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Bacteria Isolated from Cave Aquatic Environment Exhibit Significant Antifungal Activity Against Phytopathogenic Fungi and Oomycetes

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Abstract - The antifungal activity of *Serratia liquefaciens* group strains were investigated against plant pathogenic fungi (*Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Rhizoctonia solani*) and phytopathegenic oomycetes (*Phytophthora* spp. and *Pythium* spp.) *in vitro*. The bacterium was shown to produce unidentified freely diffusible compounds with fungistatic (fungicidal?) activity *in vitro.*

Key words: Serratia, Subterranean aquatic environment, Secondary metabolites, Biocontrol.

1. INTRODUCTION

The genus *Serratia* belongs to the family Enterobacteriaceae and consists of the 18 recognized species such as: *Serratia marcescens*, *Serratia liquefaciens*, *Serratia ficaria*, *Serratia rubidaea*, *Serratia fonticola*, *Serratia odorifera*, *Serratia plymuthica*, *Serratia grimesii*, *Serratia proteamaculans, Serratia quinivorans*, and *Serratia entomophila* [1] (Liu et al. 2010).

Serratia species are rod shaped opportunistic pathogens responsible for nosocomial and severe infections, Gram-negative bacteria of the c subclass of Proteobacteria and are motile, psychrophilic and facultatively anaerobic ([2] Grimont & Grimont, 2006; [3] M Mai, 2018). All *Serratia* species are ubiquitous; inhibit a variety of different environmental niches such as; water, soil, plants as well as insects and animals with some associated with food spoilage. In laboratory, *Serratia* species grow in many complex growth media, these include LB, PDA and NA at temperatures ranging from 20 °C to 37 °C ([2] Grimont & Grimont, 2006; [3] M Mai, 2018).

Prodigiosin production, a role in biology not clearly understood, has been reported in *Serratia* species. Prodigiosin (2-methyl-3-pentyl-6-methoxyprodiginine) is a red colored heterocyclic secondary metabolite that belongs to the class of tripyrrole compounds [4] (Kimyon et al. 2016). Prodigiosin appears in the later stages of bacterial growth acting as an overflow production of secondary metabolites with broad-spectrum antimicrobial activity [5] (Clements et al. 2019). 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 [4] (Kimyon et al. 2016).

During our studies in Greek cave aquatic enviroment we isolated from bat guano pile ecosystem straight rod structure gram negative bacteria designated as strains SlMC12, SlMC13 and SlMC14 identified as *Serratia liquefaciens* group. Strains SlMC12, SlMC13 and SlMC14 were able to ferment glucose, carbohydrates and Saccharose/Sucrose as a source of carbon and sugar. Bacteria were able to grow on Nutrient agar, PDA and MacConkey agar. On MacConkey agar all strains SlMC12, SlMC13 and SlMC14, after 48h of incubation, produced the Prodigiosin pigment as described by [6] Ramesh Babu et al. 2020.

In this study, we investigated the potential antimicrobial (antifungal) secondary metabolites produced by *Serratia* species in vitro.

2. MATERIAL AND METHODS

2.1. Fungi – bacterial cultures

The fungus microorganisms used in this research *F. oxysporum* f.sp. *lycopersici* and *A. alternata* were isolated from tomato plants with techniques described by [7] Vagelas (2002) and [8] Vagelas et al. 2009. All bacteria strains were isolated from bat guano pile (Figure 1), as described by García-Fraile, et al., 2015. For routine isolation of bacteria, NA (Nutrient Agar)

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and MacConkey agar (MCA, Oxoid) were used. All bacteria strains, SlMC12, SlMC13 and SlMC14 were able to ferment glucose (D-glucose) and other carbohydrates (i.e. D-mannitol, D-mannose) and Saccharose/Sucrose as a source of carbon and sugar and identified as *Serratia liquefaciens* group by the VITEK® 2 system (bioMerieux) using the VITEK 2 GN ID card as described by [9] Saeb, et al. 2016.

Figure 1: Overview of subterranean lake and bat guano pile in Malaki Cave (Central Greece).

