Broad spectrum suppression of wheat and canola fungal diseases by endophytic biocontrol agents

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Key messages

Several bacteria from the Actinobacteria phylum with antifungal properties were isolated from paddocks across WA.

These Actinobacteria are effective at suppressing a range of fungal pathogens of wheat and canola including *Fusarium* crown rot, *Pythium* damping off, take-all root rot, *Rhizoctonia* hypocotyl rot and *Sclerotinia* stem rot.

They have enormous potential for development as biocontrol agents because they can be delivered as a seed coat, they are endophytic so they will take up residence within crop roots, they are easy to culture and grow, and they form spores that allow them to survive adverse environmental conditions.

Aims

Soil/stubble-borne fungal diseases of wheat and canola cost the Australian grain and oilseed industries > $250M annually (Murray and Brennan, 2009; Murray and Brennan, 2012). The incidence of some of these diseases is on the rise in Western Australia, including *Fusarium* crown rot of wheat and *Sclerotinia* stem rot of canola. With no or limited host resistance available, novel broad spectrum control approaches are needed. Management strategies to control several fungal root and stem diseases have been largely unsuccessful to date. Fungicides used as seed dressings and foliar sprays provide variable protection depending on the pathogen and timing of application (sprays). Plant breeding has produced more tolerant varieties to some diseases but none of these show effective disease resistance across multiple pathogens. Furthermore, after maturity, fungal pathogens can persist by growing and surviving in the stubble or on the roots of summer weeds, and act as a source of infection in the following crop.

Our research is exploring the development of biological control agents for fungal diseases of wheat and canola including projects investigating control of *Fusarium* crown rot, *Pythium* damping off, take-all root rot, *Rhizoctonia* hypocotyl rot and *Sclerotinia* stem rot.

To be considered successful the agents must 1) suppress the disease effectively, 2) survive either within the plant tissues or within the rhizoplane of growing plants and 3) be easy to culture and inoculate onto plants to allow for realistic application methods in the field.

Method

Collection and isolation of actinobacteria

Endophytic, plant growth promoting Actinobacteria were isolated from the roots of wheat plants that were sourced from areas in WA where plants were healthy and were known to consistently perform well. Plant roots were surface sterilised and aseptically cut with a scalpel to expose microorganisms inside the plant and plated on agar medium selective for Actinobacteria. Further selection of isolates produced ~300 individual isolates of Actinobacteria. The isolates were then assessed for their ability to suppress individual pathogens both in agar culture and small plant assays. Based on the results of the general screening tests, a small subset of these Actinobacteria is being investigated more intensively for biocontrol capability.

Antifungal metabolite production – Agar plate assays

Agar plate assays to test for suppression of pathogenic fungi by the Actinobacteria were conducted by co-inoculating the test organism and the fungal pathogen at opposite ends of a nutrient agar plate (half strength potato-dextrose agar). A second plate was inoculated at one end with the fungal pathogen alone as a control. The plates were incubated at 28^{o}C in the dark until the control plate was completely covered by the pathogen. The level of inhibition of the fungal pathogen by the Actinobacteria was measured as the distance between the growing front of the test organism and the fungus on the day that the fungus had completely covered the control plate. Percentage inhibition was then calculated relative to the distance between the test fungus inoculation point and the test Actinobacteria growing edge.

Small plant assays

To date, small plant assays have been completed to test for suppression of *Fusarium pseudograminearum* (crown rot) by several of the most promising Actinobacteria. Small plant assays are currently underway for the control of *Sclerotinia sclerotiorum* (stem rot) and *Rhizoctonia solani* (hypocotyl rot).

