Hydrolysis of aromatic β-glucosides by non-pathogenic bacteria confers a chemical weapon against predators

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Bacteria present in natural environments such as soil have evolved multiple strategies to escape predation. We report that natural isolates of Enterobacteriaceae that actively hydrolyze plant-derived aromatic β-glucosides such as salicin, arbutin and esculin, are able to avoid predation by the bacteriovorous amoeba Dictyostelium discoideum and nematodes of multiple genera belonging to the family Rhabditidae. This advantage can be observed under laboratory culture conditions as well as in the soil environment. The aglycone moiety released by the hydrolysis of β-glucosides is toxic to predators and acts via the dopaminergic receptor Dop-1 in the case of Caenorhabditis elegans. While soil isolates of nematodes belonging to the family Rhabditidae are repelled by the aglycone, laboratory strains and natural isolates of Caenorhabditis sp. are attracted to the compound, mediated by receptors that are independent of Dop-1, leading to their death. The β-glucosides–positive (Bgl+) bacteria that are otherwise non-pathogenic can obtain additional nutrients from the dead predators, thereby switching their role from prey to predator. This study also offers an evolutionary explanation for the retention by bacteria of ‘cryptic’ or ‘silent’ genetic systems such as the bgl operon.

1. Introduction

Bacteria form an integral part of food webs in different ecological niches and face challenges from a range of predators and parasites such as protozoa, nematodes and phages. Predation plays a major role in bacterial mortality in soil, freshwater and marine ecosystems. Fitness of bacteria in their natural habitats hence depends not only on growth and reproduction but also on their ability to defend themselves against natural predators [1]. Bacteria have evolved multiple strategies to combat predators which include increased size, motility [2], secondary metabolite production [3] and biofilm formation [1]. Despite selection pressure to maintain genomes composed predominantly of functional genes, bacteria carry genes that are silent and uninducible under most laboratory growth conditions. Their maintenance in a cryptic state without the accumulation of deleterious mutations is an evolutionary puzzle [4]. The well-studied β-glucoside (bgl) operon, present in E. coli [5] and other Gram-negative bacteria, is one such silent genetic system ([6] and references therein). Upon mutational activation, the bgl operon enables the catabolism of aromatic β-glucosides such as salicin, arbutin and esculin. Composed of an aromatic moiety linked to glucose via a β-glycosidic bond, these compounds are produced by plants as secondary metabolites. Bacteria that show a Bgl+ phenotype can derive energy from the glucose released by the hydrolysis of β-glucosides.

Unlike the members of Enterobacteriaceae present in the gut environment that predominantly show a Bgl+ phenotype [7], this study shows that many of their counterparts present in the soil can use the aromatic β-glucosides salicin and arbutin as a carbon source. Characterization of the bgl operon in Klebsiella aerogenes, primarily a soil organism, revealed the loss of the negative elements...
in the regulatory region of the operon, leading to a higher basal level of expression of the bgl genes [8]. This niche-specific difference in the pattern of β-glucoside utilization is consistent with the possibility that plant-derived β-glucosides are more likely to be encountered in the soil.

Plant secondary metabolites such as salicin often serve as a defensive tool against herbivores [9]. Whether bacteria that are able to metabolize aromatic β-glucosides and derive energy can also use these compounds for defence against predators, along the same lines as plants, is an intriguing possibility.

The present study was initiated to find a possible link between β-glucoside utilization and defence from predation in members of Enterobacteriaceae. Both laboratory strains and natural isolates of bacteria, comprising β-glucoside positive and negative strains, were selected for the study. Bacteriovorous nematode strains including natural soil nematodes as well as laboratory strains of Caenorhabditis elegans, and the amoeba Dicystostelium discoideum were used as predators, and predator–prey interaction was investigated in the context of β-glucoside metabolism.