2.2. In vitro Antifungal bioassay

Antifungal activity of the isolated strains on growth of the phytopathogenic fungi *F. oxysporum* and *A. alternate* were determined on dual culture media [10] (Agarry et al. 2005). In details, on PDA plate a 40 mm streak was made from 24h culture of bacteria 30 mm away from the centre of the petri dish. A 5mm agar plug from a 5 days old fungal culture were placed at the centre of the plate with the test bacterial strain. Plates were incubated at 22 ^{0}C for 5 days and monitored for zone of inhibition daily. Further, only strain SlMC12 was further tested on dual culture media against other phytopathogenic fungi (*Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Rhizoctonia solani*) and phytopathegenic oomycetes (*Phytophthora* spp. and *Pythium* spp.). In all treatments replicates were 8 folds.

2.3. Statistical analysis

Analyses were performed using the GenStat statistical programme. ANOVA and multiple range tests (Tukey's multiple comparisons) were applied to assess differences between treatments and identify statistical differences between means, respectively.

3. Results

3.1. Bacterial antifungal activity

All bacterial strains (SlMC12, SlMC13 and SlMC14), isolated from bat guano pile showed significant antifungal activity against *F. oxysporum* and *A. alternate* in vitro (Figure 2, 3 respectively). *Serratia liquefaciens* group SlMC12 showed same results against plant pathogenic fungi (*Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Rhizoctonia solani*) and phytopathegenic oomycetes (*Phytophthora* spp. and *Pythium* spp.), (Figure 4).

