Identifying Pesticide Degrading Microorganisms for in situ Bioremediations

Introduction and Literature Review:

Since the beginning of the twentieth century, there have been numerous amounts of dangerous pesticides that have been utilized in agricultural environments for the means of protecting crop production. In the United States, pesticides that contain dangerous chemical compounds have become increasingly used in households and agricultural environments (Kiley *et al.,* 2004). As a result, groundwater, sediments and surface water may be under a threat of pesticide contamination. Among pesticides, organophosphates are known for being highly toxic to insects and some aquatic life, however human exposure to these pesticides are highly toxic and harmful to the long term health of consumers and our environment. (Bonner *et al.,* 2007) According to the National Pesticide Information Center (NPIC), organophosphate-based pesticides such as malathion affect the nervous system when exposed to humans. This exposure to malathion in most cases leads to headaches, decrease in heart rate and abdominal pains and diarrhea (Gervais et al., 2012). Therefore, certain pesticides have been banned for use in many countries as they have been deemed dangerous for any level of use (Bhattacharyya *et al.,* 2009).

Microorganisms have been used to remediate various environmental pollutants. For example, recent research has concluded the use of microorganisms as a means of bioremediation as a safe counter response to oil and plastic pollutions (Vidiali., 2001). Microorganisms that already exist in the environment are being identified and proliferated for the use of *in situ* bioremediation for oil disasters and pollution (Vidiali., 2001). The use of microorganisms for *in situ* bioremediation may also be an effective way to counteract various pesticide contaminations.

Specific Aims:

Organophosphates are toxic to humans and pose severe the use of microorganisms can be utilized as a safe remediation tool. Therefore, in this study we propose to 1) investigate the potential of organophosphate degradation by soil microbial communities from two different soil ecosystems. 2) Isolate organophosphate compound degrading bacteria, which will provide a tool for bioremediation of organophosphate compounds. If pesticide is in use in the area which the soil sample has been taken, then there should be some microorganisms which have the ability to thrive in the presence of pesticide.

The aim of this research is to identify and isolate microorganisms that have the ability to degrade organophosphates. As a result, we hope this accomplishment will degrade pesticide into less harmful and toxic arrangements. This can be used for in situ bioremediation purposes that will allow the use of microorganisms to remove harmful organophosphates. Organophosphates such as malathion and other popular products will be used in this experiment as the sole carbon and energy source for microorganisms.

Research Design and Methods:

For our first hypothesis, we predict that if the soil microbial community is acquired from organophosphate contaminated area, then these microbial communities may have potential to remediate organophosphate compound contamination. This will increase the likelihood that microorganisms in soils contaminated with pesticides will have organophosphate degrading bacteria. This will provide a tool for organophosphate degradation for the use of bioremediation. We expect that these isolated microorganisms will have the ability to degrade toxic chemical

compounds found in pesticide products such as organophosphates. We hypothesize that if soils are exposed to malathion and various organophosphates, bacteria therein may utilize these compounds as a source of energy and degrade these organophosphates as result.

Collecting soil samples.

Soil samples will be collected from two different locations, 1) a soil sample from the surface of both an agriculture area, which pesticide has been used and 2) an area within a mile distance which pesticide has not been used.

Community DNA sequencing.

Community DNA will be extracted from those soils and sent out for sequencing to determine the composition and structure of soil microbial community.

Isolation of OPC-degrading bacteria.

We will use a minimal medium containing OPC only or OPC + glucose. The glucose in the minimal medium will serve as the primary carbon and energy source for microorganisms while the organophosphate as the secondary carbon and energy source for cometabolism. Initial concentration of OPC will be 10 mg/L. A MPN method along with spread plate technique will be used to estimate the number of viable microorganisms that can grow on media containing various organophosphates. Individual colonies will be isolated using the streak plate method and further purified.

Identification of OPC-degrading isolates.

Purified isolates will be identified by extracting genomic DNA and sequencing the small subunit ribosomal RNA gene. The 16S rRNA gene sequences of all isolates will compared to those at NCBI database. Phylogenetic analysis will then be performed using Mega6.0.

HPLC analysis.

The HPLC method will be used to determine the amount of organophosphates left in the media over time to ensure the degradation of the OPC.

References:

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