2. Material and methods

(a) Strains and media

The bacterial strains used in the study are listed in table 1 and the electronic supplementary material, table S1. The D. discoideum wild-type strain NC4 was grown in standard medium (SM) (10 g l⁻¹ glucose, 10 g l⁻¹ peptone, 1 g l⁻¹ yeast extract, 1 g l⁻¹ MGSO₄·7H₂O, 2.25 g l⁻¹ KH₂PO₄, 0.66 g l⁻¹ K₂HPO₄, pH 6.4) with Klebsiella aerogenes. The axenic strain Ax2 was grown in SM medium with Klebsiella or axenically in HL5 medium (15.4 g l⁻¹ glucose, 14.3 g l⁻¹ Difco proteose peptone, 7.15 g l⁻¹ Difco yeast extract, 0.49 g l⁻¹ KH₂PO₄, 0.507 g l⁻¹ Na₂HPO₄, pH 6.4). SM/5 medium was prepared as described by Sussman [14]. The C. elegans wild-type strain N2 was grown in nematode growth medium (NGM) plates seeded with E. coli OP50 as described in WormBook available online (www.wormbook.org).

(b) Dicystostelium discoideum viability assay

The protocol for amoeba–bacteria co-culture experiment to monitor viability of D. discoideum was adopted from Sussman [14]. Briefly, bacterial cultures were grown in Luria broth to OD₆₀₀ ~ 1.0, washed with KK2 buffer (2.25 g l⁻¹ KH₂PO₄ and 0.66 g l⁻¹ K₂HPO₄, pH 6.4) and resuspended in KK2 buffer. The washed culture (100 μl) was added to 10 ml SM/5 medium without glucose. Amoebae were grown either in HL5 (for Ax2) or in SM agar + bacteria (for NC4), washed with KK2, resuspended in the buffer and counted using a haemocytometer. Approximately, 10⁷ amoebae were added to the SM/5 medium containing bacterial cells followed by addition of specific sugars. The flasks were then incubated at 22°C with moderate shaking. Viability of the amoebae was monitored at different time intervals by plating aliquots of equal volumes from the cultures on SM agar plates seeded with the laboratory strain of Klebsiella used as the normal feed for the amoebae. Viable amoebae feed on Klebsiella and form clearings in the bacterial lawn known as plaques. The number of plaques formed at different time points was plotted against time.

Viability of amoebae (as plaques on bacterial lawn) was also checked by placing them on SM plates (without glucose) along with overnight-grown bacteria (both β-glucoside positive and negative members) in the presence or absence of β-glucosides.

(c) Chemotaxis assay

Caenorhabditis elegans chemotaxis assays were performed as described by Zhang et al. [15]. Briefly, Bgl⁺ and Bgl⁻ bacteria were grown in lysogeny broth for 24 h in the presence of glucose or β-glucoside. Approximately 25 μl of the culture (test sample and control) was spotted 6 cm apart on behavioural plates (1.6% agar, 5 mM potassium phosphate, pH 6, 1 mM CaCl₂, 1 mM MgSO₄). The plates were dried for 5 h at room temperature, and 1 μl of 1 M sodium azide (anaesthetic) was added to both the spots a few minutes before the assay to fix the worms.
Chemotaxis assays were also performed using a 32 mM solution of saligenin in ethanol, using the solvent as a control.

(d) Isolation of bacteria, nematodes and amoebae from soil
All soil samples were collected from the campus of the Indian Institute of Science, Bangalore, India, from areas with dense vegetation. The collection was done in the month of May, 2012. _Enterobacteriaceae_ from soil were isolated by serial dilution and plated on an Eosin Methylene Blue (EMB) agar plate. Individual colonies from the EMB agar plate were then patched on MacConkey agar + β-glucoside plates to analyse its status of β-glucoside utilization. Nematodes were isolated by a centrifugal flotation method as described in WormBook using 1.38 M MgSO4.7H2O solution. For the isolation of amoebae, approximately 0.5 g of soil was suspended in 1 ml KK2 buffer, mixed properly and 100 µl of the mixture was spread on SM/5 plates. Plates were then incubated at 22°C, and fruiting bodies of amoebae (cellular slime moulds) were observed after 4–5 days.

(e) Saligenin avoidance assay
This is a modification of _C. elegans_ lawn avoidance assay ([16], electronic supplementary material, methods). Two conditions were created for the behavioural assays. Under the NGM condition, NGM agar plates (6 cm size) containing 28 mM saligenin were prepared. A hole of diameter 2.5 cm was made in the centre and was filled with NGM agar without saligenin. Twenty adult worms were added at the centre. The number of worms in the inner circle was counted after 3 h and plotted as percentage occupancy with respect to the total number of worms added. For the Saligenin condition, NGM agar without saligenin was used to prepare the plates, and NGM saligenin agar was used to fill the hole in the centre.

