

UW – Green Bay Research Council

Grants-In-Aid of Research

Cover Page

Name: Alma E. Rodriguez Estrada

Phone: 920-465-2247

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Address: LS-411

Budgetary Unit: Natural and Applied Sciences

Project Title: Isolation and Identification of Microorganisms from Soil Samples

This proposal is requesting funds for:

- First Priority Activities (Data/materials collection)
- Second Priority Activities (Travel to conference for research presentation)

Amount Requested: \$ 650.03

Anticipated Dates: April - June 2011

Is Institutional Review Board (IRB) approval required? Y or N

Is Institutional Animal Care and Use Committee (IACUC) approval required?

Y or N

If awarded funding, proposals are open to review upon request.

Proposals must be submitted as a single PDF document containing:

- Cover Page
- Proposal Narrative (Page limit: 3) (Refer to guidelines)
- Budget of Expenses (Page limit: 1) (Refer to guidelines)
 - Use the template provided at www.uwgb.edu/rc/giar.htm
- Curriculum Vitae (Page limit: 2) (Refer to guidelines)

Electronic submissions only

Proposals must be received by 4:30 p.m. Monday, October 25, 2010.

E-mail your completed proposal as a single PDF file to nonnl@uwgb.edu

Late and/or incomplete applications will not be considered.

Questions? Contact Charmaine Robaidek (robaidec@uwgb.edu), Lidia Nonn or a Council member.

Institute for Research
WH 303
Phone: 2784/2565
Fax: 2043

Isolation and Identification of Microorganisms from Soil Samples

Alma E. Rodriguez Estrada, Assistant Professor

Fungi are cosmopolitan organisms with unique physiological abilities. Fungi are the most important organism in organic matter degradation with exceptional abilities to break down complex polymers. Studies have shown that certain groups of fungi (mainly white-rot fungi) can degrade recalcitrant chemicals (Mougin et al. 1994, Sanyal and Kulshrestha 2003, Lee et al. 2005). The use of fungi in bioremediation *in situ* and bioreactors offers an alternative to expand the substrate ranges that prokaryotes, widely use in bioremediation, cannot degrade (Eggen 1999, Leidig et al. 1999, Pointing 2001).

Diversity of microorganisms in the soil and how organisms' communities change in response to environmental factors is crucial to understand the impacts of disturbance in biodiversity. In addition, the identification of species that have developed mechanism to overcome disturbance has broader implication. For example, certain fungi isolated from polluted soils have the ability to degrade, absorb or inactivate toxic compounds.

The state of Wisconsin is strong in agriculture. Dairy products and corn cultivation for grain are among the most important agricultural goods in the state. Therefore, soil pollution with herbicides and estradiols is significant.

I am interested in isolating soil microorganisms, mainly fungi, seeking to detect strains that might be well adapted to breakdown herbicides and estradiols. Native strains will be suitable to use during *in situ* bioremediation (bioaugmentation). Moreover soil samples collected during this study can be further processed for large-scale sequencing studies where soil microbiota is evaluated through molecular approaches. The current proposal focuses on:

- 1) Soil Collection
- 2) Isolation of microorganism
- 3) Taxonomic identification

This work is the beginning of my research at UWGB that focuses on studying the diversity and dynamics of local fungal communities and the utilization of fungi in agriculture, bioremediation and as sources of natural products.

The following table describes the materials needed for soil collection, sample processing, microorganisms isolation, microorganisms storage and taxonomic identification.

Item	Vendor / Catalog Number	Specifications	Units	Price per Unit \$	Total Price \$
Soil collection containers	VWR / 15704-012	VWR® Specimen Containers, Polypropylene	1	122.07	122.07
Petri dishes	Fisher / 08-757-13	Fisherbrand* Petri Dishes. Polystyrene, sterilized (500).	1	147.10	147.10
Potato Dextrose Agar	Fisher / R454312	Potato Dextrose Agar (500 g)	1	104.89	104.89
Cryovials	Fisher / 1050026	Polypropylene cryovials (2 mL, package of 100)	1	46.97	46.97
Antibiotics	VWR / 80058-670	Streptomycin Sulfate, <i>Streptomyces</i> sp., Calbiochem*	1	59.00	59.00
Total					650.03

References:

Eggen T. 1999. Application of fungal substrate from commercial mushroom production – *Pleurotus ostreatus* – for bioremediation of creosote contaminated soil. Int Biodeter Biodegr 44:117–126

Lee SM., Koo BW, Choi JW, Choi DH, An BS, Jeung EB, Choi IG. 2005. Degradation of Bisphenol A by white rot fungi, *Stereum hirsutum* and *Heterobasidium insulare*, and reduction of its estrogenic activity. Biol Pharm Bull 28: 201–20

