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Urease producing microorganisms under dairy pasture management in soils across New Zealand

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ABSTRACT

Urea, the most commonly used nitrogen fertiliser in New Zealand, can be quickly lost from the system via ammonia volatilisation or nitrate leaching following hydrolysis of urea by urease producing soil microorganisms (UPSMs). This study investigated UPSMs involved in urea degradation for upcoming research to reduce soil urease activity. Soils from under dairy pasture management across New Zealand, with a pasture species component of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) and aged between 9 months to 60 years old, were collected, and UPSMs were isolated and identified using both polymerase chain reaction (PCR)-based molecular and conventional methods. The fungal genera belonged to diverse taxonomical groups including the phylum Ascomycota: class: Dothideomycetes, Eurotiomycetes, Leotiomycetes and Sordariomycetes, the phylum Basidiomycota: class: Tremellomycetes and the phylum Zygomycota: order: Mucorales, all of which have a role in urea degradation in soil. Pasture soil-resident urease producing bacteria belonged to the Gammaproteobacteria and Betaproteobacteria. *Cupriavidus* sp. and *Mucor hiemalis* showed strong urease activity when cultured on urease medium. This is the first report on the urease activity of the pasture soil inhabitants *Pochonia bulbillosa*, *Mariannaea elegans* and *Gliomastix* sp. This study was part of a larger study underway to investigate control of UPSMs in soil to improve the efficiency of urea utilisation.

1. Introduction

Nitrogen (N), a key nutrient for the grass component of dairy pastures, is commonly applied as urea. However, urea N can be quickly lost from the system via ammonia volatilization or nitrate leaching because of urea hydrolysis following the activities of urease producing soil microorganisms (Juan et al., 2009). N availability to the plant is therefore reduced, and the subsequent production of nitrous oxide and leaching of soil nitrate are contributors to environmental damage (Denier van der Gon and Bleeker, 2005). A correlation between lowered nitrous oxide emissions and a low concentration of soil ammonium nitrogen (which is a product of urea hydrolysis by urease) in wheat fields has been demonstrated (Jiang et al., 2015).

Urease (urea amidohydrolase; EC 3.5.1.5) is a nickel-dependent enzyme which can catalyse the conversion of urea to ammonia and carbon dioxide (Sirko and Brodzik, 2000), although the reason why the enzyme requires nickel but not other metals is not understood (Carter et al., 2009). Urease was the first enzyme that was crystallised (in 1926) from jack bean and its substrate, urea, was also the first organic molecule ever synthesised in a laboratory (in 1828) (Sirko and Brodzik, 2000). Urease can be found in plants, fungi, bacteria and some invertebrates, but not in animals (Carlini and Polacco, 2008; Polacco and Holland, 1993; Sirko and Brodzik, 2000; Smith et al., 1993). In general, urease enables the organism to use urea as a nitrogen source. In some bacteria, such as *Klebsiella aerogenes*, urea hydrolysis has enabled them to use urea as a sole source of nitrogen (Sirko and Brodzik, 2000). Urea is also assimilated by urease in plants (Sirko and Brodzik, 2000). In many human pathogens (e.g. *Proteus mirabilis*, *Yersinia enterocolitica*, *Staphylococcus saprophiticus*, *Helicobacter pylori* and *Ureaplasma urealiticum*), urease can act as a virulence factor, and this has been used as a simple test to identify virulent isolates. Hydrolysis of urea by *Helicobacter pylori*, the agent of peptic ulceration, in the stomach, is a method for this bacterium to increase pH of the acidic environment and colonise it (Carter et al., 2009; Follmer et al., 2004; Mobley et al., 1995;

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Table 1
Urease producing microorganisms.