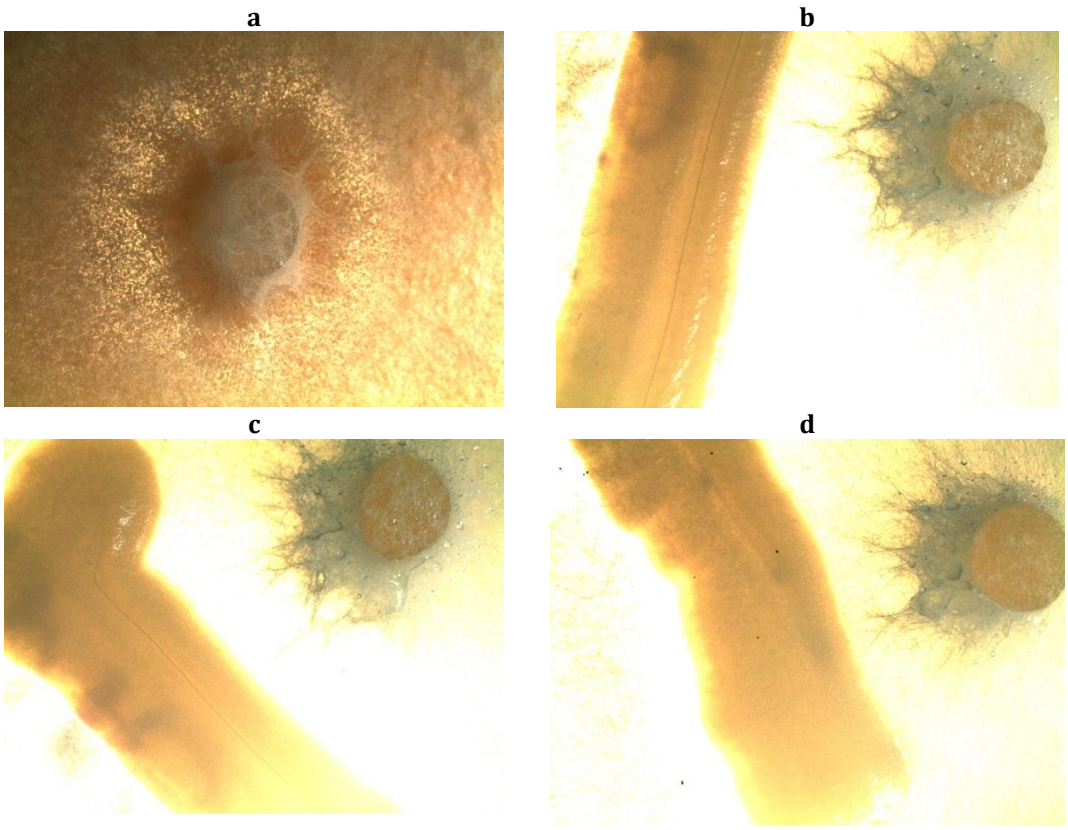

Figure 2: Antifungal activity against *F. oxysporum* of the bacteria isolated from bat guano. (a) *F. oxysporum* control; (b) strain SlMC12; (c) strain SlMC13; (d) strain SlMC14. The fungus (*F. oxysporum*), was inoculated into PDA medium.

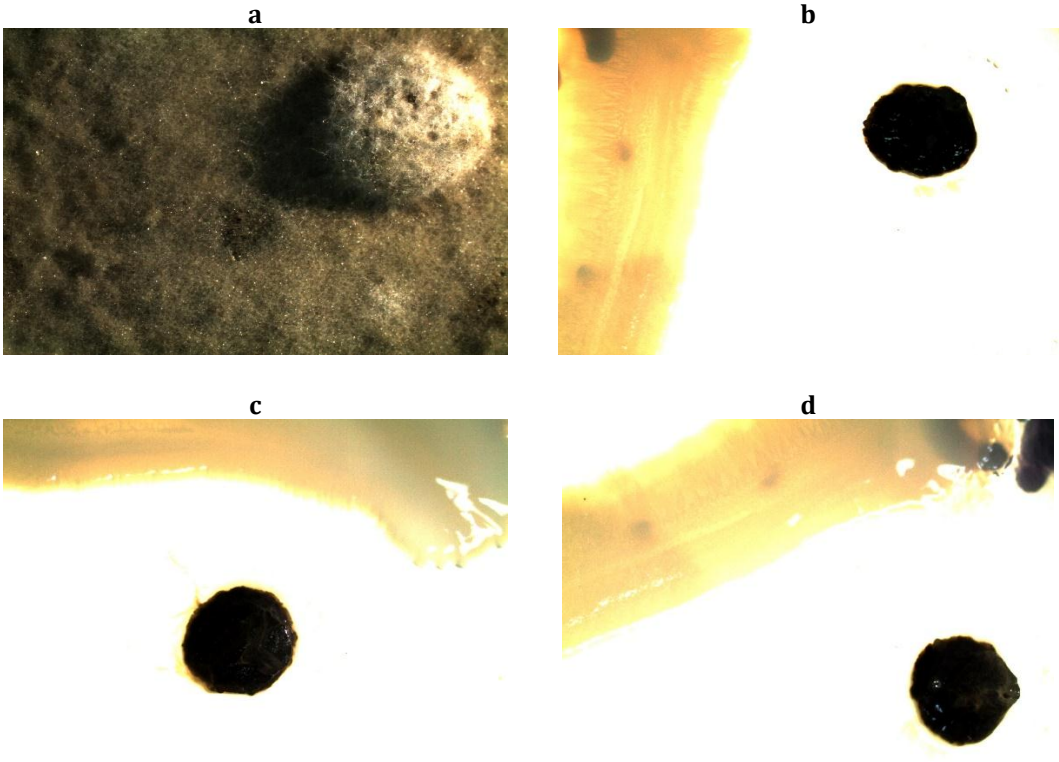

Figure 3: Antifungal (fungicidal?) activity against *A. alternate* of the bacteria isolated from bat guano. (a) *A. alternate* control; (b strain SlMC12; (c) strain SlMC13; (d) strain SlMC14. The fungus (*A.* alternate), was inoculated into PDA medium.

