Resistance of rhizobia against different pesticides and detection of nitrogen fixing protien sequences

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ABSTRACT

Pesticides are used for the increase yield of the crops, Rhizobia are bacteria which fixes the nitrogen by the formation of legumes. In this paper we had checked whether the pesticide affects the Rhizobium or not i.e. the whether the rhizobium is resistant to the particular pesticide (e.g. Monochrotophos). Chick pea i.e. Cicer arietinum is one example rhizobium leguminosarum had showed the result, i:e it is resistant to the pesticide monocrotophos in additionally we had taken the sequence of the chick pea compare with the sequence of the other sequence of the rhizobium we had codended that other sequence matched with the cicer arietinum i.e. Fixes the nitrogen taken the 2d & 3D structure.

Keywords: Rhizobia, monocrotophos, MIC, alignment, 2D structure prediction, 3D structure prediction.
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INTRODUCTION

RHIZOBIA-

This module introduces the general role of microorganisms in the soil, and specifically the rhizobia. Rhizobia are special bacteria that can live in the soil or in nodules formed on the Roots of legumes. In root nodules, they form a symbiotic association with the legume, obtaining nutrients from the plant and producing nitrogen in a process called biological nitrogen fixation, or BNF. The rhizobia are broadly classified as fast- or slow-growing based on their growth on laboratory media. Rhizobia are further classified according to their compatibility with particular legume species. Farmers can stimulate BNF by applying the correct rhizobia to their legume crops, a process called inoculation. The module describes the diversity of rhizobia and the selection of superior strains, as well as plant and environmental factors that affect rhizobia in the soil.

Soil microorganisms play many important roles. Rhizobia are special soil microorganisms that can form a symbiotic relationship with legumes resulting in biological nitrogen fixation, or BNF.Rhizobia are classified according to the legume species that they nodulate — a Concept known as "cross-inoculation" groups.

To achieve effective BNF, legumes must be inoculated with the correct rhizobia. Superior strains of rhizobia can be selected as inoculants. Plant and environmental factors affect native and introduced rhizobia in the soil.
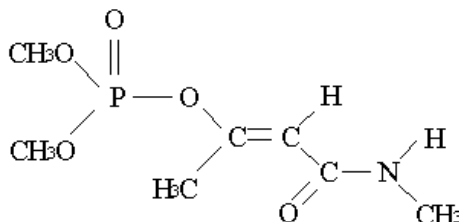
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MONOCROTOPHOS
Common Name-
Monocrotophos
CAS-No.6923-22-4
Use- Insecticide, acaricide with systemic and contact action
Formulation Types - Water miscible, soluble concentrate; Ultra Low Volume Spray

1. *Formula* : C7H14N05P

2- *Chemical Name* : Dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate (IUPAC)
3- *Chemical Type* : Organophosphate
4- *Solubility* : 1 kg/I (20°C, water) ; LogPow -0.22-0.5 (calculated)
5- *Vapour Pressure* : 0.29 mPa (20°C)
6- *Melting Point* : 54-55 °C
7- *Reactivity* - Decomposes above 38°C; unstable in short chain alcohols; half-life in aqueous solutions range from 96 days (pH 5) to 17 days (pH 9); monocrotophos is corrosive to black iron, drum steel and stainless steel. Further information in Tomlin, 1994 and IPCS, 1993

8- *General*
Mode of action : Monocrotophos affects the nervous system by inhibiting acetyl cholinesterase, an enzyme essential for normal nerve impulse transmission.

Uptake : Monocrotophos can be absorbed following ingestion, inhalation and skin contact.

Metabolism : In mammals, the primary conversion products of monocrotophos are dimethyl phosphate, O-desmethyl monocrotophos and N-desmethyl monocrotophos. N-desmethyl monocrotophos is more toxic than monocrotophos.

Concentration µg/ml	Growth of bacteria
100	+
200	+
300	+
400	+
500	+
600	+
700	-
800	-
900	-
1000	-
Blank	-

'+'indicates growth of bacteria (resistant to monocrotophos), '–'indicates inhibition of growth of bacteria (sensitive to monocrotophos).

Determination of MIC by agar plate dilution method . YEMA Agar plate dilution test was used to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent.

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Interpretation of results -
The MIC represents the concentration of antimicrobial at which there is complete inhibition of growth. In reading the end points, a barely visible haze of growth or a single colony is disregarded.

