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Characterization of bacteria isolated from maize roots: Emphasis on *Serratia* and infestation with corn rootworms (Chrysomelidae: *Diabrotica*)

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ABSTRACT

Larval corn rootworms (Coleoptera: Chrysomelidae) are soil-dwelling insect pests that damage maize (*Zea mays* L.) by consuming root tissues, thus lowering grain yield. Little is known about interactions between rootworms and soil bacteria, including potential impacts of maize rhizobacteria, such as entomopathogenic *Serratia* spp., on subterranean rootworm pests. We used selective growth medium (caprylate-thallous agar, CT) to quantify and isolate *Serratia* spp. from: (1) bulk soil, (2) roots of four field-grown maize genotypes, half of which were infested with rootworm eggs from a reared colony, and (3) non-diseased, larval rootworms from the same colony. Phenotypic testing and 16S rRNA gene sequencing were used to identify bacteria, including non-*Serratia* spp., that were successfully isolated on the CT solid medium. We also isolated and identified *Serratia* spp. associated with non-diseased and diseased *Diabrotica* adults. *Serratia* spp. associated with maize roots were more abundant than those associated with bulk soil, where they were undetectable with our methods except for *Serratia grimesii*. There was no impact of plant genotype on densities of bacteria isolated from maize roots. *S. grimesii* was frequently associated with maize roots, regardless of infestation with rootworm eggs. *Serratia marcescens* biotype A4, *Serratia plymuthica* and several other Enterobacteriaceae genera were also associated with maize roots. Infesting the soil with rootworm eggs enhance the occurrence of two strains of the *S. marcescens* biotype A1b, with comparable densities of these orange and pink strains within infested roots. However, both strains were associated with larval rootworms from the reared colony, which may indicate that rootworms were introducing these bacteria into infested maize roots. In addition, within larvae the orange strain was significantly more abundant than the pink strain, and was also associated with diseased rootworm adults. Our studies identified specific *Serratia* strains associated with diseased rootworms that may have potential as biological control agents, and additional *Serratia* biotypes associated with the maize rhizosphere that, based on the literature, may function as plant growth promoting agents via antagonistic action against plant-pathogenic fungi.

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1. Introduction

Corn rootworms (Coleoptera: Chrysomelidae) are devastating pests of maize (*Zea mays* L.). Although adult rootworms feed on developing ears and corn silks, the majority of plant damage is caused by subterranean larvae that consume maize root tissues, thus negatively impacting plant physiology and function (Riedell, 1990; Riedell and Reese, 1999). Ultimately, larval rootworm feeding reduces yield (Sutter et al., 1990; Spike and Tollefson, 1991), and can cause significant economic losses, which have been estimated at one billion U.S. dollars each year (Metcalf, 1986).

Interactions between plants, pests, and predators have been well studied for aboveground systems, but relatively little is known about subterranean ecological webs and plant-mediated protection against herbivores such as larval rootworms (Van der Putten et al., 2001). The role of rhizobacteria in plant protection against pathogenic bacteria and fungi (Elad et al., 1987; Press et al., 1997, 2001; Berg et al., 2000), plant-parasitic nematodes (Racke and Sikora, 1992; Siddiqui and Mahmood, 2001; Sturz and Kimpinski, 2004), and foliar-feeding arthropods (Zehnder et al., 2001) has received considerable attention, but information on interactions between rhizobacteria and rhizophagous insects is limited.

Maize roots have a diverse rhizobacterial community (Miller et al., 1989; Chelius and Triplett, 2001; Gomes et al., 2001; Brusetti et al., 2004) that includes several genera of Enterobacteriaceae, such as *Serratia* (Lalande et al., 1989; Lambert et al., 1987; McInroy and Kloepper, 1995). Microbes in this widespread genus are found within numerous habitats, such as soil, water, plants, and animals (Ashelford et al., 2002; Grimont and Grimont, 2006), and can have extremely different ecological functions, including plant pathogens (Bruton et al., 2003; Rascoe et al., 2003), biocontrol agents of plant pathogens (Ordentlich et al., 1988; Lottmann et al., 2000; Someya et al., 2001, 2005; Berg et al., 2006), plant growth promoters (Lalande et al., 1989; Hameeda et al., 2007), and insect symbionts (Iverson et al., 1984; Graber and Breznak, 2005; Moran et al., 2005; Lundgren et al., 2007). However, many *Serratia* strains are opportunistic pathogens of vertebrates and invertebrates (Grimont and Grimont, 2006), and are often associated with diseased and seemingly healthy insects (Lysenko, 1959; Steinhaus, 1959; Jackson et al., 2001).

