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Rhizobial galactoglucan determines the predatory pattern of *Myxococcus xanthus* and protects *Sinorhizobium meliloti* from predation

Juana Pérez¹, José I. Jiménez-Zurdo², Francisco Martínez-Abarca², Vicenta Millán², Lawrence J. Shimkets³, and José Muñoz-Dorado^{‡,1}

¹Departamento de Microbiología, Facultad de Ciencias, Universidad de Granada, Avda. Fuentenueva s/n E-18071, Granada, Spain

²Grupo de Ecología Genética de la Rizosfera. Estación Experimental del Zaidín. Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain

³Department of Microbiology, University of Georgia, Athens, Georgia, 30602, USA

Summary

Myxococcus xanthus is a social bacterium that preys on prokaryotic and eukaryotic microorganisms. Co-culture of *M. xanthus* with reference laboratory strains and field isolates of the legume symbiont *Sinorhizobium meliloti* revealed two different predatory patterns that resemble frontal and wolfpack attacks. Use of mutants impaired in the two types of *M. xanthus* surface motility (A or adventurous and S or social motility) and a *csgA* mutant, which is unable to form macroscopic travelling waves known as ripples, has demonstrated that both motility systems but not rippling are required for efficient predation. To avoid frontal attack and reduce killing rates, rhizobial cells require a functional *expR* gene. ExpR regulates expression of genes involved in a variety of functions. The use of *S. meliloti* mutants impaired in several of these functions revealed that the exopolysaccharide galactoglucan (EPS II) is the major determinant of the *M. xanthus* predatory pattern. The data also suggest that this biopolymer confers an ecological advantage to rhizobial survival in soil, which may have broad environmental implications.

Introduction

Planet sustainability depends on a high number of factors and, among others, on diverse inter kingdom interactions in the soil environment. One such interaction, the root-nodule endosymbiosis established between certain nitrogen-fixing α - and β -proteobacteria (collectively referred to as rhizobia) with leguminous plants, contributes the largest annual input of combined nitrogen into terrestrial ecosystems (Newton, 2000). Successful symbiotic associations depend on the capacity of rhizobia to survive in soil and establish competitive populations in the plant rhizosphere (Toro, 1996; Triplett and Sadowsky, 1992). The composition, structure and dynamics of rhizobial communities are shaped by diverse abiotic

[‡]Corresponding author: Tel: +34958243183. Fax: +34958249486. jdorado@ugr.es.

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(e.g. drought, salt or acidic stresses) and biotic (e.g. compatibility with the legume host and competition with other soil organisms) variables (Zahran, 1999; Rangin *et al.*, 2008; Lindström *et al.*, 2010). However, the mechanisms used by rhizobia to sense, transduce and respond to most of these environmental cues are poorly understood.

Sinorhizobium meliloti, the symbiotic partner of agronomic forage legumes of the genera *Medicago*, *Melilotus* and *Trigonella*, is a genetically tractable model species for investigating rhizobial biology (Jones *et al.*, 2007). Many of the known adaptive responses of *S. meliloti* to soil and plant stimuli are governed by the density-dependent ExpR/Sin quorum sensing system. Two transcriptional regulators (i.e. ExpR and SinR) and a synthase (SinI) of several *N*-acyl-L-homoserine lactone autoinducers (AHLs) activate the system (Marketon *et al.*, 2002; Pellock *et al.*, 2002; Gao *et al.*, 2005). Upon activation by AHLs, the LuxR homolog ExpR regulates expression of a large array of genes involved in free-living and symbiotic functions such as transport of metal ions and small molecules, motility, chemotaxis, nitrogen fixation, and exopolysaccharide (EPS) biosynthesis (Marketon *et al.*, 2003; Hoang *et al.*, 2004; Hoang *et al.*, 2008; Gurich and González, 2009).

S. meliloti produces two types of EPS; succinoglycan (EPS I) and galactoglucan (EPS II). ExpR is required for EPS II biosynthesis and fine-tunes EPS I levels. The low molecular weight fractions of these polymers have an interchangeable symbiotic function, being required in picomolar concentrations for successful colonization of *Medicago* nodules (González *et al.*, 1996a,b). These results suggest that EPSs act as a signaling molecule during symbiosis, perhaps by modulating plant defense responses triggered by the invading bacteria. As an abundant cell-surface layer, EPSs are also expected to confer much broader ecological advantages to rhizobia such as facilitating bacterial attachment to surfaces, improving nutrient acquisition, and providing protection from abiotic stresses (e.g. oxidative stress) (Lehman and Long, 2013).