RESULTS AND DISCUSSION

Plates that have concentration 700 μg/ml showed no growth hence that concentration is MIC for Rhizobia

Sequence use for alignment (GOR an chau fasman 2D and spdv for 3D)

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Accession no. from Protein Data Bank
>gi|240856680|gb|ACS54347.1| Scaffold protein Nfu/NifU [Rhizobium leguminosarum bv. trifolii WSM1325]
MFIQTEATPNPATQKFLPGKVVMENGTAEFRSTEEAQASPLAARLFEISGVTGVYFGYDFISVSKDNADW
QHLKPAILGSIMEHFMSGKPVMGDASILSEDADAGDEFFDEGDESIVLTIKELLETRVRPAVAQDGGDIT
FRGFKDGKVYLNMKGSCAGCPSSTATLKHGVQNLLRHFVPEVQEVIAA

E-value-1e.79

Display Settings: ☑ GenPept   Send to: ☑ | Change region shown ▼ |

| Customize view ▼ |

nitrogen-fixing NifU-like protein [Roseovarius sp. TM1035]

NCBI Reference Sequence: ZP_01879943.1

FASTA   Graphics

Analyze this sequence ▲
Run BLAST

Go to: ☑

| Identify Conserved Domains |

LOCUS   ZP_01879943   187 aa   linear   BCT 09-NOV-
2010
DEFINITION nitrogen-fixing NifU-like protein [Roseovarius sp. TM1035].
ACCESSION   ZP_01879943
VERSION   ZP_01879943.1 GI:149202972
DBSOURCE   REFSEQ: accession NZ_ABCL01000005.1
KEYWORDS   .
SOURCE   Roseovarius sp. TM1035
  ORGANISM Roseovarius sp. TM1035
            Bacteria; Proteobacteria; Alphaproteobacteria; Rhodobacterales;
            Rhodobacteraceae; Roseovarius.
COMMENT   PREDICTED REFSEQ: This record has not been reviewed and the
            function is unknown. The reference sequence was derived from
            EDM31554.
            Method: conceptual translation.
FEATURES   Location/Qualifiers
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                     /strain="TM1035"
                     /db_xref="taxon:391613"
     Protein   1..187
                     /product="nitrogen-fixing NifU-like protein"
                     /calculated_mol_wt=19872
     Region   3..89
                     /region_name="Nfu_N"
                     /note="Scaffold protein Nfu/NifU N terminal; cl07364"
                     /db_xref="CDD:208680"
     Region   109..185

Highlight Sequence Features
Find in this Sequence

Identical proteins for ZP_01879943.1 ▲
nitrogen-fixing NifU-like protein [Ros [EDM31554]
See all...

Related information ▲
BLink
Related Sequences
Identical Proteins
BioProject
CDD Search Results
Conserved Domains (Concise)
Conserved Domains (Full)
Domain Relatives
Genome
Nucleotide

_____

>gi|209533392|gb|ACI53327.1| Scaffold protein Nfu/NifU [Rhizobium leguminosarum bv. trifolii WSM2304]
MFIQTEATPNPATQKFLPGKVVMENGTAEFRSAEEAEASPLAARLFEIPGVTGVYFGYDFISVSKDDAEW
QHLKPAILGSIMEHFMSGKPVMGDASILSEDVDAGDEFFDEGDESIVLTIKELLETRVRPAVAQDGGDIT
FRGFKDGKVYLNMKGSCAGCPSSTATLKHGVQNLLRHFVPEVQEVIAA

Alignment Result:-

Query sequence	Subject
Sequence no.1 >gi|240858659|gb|ACS56326.1| nitrogen-fixing NifU domain protein [Rhizobium leguminosarum bv. trifolii WSM1325]	LOCUS CBI80951 145aa Linear BCT 25May2011 DEFINATION Conserved Hypothetical Protein [Bartonella sp. 1-1c] E-value 1e.40
Sequence no.2 >gi|209535198|gb|ACI55133.1| nitrogen-fixing NifU domain protein [Rhizobium leguminosarum bv. trifolii WSM2304]	LOCUS CBI179370 145aa Linear BCT 25May2011 DEFINATION Conserved Hypothetical Protein [Bartonella sp.AR 15-3] E-value 1e.40
Sequence no.3 >gi|240856680|gb|ACS54347.1| Scaffold protein Nfu/NifU [Rhizobium leguminosarum bv. trifolii WSM1325]	LOCUS ZP_01879943 187aa Linear BCT 09 Nov 2010 DEFINATION nitrogen fixing NifU-like protein [Roseovarius sp. TM1035] E-value 1e.79
Sequence no.4 >gi|209533392|gb|ACI53327.1| Scaffold protein Nfu/NifU [Rhizobium leguminosarum bv. trifolii WSM2304]	LOCUS ZP_0174087 187 aa linear BCT 09-NOV-2010 DEFINITION nifU domain protein [Rhodobacteraceae bacterium HTCC2150]. E-value 1e.79