(f) Nematode viability assay in soil
Autoclaved soil (approx. 10 g) was placed on 6 cm plates. Approximately 100 adult N2 or _Oscheitius tipulae_ worms were inoculated separately in the soil along with bacteria (10^6 cells of Bgl^+ or Bgl^-) with or without addition of salicin (approx. 1% final concentration). Both RS-Bgl^+ and RS-Bgl^- strains used are _Enterobacteriaceae_ members isolated from soil. The _Shigella sonnei_ strains AK1 (Bgl^+) and AK102 (Bgl^-) were used as controls. An equal volume of water was added in all the conditions to maintain the moisture. All soil microcosms were processed after 10 days. Nematodes were extracted as described earlier and their numbers were plotted.

3. Results

(a) Bacteria actively hydrolyzing aromatic β-glucosides are toxic to predators
Members of _Enterobacteriaceae_ comprising both β-glucoside degraders and non-degraders (isolated from different niches) were selected to test the effect of catabolism of the aromatic β-glucosides salicin, arbutin and esculin on their predators entering the spots. One-day-old adult worms (40–100) were washed with M9 buffer and placed in the behavioural plates equidistant from both bacterial spots. After 1 h, the number of worms in both bacterial spots was counted. The chemotaxis index, which is indicative of the preference of the nematode in terms of the bacterial cultures spotted, was calculated as:

\[
\text{chemotaxis index} = \frac{\text{number of worms in test spot} - \text{number of worms in control spot}}{\text{total number of worms}}
\]

D. discoideum strains NC4 (wild-type) and Ax2 (an axenic derivative of NC4), and _C. elegans_ (strain N2; table 1).

The effect of salicin metabolism on _D. discoideum_ was monitored by amoeba–bacteria co-culture experiments as described previously ([14] and see §2). NC4 amoebae showed loss of viability when they were grown in the presence of Bgl^+ bacteria and 35 mM salicin (the concentration of salicin which when used as the sole carbon source allows growth of bacteria; figure 1; electronic supplementary material, figure S1a). Bgl^- bacteria along with the same concentration of salicin or Bgl^- bacteria growing on glucose were not toxic to the amoebae. No increase in cell count was observed in the presence of salicin alone, as expected, owing to the absence of bacteria as food. Disruption of the bgl operon in the Bgl^- strain led to the loss of growth inhibition of NC4 indicating the requirement for salicin catabolism, mediated by the bgl genes, for the toxicity observed. The axenic strain Ax2 showed a similar growth inhibition in the presence of Bgl^- bacteria + salicin (see the electronic supplementary material, table S2) and Bgl^- bacteria + arbutin (see the electronic supplementary material, figure S1b).

The effect of Bgl^+ bacteria on _C. elegans_ was monitored by a killing assay. Bgl^+ bacteria were grown for 36 h at 37°C in NGM without glucose but supplemented with 35 mM salicin. One-day-old adult N2 worms were transferred to the bacterial lawn and their viability was monitored. A majority of the nematodes died within a few days on plates containing salicin and Bgl^- bacteria (figure 2). Lethality was also observed when eggs were exposed to Bgl^- bacteria growing on salicin (see the electronic supplementary material, table S4). In the control plates, i.e. Bgl^- bacteria growing on glucose and Bgl^- bacteria grown in the presence of salicin, the viability of the worms was unaltered and they showed normal growth and development.

