SIDEROPHORES TO INCREASE IRON AVAILABILITY

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ABSTRACT

Siderophores are biologically produced low molecular weight amino acids that act to chelate metals. These can be generated by soil microorganisms and some plants, most notably grasses, also produce siderophores. These compounds are associated with improved availability of iron in the soil. This manuscript presents a survey of microbial siderophores present in soils under chlorotic and non-chlorotic 'Concord' grapevines.

INTRODUCTION

Leaf yellowing, or chlorosis, occurs on in many crop plants. The yellowing of the leaves resembles classic Fe-deficiency induced chlorosis, and Fe deficiency in orchard and vineyard systems is well documented in calcareous soils around the world (see reviews by Tagliavini and Rombolá 2001, and Marschner et al. 2011). In grapes, the initial symptoms of chlorosis develop around bloom, after which time chlorotic leaves turn brown or burned and vine size, vine uniformity and productivity are reduced and can lead to vine death (Davenport and Stevens, 2006).

Many studies have attempted to determine the precise cause of this type of chlorosis in the hopes of developing effective management strategies (Korcak, 1987) and the application of synthetic iron chelates has been employed in many vineyards (Tagliavini and Rombolá, 2001). However, application of Fe-chelates is only a temporary solution, requiring re-application each year, and therefore does not represent a sustainable nor cost effective cure to iron chlorosis (Smith and Cheng, 2006). While links have been drawn between high soil pH, calcium carbonate levels, as well as high soil moisture and low soil temperature all work, to date, supports the concept that some factor beyond these is involved in grape chlorosis in areas of similar soils in Washington state.

Although Fe is relatively abundant in soils, Fe acquisition is limited by solubility because the dominant forms of Fe in soils are poorly soluble and consequently unavailable biologically (Marschner, 2003). Plants and microorganisms have developed similar mechanisms to address Fe deficiency, mobilization, and uptake of Fe in the environment and there is intensive competition for uptake (Marschner et al., 2011).

Strategy I plants, the group which perennial fruit trees and grapes belong, increase Fe availability by releasing protons that lower the soil pH and complex Fe, holding it in a soluble form that can diffuse to the root surface. However, this mechanism alone does not appear sufficient to alleviate iron chlorosis in these plants when grown in arid and semi-arid environments.

Siderophores are low molecular-weight, amino acid derivatives which chelate Fe, allowing uptake and storage.

Soil microbes produce a wide variety of different types of siderophores (Neilands, 1984b) which contain primarily hydroxamate, catecholate, or carboxylate functional groups (e.g., Miller

and Malouin, 1993) each with a different affinity for Fe and capacity to enhance Fe bioavailability. The production of microbial siderophores in the rhizosphere can result in high localized, plant-available concentrations of siderophores (Marschner et al., 2011), but their contribution to plant iron nutrition will vary greatly depending on the exact composition of the microbial community (Sullivan et al., 2013) and their associated classes of secreted siderophores. Soil microbial production of siderophores has been found to increase Fe solubility and improve plant Fe acquisition as a result, for many different plant species.

Many graminaceous plants (strategy II), along with microorganisms, release siderophores into soil to chelate the Fe. There are a number of plants that that release these phytosiderophores, most of which are grass plants including barley and wheat (Oburger et al., 2014).

However, virtually nothing is known about the soil microbial community composition of the 'Concord' grape (*Vitis labruscana* Bailey) vines of Washington or their capacity to produce siderophores. We propose to embark upon this critical knowledge gap and hypothesize those differences in the soil microbial community composition and siderophore production may be tied to chlorosis status of 'Concord' grape. The objective of this study is to compare the structure and function of rooting-zone soil microbial communities associated with chlorotic and healthy Concord grape vines in south-central Washington. We intend to identify rooting-zone microbial communities that may be beneficial to grape Fe nutrition and vine growth characteristics. In the long-term, we hope to provide a more sustainable and cost effective alternative to the application of synthetic Fe chelates.

METHODS

This project was conducted in an established (ca. 20 year old) 'Concord vineyard near Prosser, Washington (46.29 N, 119.78 W) on a Warden silt loam soil (coarse-silty, mixed, superactive, mesic Xeric Haplocambid). 'Concord' grape vines were identified as "Chlorotic" or "Healthy" based on visual assessment of the yellowing and chlorosis or necrosis of the leaves. A "site" was operationally defined as a location within the vineyard where sets of three healthy and three chlorotic vines were within 10 yards of each other. Five sites were identified for root zone sampling. In mid-June 2014, prior to bloom, three replicate soil cores were collected from within 30 cm of the base of each vine. Cores were composited by vine and by depth (0-30 cm and 30-75 cm). Total cultivable community was determined by plate-counts on potato dextrose agar (PDA) and malt extract agar (MEA) (Neilands 1984 a, b). Morphologically distinct colonies were transferred to pH-indicative media (methyl orange) to determine extent of media acidification. Acid producer organisms were then tested for siderophore production by O-CAS (overlay of chrome-azurol S. Genomic DNA was extracted from fungal and bacterial isolates using Mobio PowerSoil DNA Isolation Kit and Mobio Microbial Isolation Kit, respectively. Bacterial 16s and fungal 18s rRNA gene sequences were obtained for those organisms testing positive in both acid and siderophore production from the WSU Genomics Core facility and analyzed against the NCBI database using BLASTN.