2D structure prediction method (GOR and chau fasman)
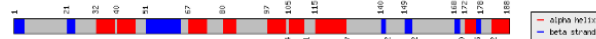
Sequence 3

Name of the sequence is *gi|240856680|gb|ACS54347.1| Scaffold protein Nfu/NifU [Rhizobium leguminosarum bv. trifolii WSM1325].*

Sequence consists of 188 amino acids.

Target Sequence:

MFIQTEATPN PATQKFLPGK VVMENGTAEF RSTEEAQASP LAARLFEISG VTGVYFGYDF ISVSKDNADO QHLKPAILGS IMEHFMSGKP VMGDASILSE DADAGDEFFD EGDESIVLTI KELLETRVRP AVAQDGGDIT FRGFKDGKVY LNMKGSCAGC PSSTATLKHG VQNLLRHFVP EVQEVIAA

Secondary Structure:

```
            *        *        *        *        *
Query 1   MFIQTEATPNPATQKFLPGKVVMENGTAEFRSTEEAQASPLAARLFEISGVTGVYFGYDF 60
Helix 1   <---->    <----------->    <------------------->    < 60
Sheet 1   EEEEEEE      EEEEEEEEEE              EEEEEEEEEEEEEEEEEEEE 60
Turns 1       T     T   T    T      T       T                    60

            *        *        *        *        *
Query 61  ISVSKDNADOQHLKPAILGSIMEHFMSGKPVMGDASILSEDADAGDEFFDEGDESIVLTI 120
Helix 61  ------------------------>       <------------------------ 120
Sheet 61  EE     EEEEEEEEEEEEEEEE                          EEEEE 120
Turns 61     TT T      T            TT        T       T   T T   120

            *        *        *        *        *
Query 121 KELLETRVRPAVAQDGGDITFRGFKDGKVYLNMKGSCAGCPSSTATLKHGVQNLLRHFVP 180
Helix 121 ------------->    <---------->          <-------------- 180
Sheet 121 EEEEEEEEE          EEEEE      EEEE          EEEEEEEEEEEEEEEE 180
Turns 121      T    T    T T       TT       T       T       T      180


Query 181 EVQEVIAA 188
Helix 181 ------> 188
Sheet 181 EEEEE   188
Turns 181         188


Total Residues: H: 139   E: 100   T: 25
       Percent: H: 73.9  E: 53.2  T: 13.3
```

Result of comparing Chou-Fasman method, GOR method and Neural Network method

Summary:

Details:

```
                .         .         .         .         .
AAseq.   1 MFIQTEATPNPATQKFLPGKVVMENGTAEFRSTEEAQASPLAARLFEISGVTGVYFGYDF
CF         EEECCCCCCCCCCCCCCEEECCCCHHHHCCHHHHHCCCHHHHHHHCEEEEEEEEEEEEEE
GOR        EEEECCCCCCCCHHECCTTCEEEETTTHHHHHHHHHHHHCHHHHHHEEETTEEEEEEEEEE
NN         EEEECCCCCCCCCCCCCCCEEECCCCCCCCCCCCCCHHHHHHHHHHHECCCEEEEEEEEE
Joint      EEEECCCCCCCCCCCCCCCCEEECCCCCCCCCHHHHHHHCHHHHHHHHCCCCEEEEEEEEE

                .         .         .         .         .
AAseq.  61 ISVSKDNADWQHLKPAILGSIMEHFMSGKPVMGDASILSEDADAGDEFFDEGDESIVLTI
CF         EECCHHHHHHHHHHEEEEHHHHHCCCCCEECCCEEHHHHHHHHHHHHHHHCCEEEEEEHH
GOR        EEETTTHHHHHHHCTHHHTHHHHHHHTTCCCCCCCHHHHHHHTHHHHHTTHHHHHHHH
NN         EEECCCCCHHHHCCCCCCCHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCHHHHHHH
Joint      EEECCCHHHHHHCCCCCCHHHHHCCCCCCCCCCCCHHHHHHHCHHHHHHCCCCHHHHHH

                .         .         .         .         .
AAseq. 121 KELLETRVRPAVAGDGGDITFRGFKDGKVYLNMKGSCAGCPSSTATLKHGVQNLLRHFVP
CF         HHHHEEEECCHHHHCCCEEEEECCCCCCEEECCCCCCCCCCCCCCEEECCCEEEEHHHHEECCC
GOR        HHHHHHHHCHHEECTTCCCEEEETCCTTTEEEETTTCETTCCTTEEEEEETTCTTHHEEECC
NN         HHHHHHCCCCCCCCCCCCCCEECCCCCCCCHEECCCCCCCCCCCCCHHHHHHHHCCCCCC
Joint      HHHHHHHCCCCCCCCCCCCCEECCCCCCCEEECCCCCCCCCCCCCCCCEECCHHHHEECCC

AAseq. 181 EVQEVIAA
CF         CEECCCCC
GOR        HHHHHHHH
NN         CHHHHHHH
Joint      CHHHHHHH
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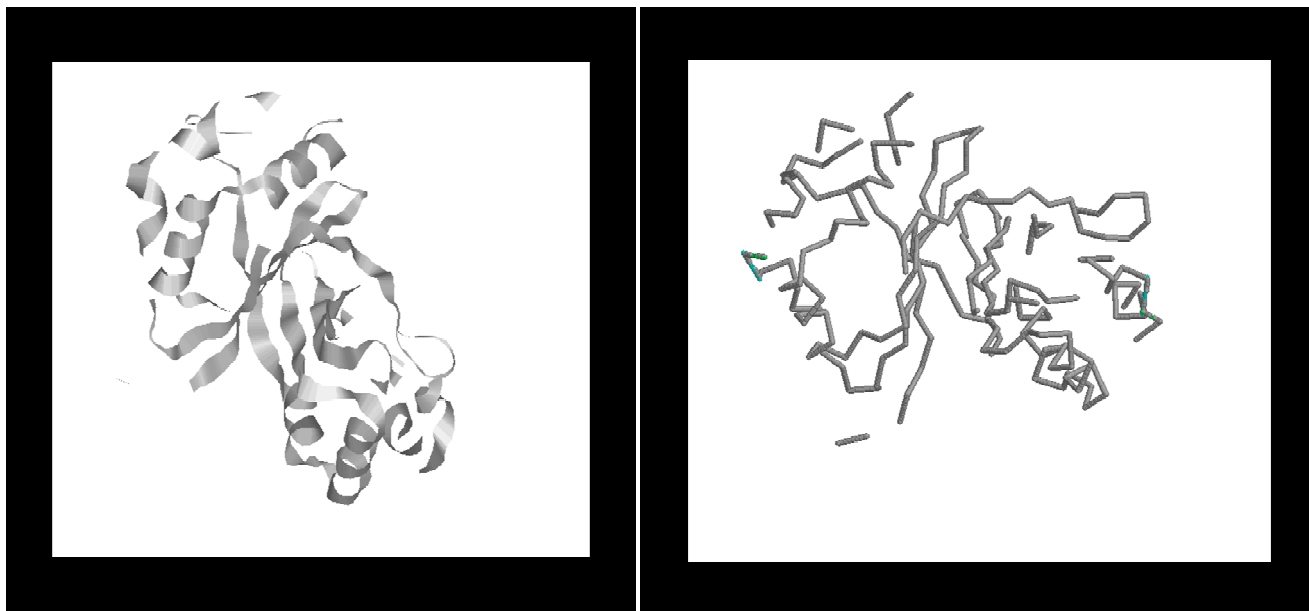
Sequence 4

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3D structure prediction by using rasmol.

Pdb 2 ribbon
2QLW Crystal structure of rhamnose mutarotase RhaU of Rhizobium leguminosarum

Pdb 3 ribbon
2QLX Crystal structure of rhamnose mutarotase RhaU of Rhizobium leguminosarum in complex with L-Rhamnose
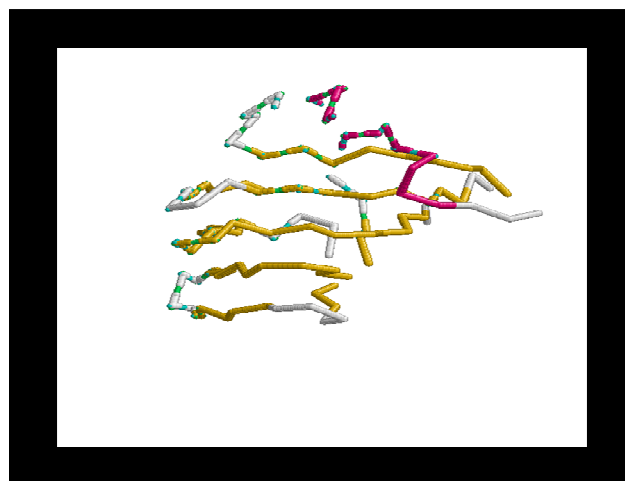
Pdb 4 ribbon
2VQR CRYSTAL STRUCTURE OF A PHOSPHONATE MONOESTER HYDROLASE FROM RHIZOBIUM
LEGUMINOSARUM A NEW MEMBER OF THE ALKALINE PHOSPHATASE SUPERFAMILY

Pdb 5 ribbon
3LWC Crystal structure of Structural Genomics, unknown function (YP_766765.1) from Rhizobium leguminosarum
BV. Viciae 3841 at 1.40 A resolution

CONCLUSION

On the basis of MIC & MBC the Nitrogen Fixing Rhizobia (Cicer arietinum) were Resistance to Pesticide