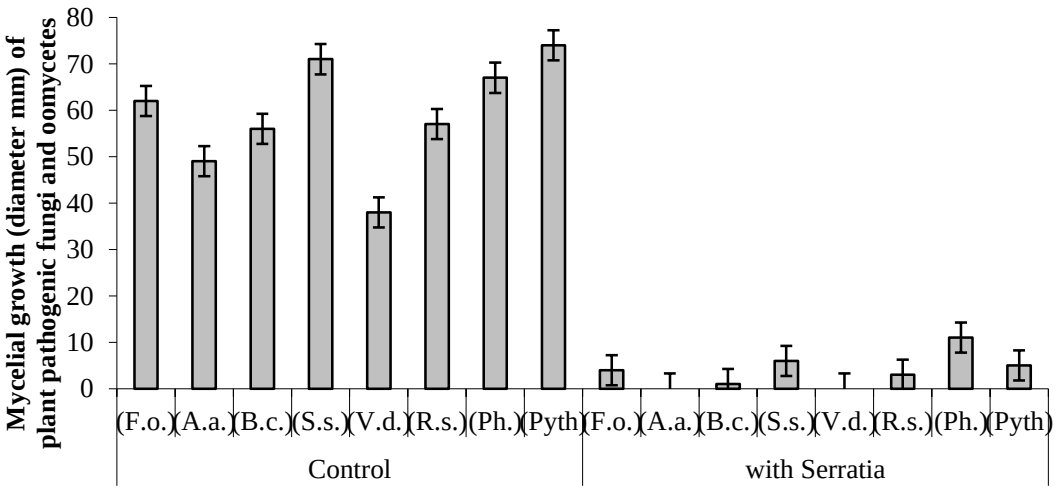

Figure 4: Mycelial growth inhibition of plant pathogenic fungi and oomycetes by *Serratia* strain SlMC12. *Fusarium oxysporum* (F.o.), *Alternaria alternata* (A.a), *Botrytis cinerea* (B.c.), *Sclerotinia sclerotiorum* (S.s.), *Verticillium dahliae* (V.c.), *Rhizoctonia solani* (R.s.), *Phytophthora* spp. (Ph.) and *Pythium* spp.(Pyth).

4. DISCUSSION AND CONCLUSIONS

It is well known that the genus Serratia can be found in many different habitats such as water, soil, plants and animals ([2] Grimont & Grimont, 2006; [11] García-Fraile, et al. 2015). *Serratia* species have been investigated as a bacterial found within bats skin or rectal swab [11] García-Fraile, et al. 2015, but only few studies have explored bacterial diversity within bat guano piles [12] (Newman, et al. 2018).

Serratia species produce commercially important compounds and enzymes such as lipases, serralysin, chitinases, nucleases, protease, haemolysin and amylases [3] (M Mai, 2018).

There are numerous studies regarding the important role of *Serratia* species as bio-control agents in agricultural crops management including strawberry, cauliflower and olives. *S. plymuthica* A30 shows potent activity against the bacterium pathogen *Dickeya solani* that cause blackleg and soft rot in potato ([13] Frankowski, et al. 2001; [14] Czajkowski & Wolf, 2012; [15] Petersen & Tisa, 2013).

The novel strain *Serratia marcescens* B4A produces potent antifungal compounds and inhibit the growth of insects and plant pathogens such as *Rhizoctonia solani* and *Alternaria raphanin* [3] (M Mai, 2018). *Serratia* species, also produce secondary metabolites such as siderophores and phytohormone and protect the plants against pathogenic infections [3] (M Mai, 2018). Some *Serratia* strains produce the halogenated secondary metabolite pyrrolnitrin which is a promising agricultural fungicide ([16] Bhadra, et al. 2005; [3] M Mai, 2018).

Based on the above, our data provides new information about the unique *Serratia* species found in a large bat guano pile. This study shows that a small number of cells are able to have a very high competitive ability through the toxin(s) that defuse into the agar against plant pathogenic fungi and oomycetes. For *Serratia liquefaciens* group these finding provide novel insights into the relationship between prodigiosin and the effects of secondary metabolites and/or the toxin(s) as promising agriculture fungicides. More work needs to be done to identify and quantify the unknown metabolite (s) and/or the toxin(s) of *Serratia liquefaciens* group strains SlMC12, SlMC13 and SlMC14.

CONFLICT OF INTEREST

The authors have no conflict of interest in preparing of this article.

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