Leidig E, Pruesse U, Vorlop KD, Winter J (1999) Biotransformation of Poly R-478 by continuous cultures of PVAL-encapsulated *Trametes versicolor* under non-sterile conditions. *Bioprocess Eng* 21:5–12

Mougin C, Laugero C, Asther M, Dubroca J, Frasse P (1994) Biotransformation of the herbicide atrazine by the white-rot fungus *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 60:705–708

Pointing SB. 2001. Feasibility of bioremediation by white-rot fungi. *Appl Microbiol Biotechnol* 57:20–33

Sanyal D. and Kulshrestha G. 2003. Degradation of metolachlor in soil inoculated with a mixed fungal culture. *Biol Fertil Soils* 38:253–256

Grants in Aid Of Research Budget

Name of Applicant Alma E. Rodriguez Estrada	Budgetary Unit Natural and Applied Sciences Name of ADA (Academic Department Associate) Carol Wautlet	Telephone No. 920-465-2247 (Rodriguez) 920-465-2370 (Wautlet)										
Budget Category	Grant Request Amount	Match (Not Required)										
Match Type (i.e., monetary, service, or supplies) & Source												
a. Research Supplies and Expenses	650.03											
<table style="width: 100%; border: none;"> <tr> <td style="width: 80%;">Soil collection containers</td> <td style="text-align: right;">122.07</td> </tr> <tr> <td>Petri dishes</td> <td style="text-align: right;">147.10</td> </tr> <tr> <td>Potato Dextrose Agar</td> <td style="text-align: right;">104.89</td> </tr> <tr> <td>Cryovials</td> <td style="text-align: right;">46.97</td> </tr> <tr> <td>Antibiotics</td> <td style="text-align: right;">59.00</td> </tr> </table>	Soil collection containers	122.07	Petri dishes	147.10	Potato Dextrose Agar	104.89	Cryovials	46.97	Antibiotics	59.00		
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Petri dishes	147.10											
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Cryovials	46.97											
Antibiotics	59.00											
b. Travel (mileage, lodging, meals)												
Name or number of people <div style="margin-left: 150px;"> Rate and distance and/or charge (Click for UW System Travel Regulations) </div>												
c. Other												
TOTAL	650.03											

Describe how funds will be utilized: Funds will be used to purchase supplies for sample collection, isolation of microorganisms and storage.

Alma Edith Rodriguez Estrada, Ph.D.

University of Wisconsin Green Bay
Department of Natural and Applied Sciences
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EDUCATION

Ph.D. Plant Pathology

2005 – 2008

The Pennsylvania State University, Department of Plant Pathology

Dissertation: Molecular phylogeny and increases of yield and the antioxidants selenium and ergothioneine in basidiomata of *Pleurotus eryngii*

M.S. Plant Pathology

2003 – 2005

The Pennsylvania State University, Department of Plant Pathology

Dissertation: Influence of substrate composition and mushroom strains on productivity and susceptibility of *Pleurotus eryngii* to bacterial blotch disease

PUBLICATIONS

Peer Reviewed

- **Rodriguez Estrada, A. E.**, Hegeman, A., and May, G. Secondary Metabolites Mediate Interactions between *Fusarium verticillioides* and *Ustilago maydis* (in review)
- **Rodriguez Estrada, A. E.**, Jimenez-Gasco, M. M., and Royse, D. J. 2010. *Pleurotus eryngii* species complex: sequence analysis and phylogeny based on partial *EF1 α* and *RBB2*. Fungal Biology 114: 421-428.
- **Rodriguez Estrada, A. E.**, Royse, D. J. and Jimenez-Gasco, M. M. 2009. Improvement of yield of *Pleurotus eryngii* var. *eryngii* by substrate supplementation and use of a casing overlay. Bioresource Technology 100: 5270-5276.
- **Rodriguez Estrada, A. E.**, Lee, H. J., Beelman, R. B., Jimenez-Gasco, M. M. and Royse, D. J. 2009. Enhancement of the antioxidants ergothioneine and selenium in *Pleurotus eryngii* var. *eryngii* basidiomata through cultural practices. World Journal of Microbiology and Biotechnology. DOI 10.1007/s11274-009-0049-8.
- **Rodriguez Estrada, A. E.** and Royse, D. J. 2007. Yield, size and bacterial blotch resistance of *Pleurotus eryngii* grown on cottonseed hulls/oak sawdust supplemented with manganese, copper and whole ground soybean. Bioresource Technology 98: 1898-1906.

Manuscripts in Conference Proceedings

- **Rodriguez Estrada, A. E.**, Royse, D. J. and Jimenez-Gasco, M. M. 2008. Nucleotide sequence polymorphisms of the partial β -tubulin gene in two varieties of *Pleurotus eryngii*. 17th Congress of the International Society for Mushroom Science. Cape Town, South Africa. Mushroom Science 17: 83-96 (CD-ROM)

