. liquefaciens* and *S. proteamaculans* colonies produced a clear reddish-orange (orange halo) colonies suggesting prodigiosin pigment production (see Fig. 2, B). The optimum prodigiosin production where obtain 72 h after incubation at 28 °C (Table 2).

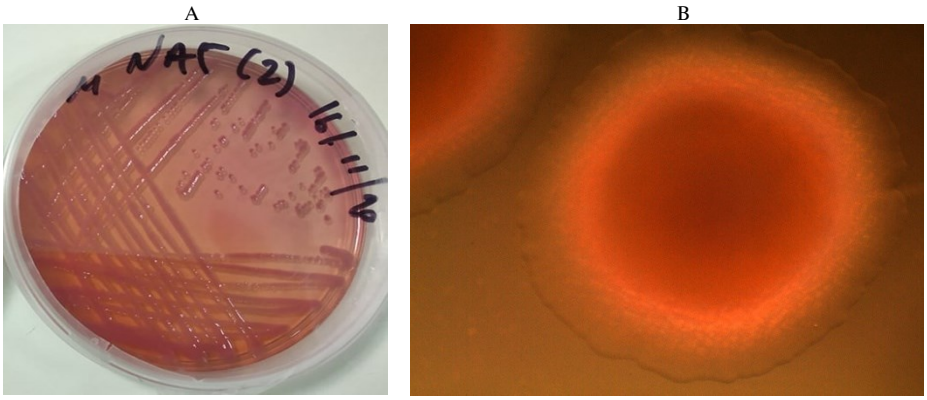

Fig. 2. Colonies of *Serratia liquefaciens* group bacteria isolated from bat guano (Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.) **after incubation on the MacConkey agar at 28 °C for 72 h:** A — lactose fermentation, B — pigmentation (prodigiosin production).

2. Effect of incubation time on prodigiosin production of *Serratia* strains isolated from bat guano (Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.)

Strain	24 h	48 h	72 h
S. proteamaculans	+/–	++	+++
S. liquefaciens	+/–	+	++

N o t e. Bacteria were incubated on the MacConkey agar at 28 °C. The mark +/– stands for very low pigment production, + for an enhanced pigment production; + for low pigment production, ++ for medium pigment production, and +++ for high pigment production.

S. proteamaculans and *S. liquefaciens* isolates exhibited significant antagonism results against *F. oxysporum*, *A. alternata*, *B. cinerea*, *S. sclerotiorum*, and *R. solani*. In details, *Serratia* species (*S. liquefaciens* and *S. proteamaculans*) were found to limit the colony growth of *F. oxysporum*, *A. alternata*, *B. cinerea*, *S. sclerotiorum*, and *R. solani* to a considerable extent (Fig. 3).

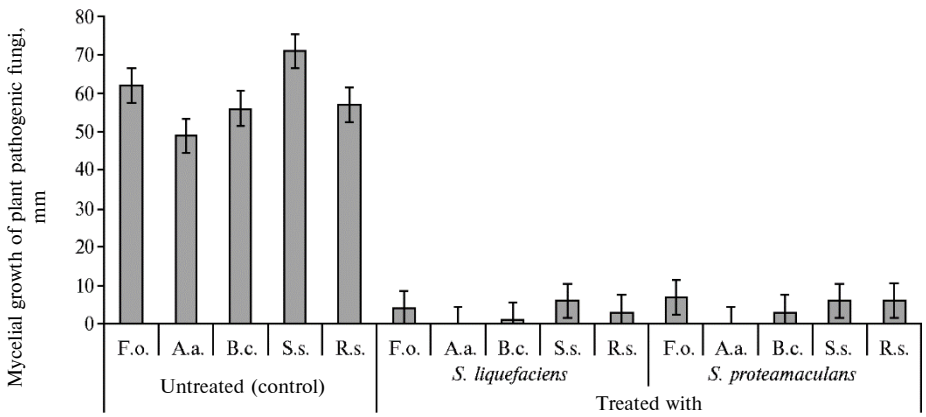

Fig. 3. Mycelial growth (mycelium diameter in mm) **of plant pathogenic fungi *Fusarium oxysporum*** (F.o.)**, *Alternaria alternata*** (A.a)**, *Botrytis cinerea*** (B.c.)**, *Sclerotinia sclerotiorum*** (S.s.)**, and *Rhizoctonia solani*** (R.s.) **treated in dual cultures with *Serratia liquefaciens* or *S. proteamaculans* isolated from bat guano** (Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.) (n = 17, M±SEM; PDA, 25 °C, 5 days).

Both bacterial *Serratia* species (*S. liquefaciens* and *S. proteamaculans*) isolated from bat guano pile showed significant antifungal activity against *A. alternata* in vitro compared to the untreated fungi plates.

In this research, we took samples of bat guano from different places from a subterrestrial Karst aquatic ecosystem which is the nesting place of several bat species. Bacteria that were isolated were grown on Nutrient agar, PDA and MacConkey agar. From these samples we cultured, isolated, and identified bacteria of the *Serratia liquefaciens* group using VITEK® 2 identification system. Using Matrix-Assisted Laser Desorption Ionization—Time of Flight Mass Spectrometry (MALDI-TOF MS) typing we have been able to classify the isolates of *S. liquefaciens* group to *S. liquefaciens* and *S. proteamaculans*. The latest breakthrough in identification of pathogens and determination of susceptibility are the techniques of mass spectrometry. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF MS) mass spectrometry is an easy to use, rapid, reliable, economical, and environmentally friendly methodology that has revolutionized pathogen identification and detection of antimicrobial susceptibility/resistance in clinical settings [36]. On MacConkey agar all strains after 48h of incubation, produced the prodigiosin pigment as described by [37].