Parasitism and predation are expected to regulate microbial diversity in natural populations (Chesson, 2000). However, their impact on rhizobial communities has remained largely unexplored. Some rhizobiophages as well as antimicrobial peptides produced by *Rhizobium etli* alter the ecological balance of competing rhizobial strains in soil (Barnet, 1980; Hashem and Angle, 1988; Robleto *et al.*, 1998). *R. vitis* and *S. fredii* strains are sensitive to predation by the ubiquitous, soil-dwelling, δ -proteobacterium *Myxococcus xanthus* (Morgan *et al.*, 2010; Mendes-Soares and Velicer, 2013). Myxobacteria constitute a phylogenetic order of microorganisms characterized by predatory behavior on a wide range of microbial species (Dawid, 2000; Bull *et al.*, 2002; Pham *et al.*, 2005; Berleman *et al.*, 2006; Morgan *et al.*, 2010; Pérez *et al.*, 2011), which lyse prey cells by a mixture of bioactive secondary metabolites and exoenzymes (Goldman *et al.*, 2006; Berleman and Kirby, 2009; Xiao *et al.*, 2011; Volz *et al.*, 2012). Although the predatory activity of myxobacteria is usually referred to as a wolfpack attack, current evidence indicates that *M. xanthus* cells do not surround the prey colonies. Instead, they penetrate the colony and lyse cells directly. Macroscopic travelling waves known as ripples are often observed and thought to maximize predatory efficiency (Berleman *et al.*, 2006; Berleman *et al.*, 2008; Berleman and Kirby, 2009; Zhang *et al.*, 2012). In contrast, results obtained about the role of the two *M. xanthus* motility

systems are contradictory, suggesting that characteristics of the prey define specific motility requirements (Pham *et al.*, 2005; Berleman *et al.*, 2006).

In this work we studied the interaction of *M. xanthus* with a series of *S. meliloti* field isolates, reference laboratory strains, and genetically engineered derivatives. The results show that *M. xanthus* exhibits two different predatory patterns that are determined by the rhizobial EPS galactoglucan. Our results also show that EPS II reduces killing rates, thus revealing a novel role for these biopolymers in the ecology of endosymbiotic bacteria.

Results

M. xanthus exhibits two predatory patterns on *S. meliloti*

The predatory activity of *M. xanthus* wild-type (WT) strain DK1622 on *S. meliloti* was examined on an agar surface by spotting suspensions of each microorganism next to each other (Fig. 1A and Fig. S1A). *M. xanthus* cells moved towards all *S. meliloti* strains, but two different interaction patterns were observed. One of them is a frontal attack during which *M. xanthus* entered the prey colony at the closest point and lysed the *S. meliloti* colonies directly (Fig. 1A and Fig. S1A). Interestingly, susceptible rhizobial prey included current reference laboratory strains (Table 1) that formed dry, non-mucoid colonies (i.e. GR4b, Rm1021, Rm2011 and RMO17b). In contrast, colonies of uncharacterized *S. meliloti* field isolates (i.e. a series of AK strains) that had not been routinely subcultured, produced highly mucoid colonies which resisted *M. xanthus* attack (Fig. 1A and Fig. S1A). No zones of clearing were evident within the AK colonies 72 h after plating. Rather, myxobacterial cells surrounded the edges of these colonies in a manner resembling wolfpack hunting.

The distinctly different predatory patterns against *S. meliloti* GR4b (non-mucoid) and AK21 (mucoid) colonies were examined in more detail by time-lapse videomicroscopy (Movies S1-S3). The cell density at the interface of the *M. xanthus* and GR4b colonies increased after 6-12 h of incubation. *M. xanthus* was able to penetrate the rhizobial colony head on and massively lyse prey cells, as evidenced by progressive clarification of the interaction zone (Movie S1). Rippling behavior consisting of macroscopic travelling waves was rarely observed during this interaction (Movies S1, S4, and S5. Movies S4-13 are available at <http://www.ugr.es/~jdorado/movies.htm>).

In marked contrast, *S. meliloti* AK21 cells responded to the approaching myxobacteria by producing EPS and retreating backwards. Upon contact, the high cell density at the interface lingered for over 24 h without evident rhizobial lysis (Movies S2 and S3). Instead, progressive migration of myxobacteria toward the distal edge of the AK21 colony was observed where the myxobacteria organized traveling waves and initiated prey lysis.