(b) Lethality is due to the aglycone formed during β-glucoside degradation
The observed killing of the amoebae and nematodes could be because Bgl^- bacteria are modified and made inedible
plate.

responding to touch and did not move when transferred to a non-toxic

adults to each plate. Worms were considered dead when they stopped

experiments showing a similar trend.

detectable plaque formation. The plots are representative of two independent

AK1 (Bgl

2

indicated that lethality is not due to modification of the Bgl

ques and subsequent normal development of the amoebae

two possibilities, Bgl

2

product of salicin hydrolysis. To distinguish between these

in the presence of salicin, thereby leading to starvation of the

Alternatively, lethality could be due to a by-

product of salicin hydrolysis. To distinguish between these
two possibilities, Bgl

2

bacteria were grown in M9 minimal medium containing 35 mM salicin, washed and plated with

D. discoideum NC4/Ax2 cells in SM medium. Formation of pla-

supernatants of Bgl

2

lysis (see the electronic supplementary material, figures S3

alcohol) was found to be the major product of salicin hydro-

saligenin concentrations against OD509, 50a, AK102, AK1 and

When compared with a standard curve obtained by plotting

Supernatants of Bgl

2

also inhibited the growth of Ax2 cells (see the electronic

In an attempt to identify the product of salicin degrada-

Bgl

1

bacteria, an organic extract of the supernatant of Bgl

1

bacterial culture grown in M9 salicin (35 mM) medium was fractionated by thin layer chromatography, and also subjected to NMR analysis (see the electronic sup-

Saligenin (2-hydroxybenzyl alcohol) was found to be the major product of salicin hydro-

lethality was observed at concentrations of 28 mM and above (figure 3).

To test if saligenin had a direct effect on viability, Ax2 cells were incubated for 12 h in HL5 growth medium containing different concentrations of the compound, and viability of the

amoebae was determined as described above. The amoebae

were incubated for 12 h in HL5 growth medium containing

0.1 and 0.1 mM, respectively.

To determine whether the toxicity associated with saligenin is reversible, adult worms exposed to 28 mM saligenin at different lengths of time were transferred to fresh NGM plates containing OP50. The sick worms exposed to saligenin for 3–4 days recovered, grew and reproduced normally when shifted to non-toxic condition. Longer exposure to sali-

genin resulted in rapid and irreversible decrease in viability as shown before (figure 3). Similarly, amoebae could be

that death associated with Bgl

1

bacteria grown in the presence of salicin is related to the presence of a toxic by-product of sali-

cin hydrolysis. This was confirmed by the observation that the concentrated supernatant obtained from Bgl

1

strains grown in the presence of salicin could induce growth inhibition of predato-

When present at a final concentration of 0.4 per cent on NGM plates seeded with OP50, one-day-old N2 worms succu-

a similar concentration of the supernatant also inhibited the growth of Ax2 cells (see the electronic supplementary material, table S2, last row).

Figure 1. Effect of β-glucoside metabolism on Dictyostelium discoideum
strain NC4. Viability of amoebae was plotted as number of NC4 plaques
formed on SM agar plates seeded with Klebsiella (S2). The culture conditions
used are: AK102 (Bgl

1

+) + 55 mM glucose, AK102 (Bgl

1

+) + 35 mM salicin, AK1 (Bgl

1

+) + 35 mM salicin, 35 mM salicin, AK102Δbgl:kan (Bgl

1

+) + 35 mM salicin, 50a (Bgl

1

) + 55 mM glucose and 50a (Bgl

1

) + 35 mM salicin. ‘Not detected’ (ND) indicates the absence of plaque formation at

Figure 2. Effect of β-glucoside metabolism on the Caenorhabditis elegans
strain N2. Survival of N2 (plotted as percentage dead worms versus time)
was monitored on NGM plates containing different combinations of sugars
and bacteria i.e. 50a (Bgl

1

) + 35 mM salicin, 50a + 55 mM glucose and

AK1 (Bgl

1

) + 35 mM salicin. Bacteria were grown on the respective
medium for 36 h at 37 °C before addition of approximately 20 one-day-old
adults to each plate. Worms were considered dead when they stopped
responding to touch and did not move when transferred to a non-toxic
plate. n = 3. Error bar indicates s.d.

Figure 3. Lethal effect of saligenin on N2. Effect of saligenin on N2 young
adults when present in their growth medium of NGM + OP50, plotted as
percentage of dead worms over 7 days. n = 3.
plates containing the bacterial strain 50a (Bgl). Caenorhabditis elegans measures bacterial lawn occupancy of and the electronic supplementary material, methods), which

saligenin can act as a chemo-attractant (see the electronic chemotaxis assay with the solvent as control, indicating that ethanol at a final concentration of 32 mM) when used in the test condition.

was also attracted towards saligenin (dissolved in arbutin when used in the assay instead of salicin (see the electronic supplementary material, figure S5). Thus, bacteria that metabolize aromatic β-glucosides are able to attract the nematodes allowing for a greater exposure to the toxic aglycones.