For the small plant assays to assess control of crown rot, surface sterilised wheat (*Triticum aestivum*) seeds were coated with spores of test Actinobacteria. Seeds were then wrapped in a wet paper towel (Yang et al., 2010) which was placed in a beaker and kept moist. Once the wheat plants protruded from the top of the paper rolls, 2 ml of a 10^6 / ml spore suspension of the test pathogen was introduced. Plants were scored for disease severity and root and shoot length after 2 weeks. For tests of the control of Sclerotinia and Rhizoctonia, canola plants were grown in sterile sand in small pots in the glasshouse from seed coated with the spores of test Actinobacteria. For testing of Rhizoctonia the soil was inoculated with the pathogen several days prior to sowing. For Sclerotinia the pathogen was introduced by inoculating the fully emerged cotyledon with 10 μL of a suspension containing 10^6 mycelial fragments/mL. Plants were scored for disease severity after 1 to 2 weeks.

Results

Antifungal metabolite production – Agar plate assays

Many of the Actinobacteria isolates showed varying degrees of pathogen suppression on agar plates, with several completely inhibiting the growth of the fungus. Four Actinobacteria isolates showed consistent inhibition of all of the tested pathogens (Table 1; Figure 1).

Table 1. Inhibition of fungal pathogen growth by four Actinobacterial isolates. (N/A = Not available)

Actinobacteria Isolate	Zone of inhibition (%) 0=no inhibition, 100=complete inhibition				
	Fusarium pseudograminearum CS5642	*Pythium irregulare*	Take all - *Gaeumannomyces graminis*	*Rhizoctonia solani* AG2-1 (ZG5)	*Sclerotinia sclerotiorum* S77
MH71	78	N/A	13	96	100
MH191	38	51	91	49	78
MH192	34	84	100	58	58
MH243	100	49	82	98	98

