Hossein Alizadeh

hossein.alizadeh@lincoln.ac.nz

Bio-Protection Research Centre
Lincoln University
Christchurch, New Zealand

Diwakar Kandula[1], John Hampton[1], Alison Stewart[2], David Leung[3] & Yasmine Edwards[4]

[1] Bio-Protection Research Centre, Lincoln University, Christchurch, New Zealand
[2] Marrone Bio Innovations Inc., Davis, CA, USA
[3] School of Biological Sciences, University of Canterbury, Christchurch, New Zealand
[4] School of Biological Sciences, University of Auckland, Auckland, New Zealand

Bio-Protection
Bioprotection science for New Zealand

Screening and identification of urease producing microorganisms from New Zealand pasture soils

Introduction

Urea is the most commonly used fertiliser in agricultural systems because of its relatively low price, high nitrogen (N) content and wide availability (Gagnon *et al*., 2012). However, urea N can be quickly lost from the system via ammonia volatilisation or nitrate leaching following urea hydrolysis by urease producing soil microorganisms (UPSMs). N availability to the plant is therefore reduced, and the production of nitrous oxide and leaching of soil nitrate contribute to environmental damage. Isolating and identifying culturable UPSMs would allow an investigation of the possibility of biological suppression of soil urease activity.

Methods

Soil preparation and inoculation

- A serial dilution of soil suspension was spread on urease detection medium and after incubation at 23°C for 2 days, colonies with a pink zone (fig. 1) were isolated, purified and later retested for urease activity.
- A method based on the chelation of components other than nucleic acids using Chelex® was applied to extract DNA from microorganisms (Walsh *et al*., 2013).
- To identify the isolated fungi to the species level, the internal transcribed spacer (ITS) region of the ribosomal operon was amplified using a standard polymerase chain reaction protocol. The amplification primers were as follows:

ITS1 (forward)	TCCGTAGGTGAACCTGCGG
ITS4 (reverse)	TCCTCCGCTTATTGATATGC

- The bacterial 16S rRNA genes were amplified using the following primers:

F8-27	AGAGTTTGATCCTGGCTCAG
R1510	GGTTACCTTGTTACGACTT

- The DNA yielded was visualized by electrophoresis in 1% (w/v) agarose gel pre-stained with RedSafe™ (fig. 2) and the samples were sequenced by an Applied Biosystems 3130xl Genetic Analyzer, with a 50cm array and POP7 installed as the standard platform. The sequence files generated from ITS sequencing were edited and assembled using ChromasPro software and compared to the nucleotide database at the US National Centre for Biotechnology Information (NCBI) to find the nearest relatives. Fungal morphological features were also observed under a light microscope and checked with published fungal descriptions (fig. 3).

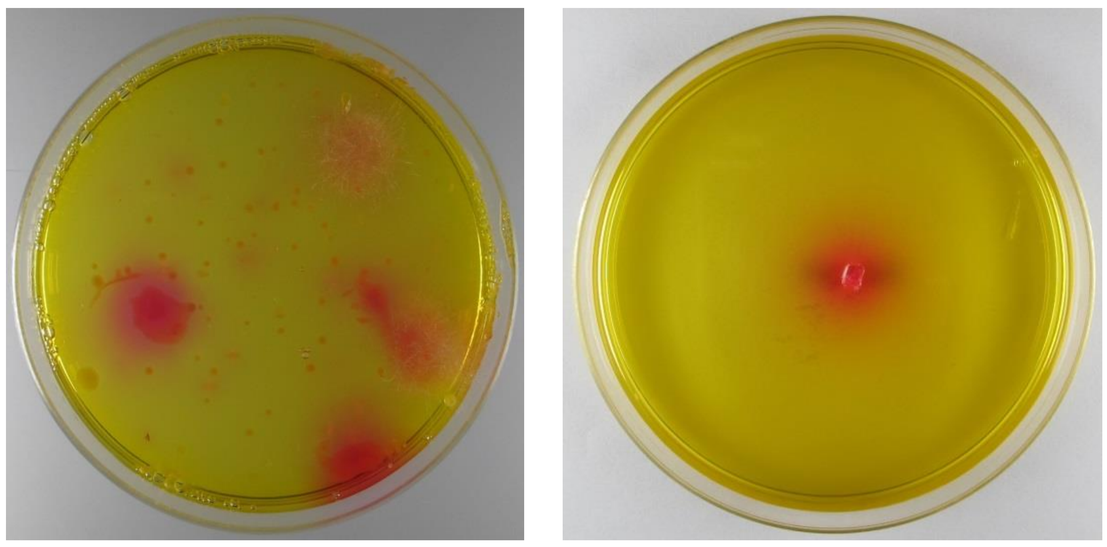

Figure 1. Urease detection medium for isolation of soil urease producing microorganisms (left) and a purified urease producing fungus (right).

Results

- Comparison of the gene sequences obtained from the test microorganisms with the sequences available in databases revealed their relationship with various species. Microorganisms isolated from New Zealand dairy pasture soils are presented in Table 1.
- This list includes a wide range of microorganisms including saprophytes (*Gliomastix* sp.), a human pathogen (*Trichosporon* sp.), an insect pathogen (*Paecilomyces marquandii*) and several plant pathogens (e.g *Fusarium solani*). *Cupriavidus* sp. and *Mucor hiemalis* showed strong urease activity on urease medium.

