Serratia marcescens isolated from red fire ants, leaf-cutter ants, and humans: their dose effect against fire ants.

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Fire ants (RIFA) native from Argentina are a worldwide distributed pest inflicting great damage to the US biodiversity and economy. Chemical control has been inefficient and contaminant. Only parasitoids from Argentina have been released in the US and, although established, their effect on ants cannot be assessed yet. Other natural enemies should be released to act synergistically with parasitoids. We report on the effect of *Serratia marcescens* isolates on RIFA mortality. This enterobacterium from water effluents, previously claimed to be completely safe for humans and animals, has been patented by others and shown to kill RIFA mounds after the third week using a bacterial concentration from 10^9 -10^{13}. We tested the specificity of several strains against *Solenopsis invicta* from Argentina. We isolated the bacterium from an *Atta vollenweideri* queen (LCA) and RIFA workers; we also used the RM66262 clinical isolate from a patient with urinary tract infection (CC). Strains were biotyped as Serratia with biochemical tests and *Serratia marcescens* by using specific 16S rRNA primers. Bacteria were cultured in YMEA and resuspended in water to count four concentration treatments: 10^5, 10^6, 10^7, and 10^8. Worker ants were sterilized and after 1-2 days inoculated and kept in Petri dishes with sugar water. All strains tested were capable of killing RIFA, with the LCA strain being the fastest and the RIFA strain the slowest. The LCA strain showed a decrease in its virulence through time. Since the strains tested were effective in killing RIFA, like the effluent strain, infection could highly likely occur the other way around. Therefore, we recommend testing any *S. marcescens* strain against a wide range of organisms before releasing it into the environment for RIFA biological control.

Introduction

Fire ants (RIFA) are native to Argentina and have spread as exotic species throughout the world. The US has changed its main strategy of fire ants control after realizing that chemicals had worsened the problem. Natural enemies of ants have only recently been investigated, probably due to the intrinsic problems that the social life history of ants poses which has delayed the development of biological control methods, especially using microrganisms.

Since we have been finding repeatedly the presence of a fuchsia bacterium in RIFA from Argentina, we decided to evaluate its performance against these ants by inoculating pure isolated bacteria that we identified as *Serratia marscesens* through biochemical and molecular biology assays.

S. marcescens have been frequently isolated from insects (Grimont et al. 1979) especially crickets, and less commonly from diptera (Mediterranean fruit flies, tse tse, and blowflies), and different isolates have shown varying virulence and specificity (O'Callaghan et al 1996). *S. marscecens* has been reported from the gut of fourth instar larvae of RIFAs, however no evaluation of mortality has been conducted (Jouvenaz et al.1996, Li et al. 2005). *S. marscecens* and other enterobacteria (isolated from effluents) have been registered to use against RIFA colonies (US2002/0064545A1). However, there are no published investigations or commercial products in the market associated to this patent.

S. marcescens is considered a facultative pathogen that has been found associated to human hospital environments (Grimont and Grimont 1978a). Therefore, before further studies or applications are proposed, a thorough evaluation of Serratia biotypes performance and specificity should be carried out in order to select appropriate and useful strains (O'Callaghan et al 1996, Li et al. 2005).

GOAL: Test the specificity and virulence of three strains of *S. marcescens* against RIFA

Results

We did not find differences among the three colonies used for LCA and RIFA strains (all cases $p > 0.016$) but we found statistical differences for CC.

We observed an effect of concentration. In all strains inoculation with the highest concentration (10^8) always showed statistical differences with the lowest one (10^5). The intermediate concentrations (10^6 y 10^7) did not show statistical differences
($p > 0,008$) (Fig.1).

We found statistical differences between strains for all concentrations ranking in the following order LCA > CC > RIFA (Fig. 2). This data is correlated with percentage mortality observed (data not shown).

The LCA strain exhibited a reduction in virulence as time proceeded, and more subcultures were done. On the first experimental set the LT_{50} was lower that on the second one (3 month later) for all concentrations tested (Table 1).

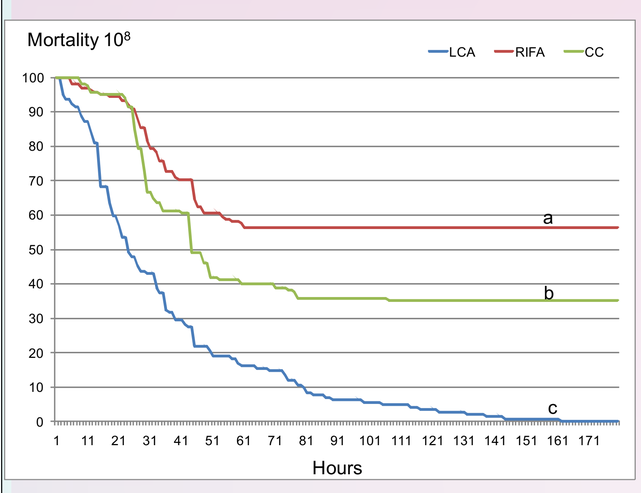
Conclusions

The tree strains were able to kill RIFA, indicating a lack of specificity, which violates one of the fundamental requisites of a biological control program.