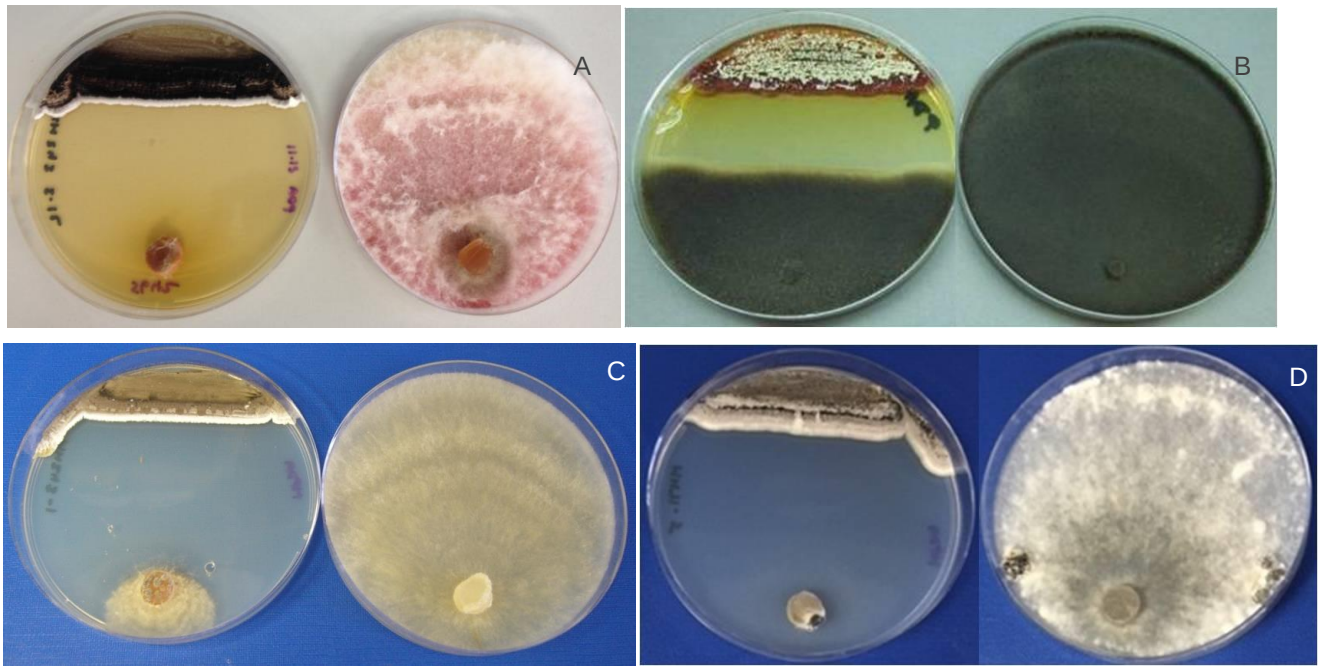

Figure 1. *In vitro* suppression of fungal pathogens by Actinobacteria. In each pair of images the plate on the left is inoculated with the anti-fungal Actinobacteria isolate at the top of the plate and the fungal pathogen at the bottom of the plate while the plate on the right is inoculated with the fungal pathogen only at the bottom of the plate. Image A shows Actinobacteria MH243 suppressing *Fusarium pseudograminearum* (crown rot). Image B shows MH191 suppressing *Gaeumannomyces graminis* (take-all). Image C shows MH243 suppressing *Rhizoctonia solani* AG2-1 (hypocotyl/root rot) and image D shows MH71 suppressing *Sclerotinia sclerotiorum* (sclerotinia stem rot).

Small plant assays

The isolates that exhibited the strongest antifungal activity in the plate tests were tested in small plant assays for their ability to suppress disease '*in planta'*. Significant disease suppression in soil-free small plant assays and in small pot assays has been measured for *Fusarium* crown rot (Table 2; Figure 2).

Small plant assays to test control of *Sclerotinia* and *Rhizoctonia* on canola are underway. Testing of plants grown to maturity in glasshouse pot trials is further informing decisions on the best selections to go to field trials.

Table 2. Suppression of root and crown disease in *Fusarium* crown rot infected wheat plants grown from seed coated with Actinobacteria in small plant assays

Actinobacteria Isolate	Suppression of disease (%)
	Fusarium pseudograminearum CS5642
MH71	83, 84[A]
MH191	28
MH192	0
MH243	75, 94[A]

[A] Suppression of disease in wheat cv. Wyalkatchem & cv. Tamaroi respectively.

Figure 2. Small plant assay showing suppression of disease in plants grown from seed coated with Actinobacteria isolate MH71. Healthy non-diseased plant (left); plants grown from seed coated with Actinobacteria and infected with *Fusarium pseudograminearum* (centre); plants infected with *Fusarium pseudograminearum* only (right).

Conclusion

Approximately 300 Actinobacteria were isolated as endophytes from within the roots of healthy plants. A small subset of these isolates demonstrated potential as biocontrol agents, expressing high-level, broad spectrum suppression of soil/stubble-borne fungal diseases in agar plate assays. This included antifungal activity against *Fusarium* crown rot, *Pythium* damping off, take-all root rot pathogens of wheat, and *Sclerotinia* stem rot and *Rhizoctonia* hypocotyl/root rot of canola. Suppression of a broad range of pathogens including *Sclerotinia* by individual Actinobacteria offers the potential to protect other susceptible crops such as legumes by reducing pathogen loads in the rotation cycle.

Assessment of antifungal activity against *Fusarium* crown rot in small plant assays demonstrated a reduction of between 75-94% in disease severity in wheat for two Actinobacteria isolates (MH71 and MH243). Smaller but significant reductions in disease were measured in glasshouse trials, so field testing is in development.

The Actinobacteria isolates are promising biocontrol candidates because they are adapted to Western Australian conditions, are readily culturable and can be introduced into the plant via a seed coat. Further wheat and canola disease assays are under investigation and, if proven successful, offer the potential for a quicker path to market than the development of disease resistant cultivars.

References

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Key words

Fusarium crown rot, Pythium damping off, Take-all, Rhizoctonia hypocotyl rot, Sclerotinia stem rot, biocontrol, Actinobacteria

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