To elucidate whether these two interaction patterns were general properties of *M. xanthus*, mucoid and non-mucoid rhizobia were assayed against another WT strain, DZ2 (Table 1). As shown in Fig. S1B and Movies S6-S9, this strain exhibits predatory patterns similar to those reported for DK1622.

Rippling and motility were examined to deepen our understanding of the two *M. xanthus* predatory patterns. As rippling depends on the accumulation of small amounts of the C-signal (Li *et al.*, 1992), a *csgA* mutant was assayed for its ability to prey on rhizobial strains. As shown in Fig. 1B and Movies S10-S13, this mutant exhibits predatory patterns similar to the parental strain against mucoid and non-mucoid rhizobia except for the absence of ripples. Furthermore, we investigated the role of motility on predation. *M. xanthus* glides on solid surfaces by using two different mechanisms, called adventurous (A) and social (S) motility (Hodgkin and Kaiser, 1979). Although these two mechanisms are genetically independent, both are controlled by the *mglBA* operon (Hartzell and Kaiser, 1991). Mutants impaired in either A or S motility as well as both systems are available (Table 1). When assayed for predator efficiency, it was observed that all had lost partial or complete ability to prey on rhizobia (Fig. 1B). This is especially evident in the case of the A[−] S[−] cells, because they cannot migrate toward the rhizobial colonies.

A functional rhizobial *expR* gene alleviates predation by *M. Xanthus*

The mucoid phenotype of *S. meliloti* colonies is primarily due to ExpR-dependent biosynthesis of EPS (Pellock *et al.*, 2002; Marketon *et al.*, 2003). PCR-amplified ExpR ORFs from the highly mucoid *S. meliloti* field isolates revealed no amino acid substitutions (Fig. S2). On the other hand, the *expR* gene of non-mucoid strains Rm1021 and Rm2011, both derived from *S. meliloti* isolate SU47, is disrupted by the 1 320 bp insertion sequence *ISRm1* (Fig. 2). The well-characterized Rm8530/1021R and 2011R strains obtained by restoration of the WT *expR* gene through allelic exchange in Rm1021 and Rm2011, respectively, exhibited a highly mucoid phenotype (Fig. 2).

Sequence analyses revealed different mutations in the *S. meliloti* GR4b and RMO17b *expR* alleles. The GR4b sequence has a frameshift mutation resulting from an internal 11-bp deletion whereas RMO17 has a single nucleotide substitution resulting in replacement of the ultraconserved Ser202 with a Leu residue (Fig. 2). Parental, mucoid isolates of the GR4b and RMO17b strains (GR4 and RMO17) (Fig. 2) were recovered from the freezer and DNA sequencing demonstrated that both strains harbor a WT *expR* gene (Fig. S2). The *expR*⁺ *S. meliloti* Rm8530, 1021R, 2011R, GR4 and RMO17 strains were then examined with the predation assay. Without exception, all exhibited the wolfpack-like predation pattern associated with mucoid, EPS-producing strains (Fig. 2).

We next monitored killing of isogenic Rm1021 and 1021R strains, which are non-mucoid and mucoid, respectively (Table 1), by WT *M. xanthus* strain DK1622. In this assay, predator cells were spotted on top of the prey colonies, which were previously grown on membrane filters placed on nutrient rich CTT agar. Survival of *S. meliloti* cells was determined with plate counts by taking advantage of prey streptomycin-resistance. Without the predator, populations of both *S. meliloti* strains increased to over 10⁹ cells (Fig. 3, black lines). In the presence of WT *M. xanthus*, the Rm1021 population decreased to ~2 × 10⁴ cells within 24 h (Fig. 3A, red line), but did not decline further with longer incubation times. In contrast, ~2 × 10⁸ 1021R cells survived at 24 h, and declined to 10⁷ cells at 96 h (Fig. 3B, red line). These findings corroborate the photographs and videos showing that mucoid

strains are more resistant to lysis. Furthermore, they demonstrate that a functional *expR* gene protects rhizobia from predation.