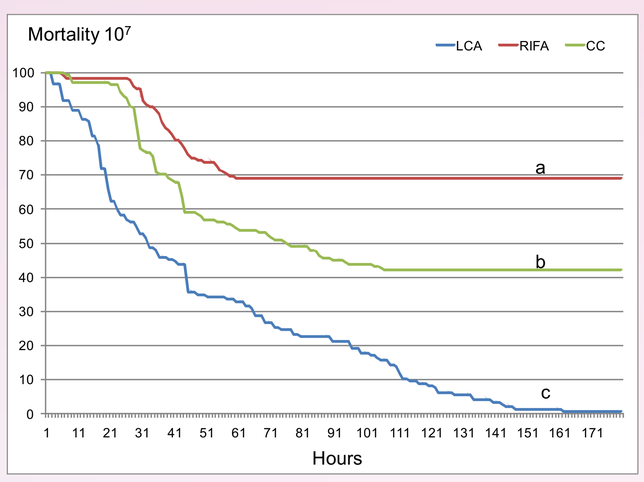
The LCA strain overall had the greatest effect by killing all RIFA in a shorter period of time. As expected, the RIFA strain produced the least mortality as the RIFA have probably been exposed to the strain and thus their immune system should protect them to some extent. In contrast, the LCA strain produced the greatest response. This may be due to the fact that the LCA strain is obtained from another ant, whereas the CC strain is a clinical strain.

The reduction in virulence through time suggests the importance of storing the bacterium in the freezer and always testing its effect before using it.

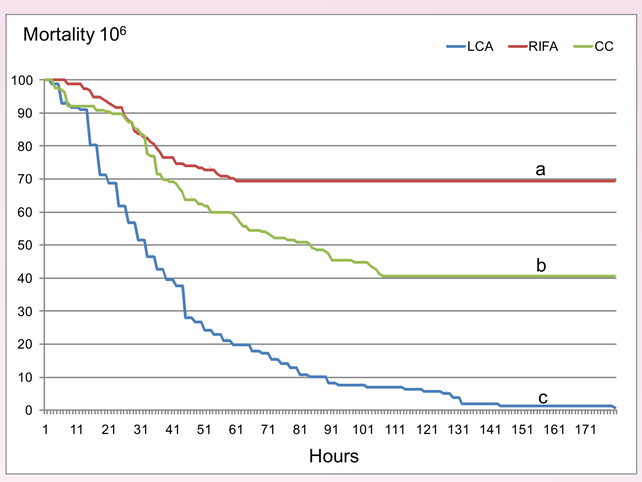
Our results highlight the negative effects that different isolates of *S. marcescens* have on non-target organisms and call into question its use in the biological control of RIFA.



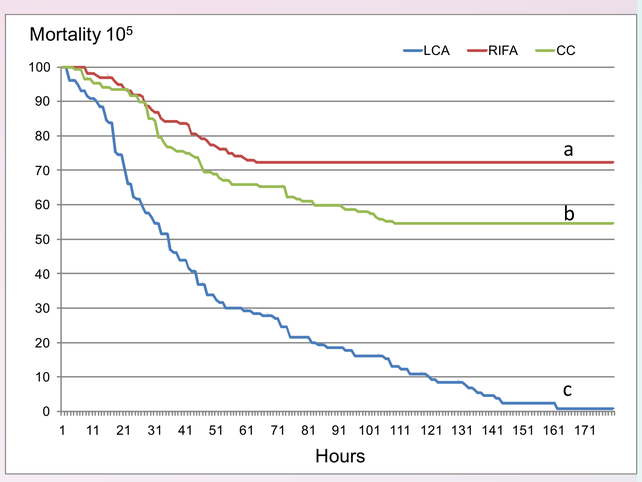
	LCA	RIFA	CC
Q1	45	-	-
Median	24	-	44
Q3	15	36	29