(c) Effect of β-glucoside utilization on predator behaviour

Caenorhabditis elegans is known to exhibit a variety of behavioural responses towards bacteria and chemicals present in the environment [15–17] which help them avoid toxic food or chemicals. To determine whether salicin metabolism influences the behaviour of C. elegans, chemotaxis assays were performed as described by Zhang et al. ([15] and see §2). Specifically, the assay involves monitoring the preference that C. elegans exhibits when placed equidistant from two compounds by moving towards the preferred compound; C. elegans preferred Bgl+ bacteria when they were grown in salicin medium over Bgl− bacteria or Bgl+ bacteria grown on glucose (figure 4). Similar results were obtained with arbutin when used in the assay instead of salicin (see the electronic supplementary material, figure S5a). Caenorhabditis elegans was also attracted towards saligenin (dissolved in ethanol at a final concentration of 32 mM) when used in the chemotaxis assay with the solvent as control, indicating that saligenin can act as a chemo-attractant (see the electronic supplementary material, figure S5a). To complement these observations, a lawn avoidance assay was conducted ([16] and the electronic supplementary material, methods), which measures bacterial lawn occupancy of C. elegans under different conditions. Caenorhabditis elegans spent more time on plates containing the bacterial strain 50a (Bgl+) grown on salicin even though it was toxic to them and the occupancy was equivalent to that for the control strain OP50 (Bgl−). However, they mostly avoided the bacterial lawn of 50a grown on glucose (see the electronic supplementary material,

(d) Mechanism of saligenin toxicity

Previous reports have demonstrated local anaesthetic and adrenergic activity of saligenin and its derivatives [18–20]. A search for DNA sequences orthologous to the mouse and rat adrenoceptors in WormBase showed sequences with significant similarity to those encoding multiple receptors in C. elegans such as Dop-1, Ser-5, Ser-7 and Tyra-3. Saligenin toxicity in the nematodes could be equivalent to an overdose of a local anaesthetic and might be mediated by orthologues of adrenergic receptors. In such a case, loss of the receptor is expected to rescue the worms from saligenin toxicity. To address this, viability of various receptor mutants in the presence of saligenin was determined with N2 as the control. Partial rescue of lethality was observed with the dop-1 (dopamine receptor) mutant, suggesting that saligenin might act via dopamine receptors in C. elegans (figure 5). The ser5 mutant appeared to be more sensitive than wild-type for reasons unknown. All mutants showed a comparable lifespan when kept in control plates without saligenin. The dop-1 mutant was also resistant to the anaesthetic action of saligenin. When suspended in 40 mM saligenin for 20 min, only 20 per cent of the dop-1 mutant stopped the thrashing movement as against 90 per cent observed with the wild-type (see the electronic supplementary material, figure S5c). This is consistent with the possibility that saligenin acts via the Dop-1 receptor. However, the mutant did not show any alteration in its chemotactic behaviour towards saligenin, suggesting that the behavioural response observed involves a different receptor and pathway independent of the Dop-1 receptor.

(e) Coexistence of nematodes and amoebae with both Bgl+ and Bgl− bacteria in the soil

The experiments described so far were carried out with bacterial strains, amoebae and nematodes maintained in the laboratory. In an attempt to gain insights on the ecological significance of the observations, we examined the distribution of
Several natural isolates of Caenorhabditis were obtained with hydroquinone (the aglycone part of arbutin). Similar results were obtained even at 4 mM saligenin concentration (see the electronic supplementary material, figure S7). In 11 out of 31 samples, amoebae and nematodes were found to coexist with both Bgl\(^+\) and Bgl\(^-\) bacteria, consistent with the universal coexistence of predator and prey in the same environment.