Adventurous and social motility, but not rippling, are required for predation

The mutants impaired in rippling and motility were also assayed to quantify their predatory efficiencies on both 1021R and Rm1021 strains. The rhizobial killing curves by the *csgA* mutant were nearly identical to those of WT strain DK1622 (compare dark blue and red lines, Fig. 3). This result was expected against non-mucoid rhizobia because DK1622 exhibits no rippling during frontal attack. But it was surprising to see that rippling did not increase predator efficiency against mucoid strains where rippling is observed.

All motility mutants exhibit a fraction of the lytic activity compared with the WT strain. As shown in Fig. 3A (green line), a difference of three orders of magnitude was observed between the WT strain and the A[−] mutant against non-mucoid rhizobia. Although the difference between these two strains was much lower with the mucoid rhizobia, significant differences were still observed (Fig. 3B, green line). These differences were even more dramatic in the case of the S[−] and the A[−] S[−] mutants (Fig. 3, light blue and brown lines, respectively). Altogether these data indicate that optimal predation requires both motility systems.

Rhizobial galactoglucan confers resistance to predation and determines the predatory pattern

The *S. meliloti* ExpR regulon is required for widely diverse functions. We examined *M. xanthus* predation on well-characterized derivatives of *expR*⁺ strain Rm8530 lacking important genes in the ExpR regulon (Table 1) in order to identify the ExpR-dependent functions determining predatory pattern. Strain Rm11601 (*expR*⁺ *flaA* *flaB*) is impaired in swarming motility because it lacks flagella. Strain 8530W (*expR*⁺ *wgeB*) is blocked in galactoglucan synthesis due to a transposon insertion in a glycosyl transferase gene. Strain 11601W (*expR*⁺ *flaA* *flaB* *wgeB*) lacks both flagella and EPS II. Strain Rm9020 (*expR*⁺ *exoY*) lacks a glycosyl transferase involved in succinoglycan synthesis. Only mutations in the *wgeB* gene (also referred to as *expE2*) rendered strains susceptible to frontal attack in a manner similar to the non-mucoid laboratory strains (Fig. 4, compare center panel with others). We therefore conclude that EPS II, rather than functional flagella or EPS I, is the ExpR-dependent factor that confers resistance to predation and determines the *M. xanthus* predatory pattern.

Crude EPS was extracted from strains 8530 (EPS II-proficient) and 8530W (EPS II-deficient). DK1622 cells rapidly spread over the area where the extracts were spotted, but rippled only in the presence of EPS II (Fig. 5, left). In contrast, *M. xanthus* cells did not invade the surface containing only buffer (Fig. 5, right) and were markedly less aggressive in the spot lacking EPS II.

Discussion

Studies on the interaction between two soil bacteria, *M. xanthus* and *S. meliloti*, carried out under laboratory conditions have revealed that *M. xanthus* exhibits two different predatory

patterns on *S. meliloti* in response to differences in the prey. In one of them, *M. xanthus* cells surround the prey and begin forming ripples at collision points (Fig. 1 and Movies S2 and S3). In the other pattern, myxobacterial cells move directly into the prey colony, lysing cells as they go (Fig. 1 and Movie S1). Rippling is not observed or is very faint in contrast to previous results with other types of prey (Berleman *et al.*, 2006; Berleman *et al.*, 2008; Berleman and Kirby, 2009; Morgan *et al.*, 2010). The differences with rippling may be due to nutrient concentrations. While other studies employed starvation media, which induce rippling independently of predation, we used a rich medium that normally suppresses rippling.

Rippling has been reported to be a predatory behavior against *E. coli* and other prokaryotic and eukaryotic cells during consumption of macromolecular substrates (Berleman *et al.*, 2006). In one approach, media with low and high nutrient concentrations were used to induce or inhibit rippling, respectively. Although predator efficiency was greater in media where *M. xanthus* ripples, the difference could have been caused by other factors such as differing levels of extracellular hydrolytic enzymes between the two media (Berleman *et al.*, 2006). In a second approach, the authors showed that a mutant impaired in S motility neither rippled nor was an effective predator. This mutant has dramatically less motility so the difference could have been due to loss of motility rather than loss of rippling. To obtain more definitive data, we performed all our assays under the same nutrient conditions with a *csgA* mutant, which has lost the ability to ripple while retaining A and S motility.