The *Serratia* strains isolated and then identified by VITEK® 2 identification system, were classified as *Serratia liquefaciens* group with four phenotypic characteristics difference between them as presented in Table 1. These characteristics (see Table 1) that were positive are the Alpha-Glucosidase, Palatinose, Gamma-Glutamyl, and the Phosphatase biochemical reaction. After the use of MALDI-TOF MS these characteristics distinguish the *S. proteamaculans* strain. *S. liquefaciens* isolate showed negative reactions to these characteristics. MALDI-TOF MS can be used for the identification of species in the *Serratia liquefaciens* group if necessary as it has been applied before with other *Serratia* species nosocomial outbreaks [14, 38]. In our study, we cultured from bat guano similar bacterial species specified as *Serratia liquefaciens* group. *S. liquefaciens* and *S. marcescens* have been described as opportunistic pathogens for many European bat species [4] but so far there has been no conclusive evidence of *S. proteamaculans* presence in either bat guano or as a part of their bacterial microflora. To the best of our knowledge, this is the first report of the presence of *S. proteamaculans* in bat guano.

Ever since its first applications for identification purposes [39, 40], MALDI-TOF MS has been proposed as a promising alternative for the dereplication of recurrent bacterial isolates [41-43] and has been used as a cost- and time-effective alternative to 16S rRNA gene sequencing [44-48]. MALDI-TOF MS-based identification of microorganisms involves the generation of mass spectra from whole-cell material or extracted intracellular content which are then matched to known database references [49, 50].

Newman et al. [51], in their recent research in caves of New Mexico, USA, presented no evidence of isolation nor identification of any *Serratia* species. Their research was based on bacteria isolation in TSA, blood agar, and bat guano medium (BGM) or by PCR amplification of 16s rRNA gene. They examined both fresh and decaying bat guano but did not find any *Serratia* spp. regardless finding different bacterial taxa on fresh and decaying guano [51]. According to our results, our findings show that there are different bacterial strains in bat microbiome and/or in guano between Europe's [4, 52, 53], India's [54, 55], America's [51] continent indigenous bats. Both European and Indian research in local bats guano revealed the presence of *Serratia* spp. but were not present in America's

bat microbiome.

Serratia marcescens B4A, a novel *Serratia* strain, produces potent antifungal compounds and inhibits the growth of insects and plant pathogens such as *R. solani* and *A. raphanin* [56]. *Serratia* spp. also, are responsible for the production of secondary metabolites such as siderophores and phytohormone and shield the plants against pathogenic infections [56]. Some *Serratia* strains produce the halogenated secondary metabolite pyrrolnitrin which is a promising agricultural fungicide [56, 57]. *Serratia* species produce essential compounds and enzymes such as nucleases, chitinases, lipases, proteases, amylases, serralysin, and haemolysin [56]. There are quite a few studies regarding the important role of *Serratia* species as bio-control agents in agricultural crops management including strawberry, cauliflower, and olives. *S. plymuthica* A30 presents exceptional activity against the pathogenic bacteria *Dickeya solani* that cause blackleg and soft rot in potatoes [58-60].

In our study, both *Serratia* strains (*S. liquefaciens* and *S. proteamaculans*) were able to produce the red pigment prodigiosin and display significantly activity against *F. oxysporum*, *A. alternata*, *B. cinerea*, *S. sclerotiorum*, and *R. solani*. Literature shows that prodigiosin compound, that plays a role in biology not clearly clarified, has been produced by many *Serratia* species. Prodigiosin (2-methyl-3-pentyl-6-methoxyprodiginine) is a red-colored heterocyclic secondary metabolite that belongs to the class of tripyrrole compounds [61]. Prodigiosin appears in the later stages of bacterial growth acting as an overflow production of secondary metabolites with broad-spectrum antimicrobial activity [11]. The biosynthesis of prodigiosin is controlled by numerous environmental and physiochemical factors including temperature, oxygen and pH with maximum production yields achieved in the absence of light [61]. Other species within the *Serratia* genus such as *Serratia plymuthica, S. rubidaea and S. nematodiphila* are also capable of producing the non-diffusible red pigment, prodigiosin, during secondary metabolism [10] Prodigiosin has been reported to display antimalarial, antibacterial, antifungal, antiprotozoal, antitumour and immunosuppressant activities [62]. This is the first report that *S. liquefaciens* and *S. proteamaculans* isolated from bat guano are showing clear and distinct antifungal activity.

Overall, our data provide new information about the presence of *Serratia proteamaculans* found in bat guano from a subterrestrial aquatic ecosystem. This study shows that a small number of bacteria cells are able to have a highly competitive ability through the compounds that defuse into the agar against plant pathogenic fungi. Our study also provides novel data that *S. proteamaculans* and *S. liquefaciens* isolates are capable of prodigiosin production and new insights into the relationship between prodigiosin and other secondary metabolites as promising agriculture fungicides.

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