(f) Saligenin is toxic to natural isolates of nematodes

In an attempt to extend the observations with laboratory strains to natural isolates, the toxicity studies carried out with C. elegans were repeated with soil nematodes Mesorhabditis sp., O. tipulae and Rhabditis rainai (see the electronic supplementary material, table S5). Both Bgl\(^+\) and Bgl\(^-\) bacteria independently coexist with both predators in 17 out of 31 soil samples (see the electronic supplementary material, figure S6). All soil nematodes of genera Mesorhabditis, O. tipulae and Rhabditis robustly avoid saligenin (figure 7). For instance members of Mesorhabditis could sense saligenin concentrations as low as 4 mM and avoid it (see the electronic supplementary material, figure S9). Even though the plots show some occupancy after 3 h, avoidance behaviour could be seen as early as 15 min. However, no avoidance was observed against hydroquinone and salicin (see the electronic supplementary material, figure S10).

Interestingly, none of the wild isolates of Caenorhabditis avoided saligenin. Instead, they showed a positive chemotaxis towards saligenin similar to N2 in a chemotaxis assay performed as described before (see the electronic supplementary material, figure S11). Hence, members of Enterobacteriaceae exhibit two strategies against their predators via β-glucoside metabolism; i.e. repulsion of Oscheius and Mesorhabditis species and attraction followed by killing of Caenorhabditis species.

(h) Salicin metabolism by Bgl\(^+\) bacteria is toxic to nematodes in soil

In an attempt to simulate the soil environment in the laboratory, salicin was externally added to soil that was seeded with bacteria and nematodes, and the effect of its metabolism on nematodes was analysed in situ (§2). Salicin utilization by Bgl\(^+\) bacteria in soil was toxic to nematodes indicated by a decline in nematode count, whereas the count increased in the presence of Bgl\(^-\) bacteria (figure 8 and electronic supplementary material, figure S12). Growth of nematodes was also inhibited owing to starvation when no bacteria were added to the soil. These studies indicate that saligenin toxicity can manifest in the soil environment.

4. Discussion

Bacteria are known to adopt multiple strategies to combat predation under different environmental conditions. The results reported here shed light on one such strategy—release of toxic aromatic compounds by the hydrolysis of β-glucosides by members of Enterobacteriaceae. In contrast to the

\[ \text{Figure 6. Effect of saligenin on the viability of soil nematodes. Survival of soil nematodes (plotted as percentage live worms versus time) was monitored on NGM plates containing 28 mM saligenin and OP50. Approximately 20 adult nematodes were used in each experiment.} \]

\[ \text{Figure 7. Effect of saligenin on the behaviour of soil nematodes. Avoidance assays with soil nematodes were performed as described in §2 and plotted as } \]

% occupancy versus time. N\(\text{2}\) and Bgl\(^+\) bacteria independently coexist with both predators in 17 out of 31 soil samples (see the electronic supplementary material, figure S6). In 11 out of 31 samples, amoebae and nematodes were found to coexist with both Bgl\(^+\) and Bgl\(^-\) bacteria, consistent with the universal coexistence of predator and prey in the same environment.

\[ \text{Figure 8. Salicin utilization by Bgl\(^+\) bacteria is toxic to nematodes in soil.} \]

\[ \text{Figure S10. \textit{Oscheius} and \textit{Mesorhabditis} robustly avoid saligenin (figure 7). For instance members of \textit{Mesorhabditis} could sense saligenin concentrations as low as 4 mM and avoid it (see the electronic supplementary material, figure S9). Even though the plots show some occupancy after 3 h, avoidance behaviour could be seen as early as 15 min. However, no avoidance was observed against hydroquinone and salicin (see the electronic supplementary material, figure S10).} \]
antipredatory actions of pathogenic bacteria that have evolved specific tools such as extracellular proteases targeted against predators [21], our studies involved non-pathogenic bacteria that are armed only with genes that enable the uptake and hydrolysis of aromatic β-glucosides. The energy investment is minimal for the bacterium and a part of it could be derived from the glucose generated during the hydrolysis of β-glucosides. Hence, this strategy appears to confer dual advantage for the bacteria as it provides escape from predation in addition to a carbon source for growth.