Although, it has been postulated that rippling increases the rate of spreading over prey cells and allows the predator to remain on the prey longer (Zhang *et al.*, 2012), the data presented here with the *csgA* mutant indicate that rippling does not help overcome the physical and chemical barrier conferred by galactoglucan nor does it improve prey lysis (Fig. 1B and 3). The induction of traveling waves by EPS extracts containing EPS II (Fig. 5) suggests that rippling might be a general response to certain macromolecules, as it has been reported for other polymers such as peptidoglycan, proteins, or chromosomal DNA (Berleman *et al.*, 2006; Shimkets and Kaiser, 1982), which may be useful against some prey species but useless against others.

The role of each type of motility on predation was examined with mutants. S motility is important for both prey attack and prey lysis (Fig. 1B and 3), which is in agreement with the data obtained by Berleman *et al.* (2006). We also observed reduced prey lysis by A[−] mutants. Although it is expected that motility is necessary to attack the prey, it is not clear why A[−] S[−] cells or cells impaired in only one of the two motility systems have lost much of their capacity to lyse prey. We speculate that each motility system improves contact with the prey and allows distribution of hydrolytic enzymes. Surrounding the prey colony during a wolfpack-like attack seems to be the response of fully motile predator cells against highly resistant prey cells, even after the induction of ripples by galactoglucan.

We also focused on the prey defense mechanisms. Rhizobial cells producing EPS II are much more resistant to predation than those impaired in EPS II synthesis (Fig. 1-3). Muroid cells are still able to grow in the presence of *M. xanthus* 24 h after the addition of the predator. In contrast, the number of non-muroid rhizobial cells decreases about four orders of magnitude during the same period of time. However, it should be noted that *S. meliloti*

must use other unidentified mechanisms to escape predation, because $\sim 10^4$ non-mucoid cells persist for the next two days (Fig. 3). As EPS II production depends on ExpR, protection against predation can be added to the list of ExpR and EPS II functions. EPS II is commonly lost by rhizobia during subculture in the laboratory because of spontaneous mutations in the *expR* gene. Our data add new evidences supporting that EPSII provides an ecological advantage for successful survival of rhizobia in soil.

In summary, our findings show that *M. xanthus* approaches the nitrogen-fixing symbiont *S. meliloti* using two different predatory patterns that vary depending on the production of the EPS galactoglucan. Furthermore, galactoglucan protects rhizobia from lytic attack despite inducing rippling. Although fully motile cells are more efficient predators than mutants impaired in either of the two motility systems, identification of all the *M. xanthus* determinants mediating confrontation as well as the *S. meliloti* compensatory defense mechanisms are new challenges that will clarify the role of the predator in the dynamics of microbial soil populations. This observation may extend to other rhizobacteria. Since our experiments have been conducted on agar plates and, to our knowledge, there are no studies about the interaction between these two groups of bacteria in natural environments, the environmental significance of our findings remains to be elucidated.

Experimental Procedures

Bacterial strains, media and growth conditions

The bacterial strains used in this work, their source and relevant characteristics are listed in Table 1. CTT solid (1.5% Bacto-Agar) and liquid media were used to grow *M. xanthus* (Hodgkin and Kaiser, 1977) and for the predation experiments. Tryptone yeast (TY) solid (Beringer, 1974) or defined minimal media (Robertsen *et al.*, 1981) were used for maintenance and growth of *S. meliloti* strains. When required, antibiotics were added to the media at a final concentration of 200 $\mu\text{g ml}^{-1}$ streptomycin, 0.75 $\mu\text{g ml}^{-1}$ oxytetracycline, 100 $\mu\text{g ml}^{-1}$ hygromycin, or 120 $\mu\text{g ml}^{-1}$ neomycin.

EPS isolation

Crude EPS extracts were obtained using a previously described protocol with some modifications (May and Chakrabarty, 1994). *S. meliloti* 8530 (WT for EPS II production) and 8530W (impaired in EPS II synthesis) were grown on minimal medium agar for 3 days at 30°C. Bacteria were recovered from each plate (5 plates per strain) by resuspension in 2 ml 0.9% NaCl, and centrifugation at room temperature for 5 min at $12\,000 \times g$. The supernatant was carefully collected and EPS was precipitated at 4°C overnight with three volumes of ice-cold 95% ethanol. The precipitated material was centrifuged at 4°C for 15 min at $13\,700 \times g$ and the ethanol was carefully removed. The pellet was washed three times with ethanol, each time centrifuged for 15 min at $13\,700 \times g$. Finally, the pellets were dried under vacuum, UV sterilized, and resuspended in 2 ml TM buffer (10 mM Tris-HCl [pH 7.6], 8 mM MgSO_4) for biological assays.