Red-colored *Serratia* biotypes are associated with insects more often than non-pigmented biotypes (Grimont et al., 1979), while the former are rarely isolated from plants (Grimont et al., 1981). Although *Serratia* is highly toxic if it contaminates the hemocoel (Steinhaus, 1959; Grimont and Grimont, 2006), many strains also cause mortality when ingested (Steinhaus, 1959; Lysyk et al., 2002), or have sublethal effects such as reduced adult longevity and decreased oviposition and egg hatch (Sikorowski and Lawrence, 1998; Inglis and Lawrence, 2001; Sikorowski et al., 2001). *Serratia* can be transmitted vertically between one generation and the next, as well as horizontally between individuals within a generation (Sikorowski and Lawrence, 1998; Sikorowski et al., 2001). While records of mortality due to *Serratia* in natural insect populations are uncommon (Steinhaus, 1959), specific strains of *S. entomophila* and *S. proteamaculans* can kill grass grubs [*Costelytra zealandica* (White)], which are subterranean insect pests that damage plant roots (Jackson et al., 1993, 2001). Commercial pesticide formulations using *S. entomophila* have been developed for grass grub control (O'Callaghan and Gerard, 2005). However, there is little information about the extent and impact of sublethal *Serratia* infections in nature.

With regard to the maize rhizosphere, direct and indirect interactions between *Serratia*, host plants, and rootworms are poorly understood, and depending on bacterial strain identity, *Serratia* could be detrimental or beneficial to the plant. A strain of *Serratia marcescens* that causes cucurbit yellow vine disease (Rascoe et al., 2003) is vectored by the squash bug *Anasa tristis* (DeGeer) (Bruton et al., 2003). Rootworms can also vector plant pathogens (Palmer and Kommedahl, 1969; Gilbertson et al., 1986; Zehnder et al., 2001) and transfer rhizobacteria between plants (Snyder et al., 1998). Other rhizobacterial *Serratia* strains protect cucumber plants from bacterial wilt disease because the foliage of inoculated plants has lower densities of adult rootworms, which vector the disease (Zehnder et al., 2001).

Because some *Serratia* strains are insect pathogens (Steinhaus, 1959; Grimont and Grimont, 2006) that have been isolated from rootworms (Schalk et al., 1987; Tran and Marrone, 1988; Lance, 1992), and rootworms can acquire rhizobacteria when they feed on maize roots (Tran and Marrone, 1988), *Serratia* strains that colonize the maize rhizosphere may potentially function in defense against rootworms. In addition, it is possible that maize plants attacked by larval rootworms could actively defend themselves by selectively encouraging growth of entomopathogenic *Serratia* strains, because rhizobacterial communities are affected by root exudates (Richards, 1987; Walker et al., 2003; Brusetti et al., 2004; Bais et al., 2006), some of which are released in response to root damage (Denton et al., 1999; Grayston et al., 2001; Treonis et al., 2005; Ayres et al., 2007). Furthermore, the plant cultivar can influence root bacteria of some plant species (Miller et al., 1989; Germida and Siciliano, 2001), and thus maize genotype could potentially impact the resident rhizobacteria, and in turn affect host plant resistance to rootworms.

In order to elucidate interactions between maize roots, *Serratia* spp. rhizobacteria, and rootworms, and assess the pest biocontrol and/or plant growth promotion potential of associated microbes, the relevant bacteria must first be characterized. Our objectives were to: (1) quantify, isolate, and identify *Serratia* spp. associated with bulk field soil, maize roots, and larval western corn rootworms, *Diabrotica virgifera virgifera* LeConte, (2) determine if maize genotype and infesting soil with the eggs of *D. v. virgifera* impacts the presence or density of *Serratia* species and biotypes associated with bulk soil and maize roots, and (3) identify *Serratia* spp. associated with non-diseased and diseased adult rootworms. Our hypotheses were that: (1) a greater diversity of *Serratia* species and higher bacterial densities of these species would be associated with maize roots versus bulk soil, (2) plant genotype would influence the *Serratia* flora of maize roots, and (3) infestation with rootworm eggs would have a significant impact on the maize rhizobacterial flora.