The aglycone molecules produced in the course of hydrolysis of aromatic β-glucosides were found to be the causative agents of mortality of predators. Previous studies have reported the inhibitory action of plant glucosides against protozoa [22]. The results presented in our report are novel as the generation of the toxic aglycone involves catabolic activity mediated by a micro-organism. As many soil-dwelling bacterial species have the capacity to break down aromatic β-glucosides, typified by K. aerogenes [8], this is likely to be a general mechanism of self defence.

The response to saligenin by soil nematodes belonging to the Mesorhabditis genus and O. tipulae is distinctly different from that exhibited by Caenorhabditis species. While the former avoid the compound, the latter are attracted by the compound with lethal consequences. Aversive learning behaviour against toxic chemicals owing to repeated exposure has been observed in N2. However, this behaviour is short-term and does not pass through generations. The avoidance behaviour exhibited by the soil nematodes appears to be genetically based, because it was shown through multiple generations of all the genera analysed in the study. One can speculate that soil nematodes are often exposed to the toxic aglycones of β-glucosides (produced by plants and deposited on the soil via the shedding of leaves) and hence have evolved a dedicated genetic system to actively sense and avoid these chemicals.

The observation that worms of C. elegans and O. tipulae respond in the same manner to signals from the predator, which in turn results in the killing of the predator, provides strong support that the avoidance behaviour we are reporting is relevant in the natural context.

In addition to the metabolic advantage gained from the hydrolysis of β-glucosides, the ability to repel or lure predators to their death and thereby gain a nutritional advantage is likely to be a strong selective force to retain the bgl genes in the genome. Expression of the E. coli bgl operon showed a modest increase in the presence of predator/predator culture supernatant, indicating an active response of the bacterium to unknown signals from the predator (see the electronic supplementary material, figure S14). Toxicity genes in bacteria have been shown to respond in the same manner to signals from the predator, which in turn results in the killing of the predator [25].

The toxicity associated with saligenin that we have observed requires a reasonably high concentration of the aglycone. Are such concentrations physiologically relevant? Our attempts to detect β-glucosides in soil were not successful. This could be attributed to the fact that most of the β-glucosides are transient in soil and are readily converted to other forms by biotic or abiotic factors [26]. Compared with other secondary metabolites, β-glucosides are quite abundant in plants, especially in Salicaceae members [9,27,28], and are more likely to be encountered in the soil (derived from decomposing vegetation) than in the animal gut. This is consistent with the niche-specific difference in the pattern of β-glucoside utilization by different members of Enterobacteriaceae mentioned before. Our observations also indicate that in situations where multiple β-glucosides are present in the same environment, the concentrations of individual β-glucosides can be as low as 3 mM (see the electronic supplementary material, table S3).

The distribution of predator and prey analysed in this study showed coexistence of predator and prey in many soil samples. A model based on the interspecies interactions observed is depicted in figure 9. The stable coexistence of predator and prey over a long time range could be related to the oscillatory output [30] expected from the model. As evasion of predators is related to the ability to hydrolyze aromatic β-glucosides that rest with Bgl+ strains, this leads to the interesting questions as to who dies and who benefits in mixed populations that have both Bgl+ and Bgl− strains.

Figure 8. Effect of salicin metabolism on nematodes in the soil environment. Soil microcosm experiments were performed as described in §2. Briefly, approximately 10^6 cells of either RS-Bgl− or RS-Bgl+ bacteria (soil isolates) were mixed with sterile soil along with approximately 100 nematodes (either N2 or O. tipulae). The number of adult nematodes extracted after 10 days were plotted in each case. n = 3. Error bars represent s.d. (Online version in colour.)
Bgl\(^-\) bacteria might derive an advantage from Bgl\(^+\) bacteria in terms of protection from predators and hence may tend to occupy the same niche with Bgl\(^-\) in terms of protection from predators and hence may tend to occupy the same niche with Bgl\(^+\) whenever predators are present. Our observation that in 11 out of 31 soil samples, Bgl\(^-\) and Bgl\(^+\) strains coexist with predators supports this possibility. However, further experiments are necessary to validate this observation.

These studies also suggest possible strategies for the biocontrol of parasitic amoebae and nematodes by using aglycones from plant-derived aromatic β-glucosides.

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References


