Secondary metabolites produced by a novel isolate of *Metarhizium robertsii* (CPD006) during mass production

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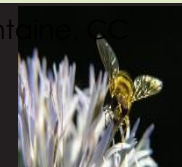
institute for sustainable horticulture



Outline

- Background
- Objective
- Materials and Methods
- Results
- Conclusions
- Acknowledgement

Background

- The Institute for Sustainable Horticulture (ISH) at Kwantlen Polytechnic University (KPU), Canada
- Secondary metabolites of *Metarhizium* anisopliae complex species
- Crop Defenders Ltd.

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Objectives

- To develop lab protocols for identifying key secondary metabolites of *Metarhizium robertsii* (CPD006)

- To quantify important metabolites produced at different time points during mass production

Materials and Methods

- Mass production
- Extraction
- Analysis

Materials and Methods - Mass production

Strains used:

Negative control

CPD006: *Metarhizium robertsii*

Positive control: ARSEF 3643 (*Metarhizium anisopliae* s.l.)

ARSEF 1724 (*Metarhizium anisopliae*)

Materials and Methods - Mass production

liquid culture phase Precultures → Inoculate → Incubate (5 d)

solid media phase Inoculate substrate → Incubate (12 d) → Drydown

Materials and Methods - Extraction

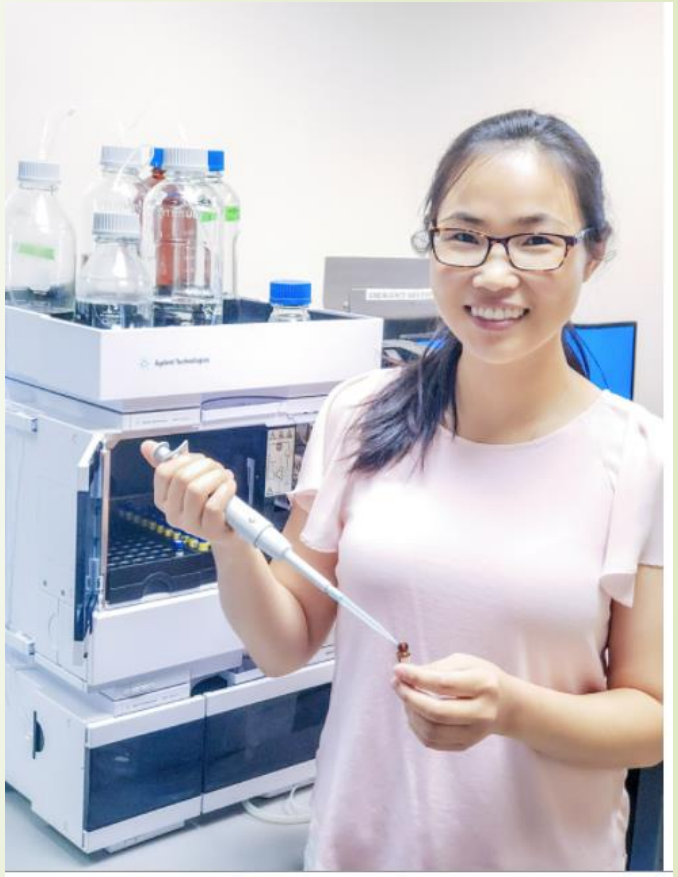
Extract at three time points

liquid culture	filter, ethyl acetate, dry (Walsh et al., 2019)
solid substrate	ethyl acetate for destruxin and cytochalasin and methanol for swainsonine (Amaral et al., 2014)
conidia	same as that for solid substrate

Materials and Methods – analysis

Agilent 1260 infinity II HPLC system

Destruxins	Xterra C18 column MilliQ water and acetonitrile (Golo et al., 2014)
Cytochalasins	Xterra C18 column (Amaral et al., 2014) MilliQ water and methanol
Swainsonine	Phenomenex Luna® HILIC column (Li et al., 2013) isoproponal



Results

Table 1. Quantity of destruxins A, B detected at three time points during mass production.

Treatment	Liquid culture		Solid substrate		Conidia	
	Dtx A (ppm)	Dtx B (ppm)	Dtx A (ppm)	Dtx B (ppm)	Dtx A (ppm)	Dtx B (ppm)
Negative Control	-[a]	-	-	-	N/A	N/A
ARSEF 3643	2.06[b] ± 0.254 a	1.51 ± 0.312 a	1.94 ± 0.572 a	0.76 ± 0.226 a	1.82 ± 0.433	3.45 ± 0.156
CPD006	0.23 ± 0.033 b	0.56 ± 0.026 b	0.39 ± 0.051 b	0.30 ± 0.078 a	-	-

[a] Not detected
[b] Means ± S.E. based on 4 replicates for each treatment.

Results

Table 2. Quantity of cytochalasin C (ppm) detected during mass production.

Treatment	Liquid culture	Solid substrate	Conidia
Negative control	-[a]	-	N/A
CPD006	0.004[b] ± 0.0013	0.027 ± 0.0063	-

[a] Not detected
[b] Means ± S.E. based on 4 replicates for each treatment.

Results

Table 3. Quantity of swainsonine (ppm) detected during mass production.

Treatment	Liquid culture	Solid substrate	Conidia
Negative control	-[a]	-	N/A
ARSEF 1724	0.79[b] ± 0.133 a	1.09 ± 0.483 a	-
CPD006	2.29 ± 1.075 a	0.75 ± 0.235 a	-

[a] Not detected
[b] Means ± S.E. based on 4 replicates for each treatment.

Conclusions

- CPD006 does produce destruxins A, B, cytochalasin C, and swainsonine;
- Conidia had none of these metabolites identified;
- The amount of these metabolites detected can vary.

Acknowledgment

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- Dr. Jean N.K. Maniania, Crop Defenders

References

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Questions?

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