Predation experiments

All *M. xanthus* and *S. meliloti* strains were grown in CTT broth (with appropriate antibiotic, when necessary) with vigorous shaking (300 rpm) at 30°C to an optical density at 600 nm (OD₆₀₀) of 1. Cells were centrifuged, washed in CTT, and concentrated in fresh CTT to a final OD₆₀₀ of 15 for *M. xanthus* and 5 for *S. meliloti* strains. Drops of 10 µl of the *S. meliloti* suspensions were deposited on the surface of CTT agar plates and allowed to dry. Next, drops of 10 µl of the different *M. xanthus* suspensions were spotted close to the *S. meliloti* spot, leaving a separation of no more than 1 mm between spots. Plates were incubated at 30°C and images were taken at different time-points (usually 24, 48, 72 and 96 h after plating) with a digital camera and an Olympus SZX7 dissecting microscope. The videomicroscopy experiments were performed as described in Pérez *et al.* (2011). Additionally, 10 µl drops (equivalent to an OD₆₀₀ of 10 for rhizobial cultures) of crude *S. meliloti* EPS extracts were assayed on plates against *M. xanthus* as described above.

For cell counting and viability determinations, *M. xanthus* DK1622 and the reference strains *S. meliloti* Rm1021 (non-mucoid) and 1021R (mucoid) were grown as indicated above. Rhizobial cultures were diluted to a final OD₆₀₀ of 0.2. Drops of 10 µl were deposited onto membrane filters (Isopore, Millipore) placed on the surface of CTT agar plates, and incubated at 30°C for 24 hours. After that time, 10 µl of concentrated cultures (OD₆₀₀ of 15) from the different *M. xanthus* strains were deposited on top of the rhizobial colonies. At different time-points, filters were deposited in an eppendorf tube, trapped with the tube stoppers, and thoroughly washed with 1 ml of TM buffer. The filters were then centrifuged for 3 min at 12 000 rpm. Supernatants and filters were discarded and the pellets resuspended in 500 µl TM buffer. *S. meliloti* viable cells were counted by using the dilution method. TY agar supplemented with streptomycin was used for counting because it allows growth of *S. meliloti* while inhibiting *M. xanthus* growth.

DNA methods

The *expR* gene of the different *S. meliloti* strains used in this work was amplified with the proofreading DNA polymerase Phusion® (New England Biolabs, Ipswich MA, USA) using bacterial lysates as template and primers ExpR1 (5'-ATCCGATACCATGGGAGG-3') and ExpR2 (5'-GGGCTGGCCGGATTC-3') in standard PCR reactions as specified by the enzyme supplier. PCR products were cloned into the pGEM-T Easy vector (Promega, Fitchburg WI, USA) for Sanger sequencing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Predatory patterns of *M. xanthus* on *S. meliloti*. The non-mucoid reference laboratory strain (ls) GR4b and the mucoid *S. meliloti* field isolate (fi) AK21 were exposed to the *M. xanthus* (*Mx*) WT strain DK1622 (A) or mutants (B) impaired in rippling (*csgA*), A motility (A⁻), S motility (S⁻), and A⁻ S⁻ (*mglBA*) on CTT agar plates. Microscopic images of the *S. meliloti*-*M. xanthus* cocultures were taken 72 h after bacterial spotting. Images on the right of panel a were taken with a digital camera.

Fig. 2.

The wolfpack-like attack of *M. xanthus* DK1622 on *S. meliloti* requires a functional rhizobial *expR* gene. Diagrams of the spontaneous mutations of *expR* in the *S. meliloti* laboratory strains Rm1021 and Rm2011 (insertion of *ISRm1*), GR4b (frameshift deletion) and RMO17b (*S*₂₀₂L), and their associated non-mucooid phenotypes on plates are shown in the left panels. *M. xanthus* preys on these strains by a frontal attack as shown in Fig. 1. The *expR*⁺ engineered derivatives (Rm8530, 1021R, 2011R) or natural variants (GR4 and RMO17) of these strains exhibit a mucooid phenotype and a wolfpack-like pattern in co-culture with *M. xanthus* (*Mx*) as shown in the images to the right.

Fig. 3.