	LCA	RIFA	CC
Q1	72	-	-
Median	33	-	61
Q3	18	46	33

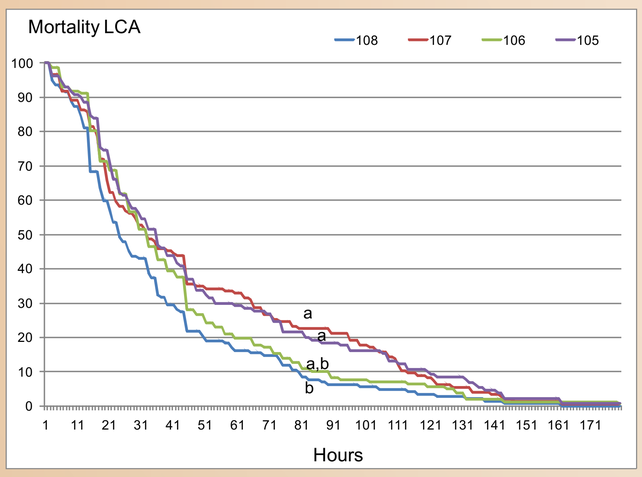


	LCA	RIFA	CC
Q1	51	-	180
Median	33	-	85
Q3	18	42	36

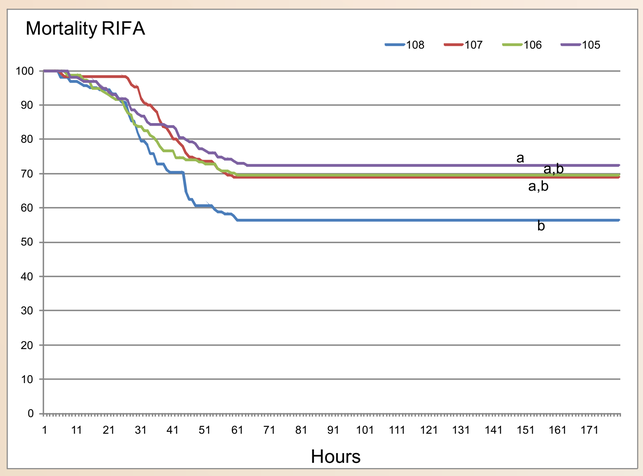


	LCA	RIFA	CC
Q1	72	-	-
Median	36	-	-
Q3	19	55	41

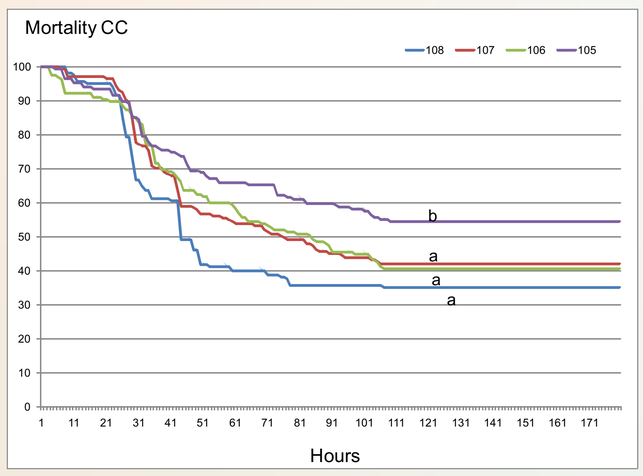
Fig 1. Survival of ants treated with all strains (LCA, RIFA and CC) at the same concentration. showing strain effect. For all concentrations we founded statistical differences (10^8 p = 0,000, chi 2 = 152,293; 107 p = 0,000, chi 2 =178,065; 10^6 p = 0,000, chi 2 = 207,568; 10^5 p = 0,000, chi 2 = 199,481) Tables show Median and Quartiles (Q1 and Q3)



	108	107	106	105
Q1	45	72	51	72
Median	24	33	33	36
Q3	15	18	18	19



	108	107	106	105
Q1	-	-	-	-
Median	-	-	-	-
Q3	36	46	42	55



	108	107	106	105
Q1	-	-	180	-
Median	44	61	85	-
Q3	29	33	36	41

Lt_{50} LCA treatment

	108	107	106	105
IS541	22	24,5	24	28
IS571	24	36	36	42
IS572	33	40	33	34,5

Table 1: Time mortality response (LT_{50}) values for each colony treated with LCA at all concentrations, showing the reduction in virulence. Both IS 571 and IS 572 were tested 3 months later than IS 541.

Fig 2: Survival of ants treated with all concentration (10^8, 10^7, 10^6 and 10^5) for each strain (LCA, RIFA and CC) showing concentration effect. For all strain we founded statistical differences (LCA p = 0,004, chi 2 = 13,085; RIFA p = 0,008, chi 2 =11,772; CC p = 0,000, chi 2 = 27,932). Tables show Median and Quartiles (Q1 and Q3)

Methods

We isolated the bacterium from an *Atta vollenweideri* (LCA) queen and RIFA workers; we also used the RM66262 clinical isolate from a hospital-patient with urinary tract infection (CC). RIFA were collected in Santiago del Estero, Argentina, and sterilized by dripping them for 1min in a 1.2% sodium hipochlorite solution. Two days later, the ants were divided into groups of 50 and each group inoculated by immersing the ants in one of 4 solutions (10^5, 10^6, 10^7, 10^8 bacteria/ml.). Ants were maintained in subgroups of ten in petri dishes with 20% water-sugar solution at 25°C for 10 days. Dead ants were recorded every 3 hours. Three colonies were used for each bacterium strain. In the case of the LCA strain we ran the same experiment at two different times at intervals of 3 months, when the bacterium was kept alive at room temperature doing successive subcultures on YMEA. We used non-parametric Kaplan-Meier survivorship tests with a posteriori contrast based on log rank Mantel Tests corrected by Bonferroni (SYSTAT 2010). We also calculated the percentage of ants dead at the end of each experiment.