No.	Scientific name	Reference
A. Fungi, actinomycetes and yeast species		
1	*Absidia* sp.	Donnison et al., 2000
2	*Alternaria tenuissima*	Hasan, 2000
3	*Aspergillus flavus*, *A. fumigatus*, *A. niger*	Hasan, 2000 & Smith et al., 1993
4	*Aureobasidium pullulans*	Hasan, 2000
5	*Botrytis cinerea*	Strope et al., 2011
6	*Cladosporium herbarum*	Hasan, 2000
7	*Coccidioides immitis*	Sirko and Brodzik, 2000
8	*Cochliobolus heterostrophus*	Strope et al., 2011
9	*Coprinus* sp.	Hasan, 2000
10	*Cryptococcus neoformans*	Hasan, 2000
11	*Emericella nidulans*	Hasan, 2000
12	*Fusarium nivale*	Hasan, 2000
13	*Geomyces destructans*	Reynolds and Barton, 2014
14	*Humicola grisea*	Hasan, 2000
15	*Magnaporthe oryzae*	Strope et al., 2011
16	*Malassezia furfur*	Hasan, 2000
17	*Mucor racemosus*	Hasan, 2000
18	*Mycosphaerella graminicola*	Strope et al., 2011
19	*Nectria haematococca*	Strope et al., 2011
20	*Neurospora* sp., *N. crassa*	Hasan, 2000 & Strope et al., 2011
21	*Paecilomyces silvatica*	Hasan, 2000
22	*Penicillium* spp., *P. brevicompactum*, *P. notatum*	Hasan, 2000
23	*Rhizopus oryzae*	Strope et al., 2011
24	*Saccharomyces cerevisiae*	Hasan, 2000
25	*Saccharomycopsis Malanga*	Hasan, 2000
26	*Schizosaccharomyces pombe*	Sirko and Brodzik, 2000
27	*Sepedonium chrysospermum*	Hasan, 2000
28	*Stagonospora nodorum*	Strope et al., 2011
29	*Streptomyces aureofaciens*	Hasan, 2000
30	*Trichophyton mentagrophytes*	Hasan, 2000
31	*Ustilago* spp.	Hasan, 2000
B. Bacteria		
32	*Anabaena cycadeae*	Hasan, 2000
33	*Arthrobacter crystallopoietes*	Wen et al., 2015
34	*Bacillus lentus*, *B. subtilis*, *B. sphaericus*	Hasan, 2000
35	*Bordetella pertussis*	Strope et al., 2011
36	*Brevibacterium ammoniagenes*, *B. stationis*	Burbank et al., 2012 & Hasan, 2000
37	*Burkholderia* sp.	Strope et al., 2011
38	*Corynebacterium glutamicum*	Wen et al., 2015
39	*Enterobacter cloacae*	Kang et al., 2015
40	*Helicobacter pylori*	Carter et al., 2009
41	*Klebsiella aerogenes*	Mobley et al., 1995
42	*Kocuria marina*	Wen et al., 2015
43	*Lactobacillus reuteri*	Hasan, 2000
44	*Lysinibacillus sphaericus*	Burbank et al., 2012
45	*Morganella morganii*	Mobley et al., 1995
46	*Mycobacterium tuberculosis*	Strope et al., 2011
47	*Nitrosomonas* sp.	Hasan, 2000
48	*Nitrosospira* sp.	Hasan, 2000
49	*Proteus mirabilis*	Carter et al., 2009
50	*Providencia vermicola*	Burbank et al., 2012
51	*Pseudomonas aeruginosa*, *P. fluorescens*	Wen et al., 2015 & Strope et al., 2011
52	*Rhodobacter capsulatus*	Hasan, 2000
53	*Sarcina urea*	Hasan, 2000
54	*Sinorhizobium medicae*	Strope et al., 2011
55	*Sporosarcina pasteurii*, *S. ureae*	Burbank et al., 2012 & Mobley et al., 1995 & Wen et al., 2015
56	*Staphylococcus saprophiticus*	Mobley et al., 1995
57	*Streptococcus salivarius*	Hasan, 2000
58	*Thiocapsa roseopersicina*	Hasan, 2000
59	*Ureaplasma urealyticum* (with the highest urease activity demonstrated)	Mobley et al., 1995
60	*Vibrio parahaemolyticus*	Sirko and Brodzik, 2000
61	*Yersinia enterocolitica*, *Yersinia pestis*	Strope et al., 2011 & Mobley et al., 1995

Sirko and Brodzik, 2000). Recently, it was demonstrated that blocking urease could improve plant tolerance to salt during seed germination as a result of reduced ammonium production (Bu et al., 2015).

Soil urease activity can be affected by factors including moisture, temperature, soil organic matter, pH and the rate of fertiliser applied (Carter et al., 2009). A number of microorganisms have been reported to produce urease (Table 1).

Around 90% of applied urea can be hydrolysed in the soil within 1–2 days of the application (Hojito et al., 2010). The half-life of urea degrading spontaneously (even at 57 °C) is around 3.6 years, (Hasan, 2000) but in the presence of urease it can be degraded 10,000 times faster (Amtul et al., 2002) suggesting a need for urease inhibitors. A number of enzymatic chemical inhibitors have been developed (Mobley et al., 1995). The use of Agrotain, a commercial urease inhibitor, in New Zealand ryegrass pastures has been reported to increase N uptake and herbage dry matter (Dawar et al., 2011).

New Zealand's pastoral production industries are heavy users of nitrogen (N) fertilisers (Moir et al., 2007). Despite the importance of urea and the environmental implications of its degradation in agricultural soils, the potential contribution of the urease producing microorganisms inhabiting pasture soils has not been investigated. The current study was designed to isolate and identify New Zealand's urease producing soil microorganisms. The results can be used for future studies to work on urease reduction strategies in pastures.

2. Materials and methods

2.1. Soil sample collection and processing

Soil samples were obtained from ryegrass (*Lolium perenne* L.) - white clover (*Trifolium repens* L.) based pastures under dairy management (hereafter referred to as pasture soils) in New Zealand located in Auckland, Canterbury, Manawatu, Marlborough, Nelson, Otago, Taranaki, Waikato, Wairarapa and West Coast (Table 2). To take samples, a straight sampling line (mostly a diagonal from corner to corner of a field) was selected and along the line six samples of similar size (400 g) were taken using a soil corer to a depth of 15 cm. The soil samples were thoroughly mixed and approximately 1 kg soil for each sample at each sampling time was placed in a sealed bag which was transferred to the laboratory and kept in a refrigerator until used for the experiments.

2.2. Urease detection medium

To detect microbial urease activity, a urease detection medium was prepared according to Christensen (1946) and MacFaddin (2000) with some modifications. Briefly, 1 g peptone, 1.5 g dextrose, 1.2 g sodium chloride, 0.016 g phenol red, 0.1 g nickel (II) sulphate, and 0.8 g monopotassium phosphate were dissolved in distilled water, pH was adjusted to 6.8 and after adding 18 g agar to the medium, the final volume was made to 800 ml and autoclaved. Urea (20 g product) was separately dissolved in distilled water up to a volume of 200 ml. The solution was sterilised using a Millipore membrane (0.22 μm sterile filter units), added to the autoclaved medium prior to agar setting (45 °C) and dispensed to 8 cm Petri plates.

2.3. Isolation of UPSMs from soil

One gram of each soil was placed in 9 ml sterile water in a Universal container and shaken for 10 min. A serial dilution was prepared and 100 μl from each dilution was spread on urease detection medium. The Petri plates were incubated at 23 °C and colonies with a pink zone (Christensen, 1946) were isolated, cultured and purified (fungi using either a single spore or hyphal tip method; Rangaswami and Bagyaraj,