Survival of *S. meliloti* Rm1021 (A) or its *expR*⁺ derivative 1021R (B) upon coculture with WT *M. xanthus* strain DK1622. *M. xanthus* WT or different mutant strains (Table 1) were spotted on top of rhizobial colonies previously grown on membrane filters. As controls (no Mx, black lines), similar experiments were conducted in the absence of myxobacteria. At the times shown, *S. meliloti* cells on the filters were measured as colony-forming units (CFU). Results are means of counts from three different filters at each time-point and three independent dilution sets. Error bars indicate standard deviations.

Fig. 4.

The rhizobial galactoglucan determines the predatory pattern of *M. xanthus* on *S. meliloti*. Simplified outline of the rhizobial functions influenced by the ExpR/SinI quorum sensing system. *S. meliloti* Rm8530 (*expR*⁺) and derivative strains harboring mutations impairing biosynthesis of EPS II/EPS I or motility (specified in brackets under the name of each strain) were subjected to predation tests on CTT agar. Images of the *S. meliloti*-*M. xanthus* cocultures taken 72 h after spotting are shown below the name of each rhizobial strain. Microscopic images of the *S. meliloti* colonies are shown to the left in each case. Only strains devoid of EPS II (inset) suffered a prolonged frontal attack.

Fig. 5.

EPS II induces rippling. Crude EPS extracts obtained from *S. meliloti* 8530 (which synthesizes EPS II) and 8530W (impaired in EPS II production), or TM buffer were spotted next to *M. xanthus* DK1622 on CTT agar plates. Pictures were taken under a dissecting microscope after 72 h of incubation. The red box indicates an area of rippling. The arrows in the enlargement of the red box point to wave crests.

Table 1
Bacterial strains used in this study

Strains	Relevant characteristics	Source/ reference
<i>M. Xanthus</i>		
DK1622	Wild-type	(Kaiser, 1979)
DZ2	Wild-type	(Campos and Zusman, 1975)
LS2442	<i>csgA</i>	(Curtis <i>et al.</i> , 2007)
MXH1777	<i>aglU</i> , A ⁻ , no adventurous motility	(White and Hartzell, 2000)
DK10410	<i>pilA</i> , S ⁻ , no social motility	(Wu and Kaiser, 1996)
DK6204	<i>mglBA</i> , A ⁻ S ⁻	(Hartzell and Kaiser, 1991)
<i>S. meliloti</i>		
AK21	Field isolate (Aral Sea region, Russia)	RIAM ¹
AK27	Field isolate (Aral Sea region, Russia)	RIAM
AK70	Field isolate (Aral Sea region, Russia)	RIAM
AK83	Field isolate (Aral Sea region, Russia)	RIAM
GR4	Field isolate (Granada, Spain)	(Casadesús and Olivares, 1979)
RMO17	Field isolate (León, Spain)	(Villadas <i>et al.</i> , 1995)
GR4b	GR4 spontaneous non mucoid variant	(Casadesús and Olivares, 1979)
RMO17b	RMO17 spontaneous non mucoid variant	(Villadas <i>et al.</i> , 1995)
Rm1021	SU47 derivative (reference strain); Sm ^{r(2)}	(Meade and Singer, 1977)
Rm2011	SU47 derivative; Sm ^r	(Case <i>et al.</i> , 1979)
1021R	Rm1021 <i>expR</i> ⁺ ; Sm ^r	(Nogales <i>et al.</i> , 2012)
2011R	Rm2011 <i>expR</i> ⁺ ; Sm ^r	(Nogales <i>et al.</i> , 2012)
Rm8530	Rm1021 <i>expR</i> ⁺ ; Sm ^r	(Pellock <i>et al.</i> , 2002)
Rm11601	Rm8530 <i>flaA flaB</i> ; Sm ^r Hyg ^r	(Gurich and González, 2009)
11601W	Rm11601 <i>wgeB::mini-Tn5</i> ; Sm ^r Nm ^r , Hyg ^r	(Nogales <i>et al.</i> , 2012)
8530W	Rm8530 <i>wgeB::mini-Tn5</i> ; Sm ^r Nm ^r	(Nogales <i>et al.</i> , 2012)
Rm9020	Rm8530 <i>exoY::Tn5-132</i> ; Sm ^r Otc ^r	(Glazebrook and Walker, 1989)

¹ Culture collection of All-Russia Institute of Agricultural Microbiology (St. Petersburg, Russia).

² Sm^r, Hyg^r, Nm^r, and Otc^r indicate streptomycin, hygromycin, neomycin, and oxitetracycline resistance, respectively.