RESULTS

Bacterial abundance, as measured by plate counts, was 19% greater at chlorotic sites than healthy sites, while fungal abundance was not significantly different between the soils of chlorotic and healthy vines. Phyla of bacterial isolates were dominated by *Pseudomonas* genus, many species of which are known plant-growth promoters and prolific siderophore producers. *P. poae* is a fluorescent Gram-negative bacteria associated with the phyllosphere of grasses, while the

diverse metabolism of *P. putida* has been useful in biocontrol of plant pathogens and also remediation of soil contaminants. *Burkholderia fungorum* is a formidable degrader polychlorinated biphenyls (PCBs), and is thereby useful in soil bioremediation. *Aureobasidium pullans* adapts to highly stressful conditions via enzyme, siderophore, and pullan secretion and is notable for its biological control applications. Frequent isolation of the common apple (*Malus domestica* Borkh.) rot fungus, *Penicillium expansum*, may have originated from apple orchards bordering the 'Concord' vineyard.

Chlorotic Vines: Siderophore and Acid Producing Isolates

Isolate ID	Closest BLAST Match	Identity %	Accession Number	Depth
1	*Pseudomonas fluorescens* strain HC1-07	99%	JQ664548.1	S
2	*Pseudomonas fluorescens* strain SWFU21	99%	KJ756336.1	S / SS
3	*Pseudomonas moorei* strain OR108	99%	KF424282.1	S
4	*Pseudomonas* sp. IK-S1	98%	KC012939.1	SS
5	*Pseudomonas* sp. JDG23	99%	JX035946.1	S
6	*Pseudomonas* sp. P-J-1	100%	KC991304.1	S / SS
7	*Pseudomonas* sp. XBBSY4	99%	KJ184927.1	S
8	*Aspergillus niger* strain A-3204	99%	JQ316522.1	S
9	*Aspergillus* sp. AHBR2	99%	KF305741.1	S
10	*Aureobasidium pullulans* strain R124	99%	HG532077.1	S
11	*Penicillium expansum* strain P63	94%	KJ933295.1	S
12	*Penicillium* sp. F731	99%	KM249071.1	S / SS

Healthy Vines: Siderophore and Acid Producing Isolates				
Isolate ID	**Closest BLAST Match**	**Identity %**	**Accession Number**	**Depth**
100	Bacterium 28S434	98%	KC734127.1	S
101	*Burkholderia fungorum* strain BH113	99%	JQ765430.1	SS
102	*Burkholderia* sp. AaM271	99%	JQ314018.1	SS
103	*Pseudomonas fluorescens* strain HC1-07	99%	JQ664548.1	SS
104	*Pseudomonas fluorescens* strain hp13	99%	JN637318.1	S
105	*Pseudomonas fluorescens* strain SWFU21	99%	KJ756336.1	S
106	*Pseudomonas* sp. JDG23	99%	JX035946.1	S / SS
107	*Pseudomonas* sp. JSPB3	98%	JQ308616.1	S
108	*Pseudomonas* sp. PON2A	99%	FN397664.1	SS
109	*Pseudomonas syringae* strain cc 1450	96%	DQ295008.1	S
110	*Aspergillus niger* strain A-3204	99%	JQ316522.1	S
111	*Aspergillus niger* strain ATCC 16888	99%	AY373852.1	S
112	*Eucasphaeria capensis* isolate OTU550	99%	GU934520.1	S
113	*Penicillium* sp. F731	99%	KM249071.1	S / SS

*S indicates an organism cultivated from the surface soils (0-30 cm).
**SS indicates an organism cultivated from the sub-surface soils (30-60cm).

CONCLUSIONS

The results from this limited trial suggest that microbial siderophores production is not limited in the soils associated with chlorotic 'Concord' grapes when compared to non-chlorotic vines. However, the vines in the study were more of a continuum from non-chlorotic to several chlorotic, suggesting that additional research on the native soil microbial populations plus studies including between row cover crops may impact the incidence of chlorosis in 'Concord' vineyards.

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