(Monocrotophos),up to 600 μg/ml concentration, and Its growth is inhibited at concentration 700 μg/ml.

After the NCBI searching we found four protein sequences that are similar to that of nitrogen fixing protein found in
Rhizobia.

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From observation and results we can conclude that other than Rhizobia, there are four protein sequences that fix nitrogen, In future we plan to study these sequences for nitrogen fixation in other than leguminous plants.

REFERENCES

[1]- Andrews,J.M. *Journal of Antimicrobial Chemotherapy* 48 (Suppl. 1):5-16, (**2001**).PMID 11420333.

[2]- Turnidge JD, Ferraro MJ, Jorgensen JH (**2003**) Susceptibility Test Methods: General Considerations. In PR Murray, EJ Baron, JH Jorgensen, MA Pfaller, RH Yolken.Manual of Clinical Microbiology.8th Ed. Washington. American Society of Clinical Microbiology. p 1103

[3]- Medical microbiology, Mims and Playfair, Mosby Europe, **1993**, 35.31.

[4]- French GL (December **2006**). *J.Antimicrob.Chemother*. 58 (6): 1107–17.doi:10.1093/jac/dkl393. PMID 17040922.

[5]http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=Blast Search&SHOW_DEFAULTS=on&LINK_LOC=blasthome

http://www.rcsb.org/pdb/results/results.do?outformat=&qrid=DFB246C4&tabtoshow=Current

[6]-http://spdbv.vital-it.ch/

[7]- "Current taxonomy of rhizobia".Retrieved **2006**-08-07.

[8]- "Taxonomy of legume nodule bacteria (rhizobia) and agrobacteria".Retrieved **2008**-11-14.

[9]- "What is Rhizobia". Retrieved **2008**-07-01.

[10]- Giraud, Eric; et al., L; Vallenet, D; Barbe, V; Cytryn, E; Avarre, JC; Jaubert, M; Simon, D et al (**2007**). "Legumes symbioses: absence of nod genes in photosynthetic bradyrhizobia". *Science* 316 (5829): 1307–12. doi:10.1126/science.1139548.PMID17540897.

[11]- Denison, R. F. 2000. *American Naturalist* 156:567-576

[12]- Kiers ET, Rousseau RA, West SA, Denison RF **2003**. *Nature* 425 : 79-81

[13]- Simms et al. **2006**.An empirical test of partner choice mechanisms in a wild legume-rhizobium interaction. *Proc. Roy. Soc.* B 273:77-81.

[14]- Heath, K. D., and P. Tiffin. **2009**. *Evolution* 63:652-662

[15]- Marco, D. E., R. Perez-Arnedo, A. Hidalgo-Perea, J. Olivares, J. E. Ruiz-Sainz, and J. Sanjuan. **2009**. *Acta Oecologica-International Journal of Ecology* 35:664-667