Results (continued)

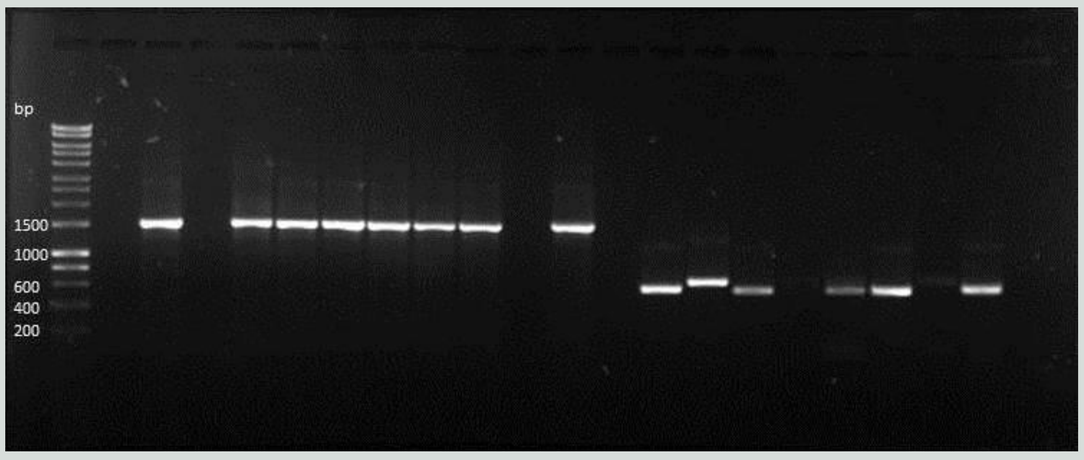

Figure 2. PCR amplification of 16S rRNA genes of bacteria and ITS regions of fungi using specific primers. The amplified fragments of 16S rRNA genes (from bacteria) and ITS regions of fungi represent 1500 and 600bp, respectively, when analysed by electrophoresis on a 1% agarose gel.

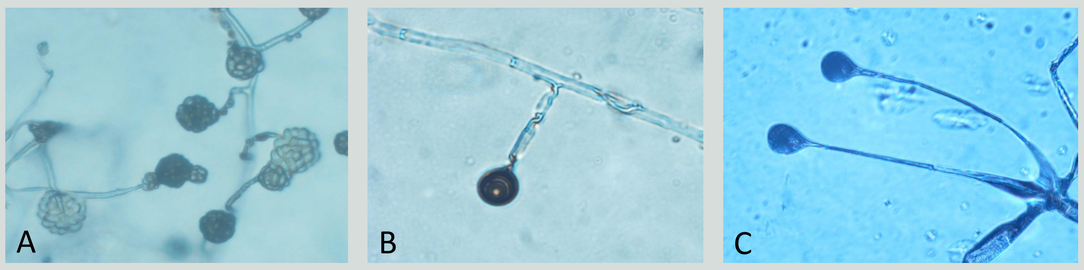

Figure 3. (A) Large spore balls of *Gliomastix* sp., (B) Aleuriospore of *Humicola grisea* and (C) Sporangia of *Absidia* sp.

Table 1. Culturable urease producing microorganisms isolated from New Zealand dairy pasture soils.

Scientific name	Location (s)
A. Fungi	
Absidia sp.	Canterbury (Oxford)
Chaetomium sp.	Marlborough
Cladosporium cladosporioides	Taranaki
Cordyceps sp.	Canterbury (Leeston)
Fusarium culmorum	Canterbury (Leeston)
Fusarium graminearum	Manawatu (Palmerston North)
Fusarium oxysporum	Auckland (Pukekohe), Canterbury, Otago, Waikato
Fusarium solani	Marlborough, Nelson
Geomyces sp.	Canterbury (Lincoln University)
Gliomastix sp.	Otago
Humicola grisea	Canterbury (Leeston & Lincoln University)
Lewia infectoria	Canterbury (Oxford)
Mariannaea sp.	Nelson
Mucor hiemalis	Taranaki, Wairarapa
Nectria sp.	Westport
Paecilomyces carneus	Canterbury (Lincoln University), Otago, Westport
Paecilomyces lilacinus	Waikato
Paecilomyces marquandii	Manawatu (Palmerston North), Waikato
Penicillium spinulosum	Palmerston North
Phoma exigua	Otago
Phoma paspali	Waikato
Pochonia bulbillosa	Auckland (Pukekohe), Manawatu (Palmerston North)
Thelonectria veuillotiana (*Cylindrocarpon candidulum*)	Canterbury (Oxford)
Trichosporon sp.	Taranaki
B. Bacteria	
Citrobacter freundii	Nelson
Cupriavidus sp.	Westport
Enterobacter ludwigii	Nelson
Pseudomonas chlororaphis	Canterbury (Lincoln University)
Rahnella aquatilis	Marlborough
Serratia proteamaculans	Wairarapa
Yersinia kristensenii	Taranaki

Conclusion and future work

Culturable microorganisms involved in urea degradation in New Zealand dairy pasture soils belong to a diverse genera of fungi and bacteria. Urease activity of these microorganisms differed amongst species. Biological suppression of culturable UPSMs is being investigated as a method to reduce soil urease activity.

References

Gagnon, B., Ziadi, N., Grant, C. (2012). Urea fertilizer forms affect grain corn yield and nitrogen use efficiency. *Canadian Journal of Soil Science*, 92: 341-351.
Walsh, P.S., Metzger, D.A., Higuchi, R. (2013). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, 54: 134-139.

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