

SUMMARY

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STUDY TITLE

Toxicity Similarity Assessment of AAD-12 Protein Expressed in Soybean Event DAS-68416-4  
by Bioinformatics Analysis (Update, March, 2010)

DATA REQUIREMENTS

N/A

AUTHOR(S)

P. Song

STUDY COMPLETED ON

23-April-2010

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101573

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SUMMARY

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Compound: AAD-12 Protein

Title: Toxicity Similarity Assessment of AAD-12 Protein Expressed in Soybean Event DAS-68416-4 by Bioinformatics Analysis (Update, March, 2010)

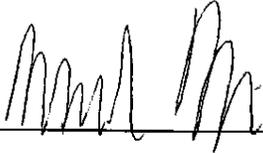
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Title: Regulatory Manager

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Date: 21 April 2010

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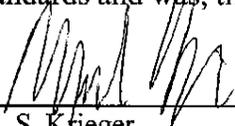
Study Initiation Date: 18/03/2010

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

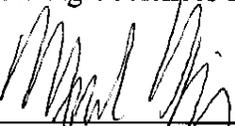
United States Environmental Protection Agency  
Title 40 Code of Federal Regulations Part 160  
FEDERAL REGISTER, August 17, 1989

Organisation for Economic Co-Operation and Development  
ENV/MC/CHEM(98)17, Paris January 26, 1998

At the time this study was conducted, it was not subject to the Good Laboratory Practice Standards and was, therefore, not monitored by the quality assurance unit.

  
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## QUALITY ASSURANCE STATEMENT

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**NON-GLP STUDY**

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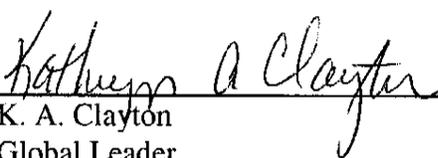
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DAS-68416-4 by Bioinformatics Analysis (Update, March, 2010)

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ABSTRACT

A plant-optimized *aad-12* gene, originally from common soil bacterium *Delftia acidovorans*, was integrated into Soybean (*Glycine max*) to produce event DAS-68416-4 by *Agrobacterium*-mediated transformation of a variety “Maverick” with plasmid pDAB4468, followed by conventional breeding. Aryloxyalkanoate dioxygenase-12 (AAD-12 protein), encoded by the *aad-12* gene, provides tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D), fluroxypyr, and triclopyr-based herbicides. In this study, the amino acid sequence of the AAD-12 protein was evaluated for sequence similarity to known protein toxins using a BLASTp search against an up-to-date GenBank non-redundant protein database (update to March 18, 2010). All the sequence alignments returned by the search were associated with aryloxyalkanoate dioxygenase, or similar proteins. The results indicated that the AAD-12 protein expressed in soybean event DAS-68416-4 contains no significant sequence similarity with any known toxic protein that is harmful to humans or animals.

## INTRODUCTION

A plant-optimized *aad-12* gene, originally from common soil bacterium *Delftia acidovorans*, was integrated into Soybean (*Glycine max*) to produce event DAS-68416-4 by *Agrobacterium*-mediated transformation of a variety “Maverick” with plasmid pDAB4468, followed by conventional breeding. Aryloxyalkanoate dioxygenase-12 (AAD-12 protein), encoded by the *aad-12* gene, provides tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D), fluroxypyr, and triclopyr-based herbicides.

The safety assessment of proteins expressed in transgenic plants may include an evaluation of whether or not the protein can function as a potential toxin when present in the human diet. It has been reported that assessing the potential toxicity of a protein may include comparison of the protein sequence to known protein toxin sequences (1). Since there is no commonly recognized definition of a protein toxin based on its sequence, a comparison should be made to a database of all available protein sequences as a conservative approach. Proteins identified with statistically significant similarity using a local alignment algorithm should then be evaluated for their relevance as potential toxins. For small-scale analyses, such as a single search of a query protein against a large database, a statistically significant similarity that ensures avoidance of false positives (labeling a sequence as related to something in the database when it is not) typically employs an expectation value (E-value) threshold between 0.001 and 0.01. The E-value corresponds to the frequency one would expect to see a match of equal or greater quality by chance (false positive) in a search of the database. For E-values of 0.001 or 0.01, one would expect to find a false positive result once in 1000 or 100 searches, respectively (2). For  $E() = 1$ , there is a statistically equal chance of a hit occurring due to homology or by random chance in a search of a given database. Although little can be done to avoid false negatives (labeling a sequence as unrelated to something in the database when in fact a homolog is present), visual inspection of the quality of less conservative alignments with  $E() < 1$  may minimize false negative results (2).

The objective of this study was to compare the amino acid sequences of the AAD-12 protein expressed in soybean event DAS-68416-4 with known protein toxins in the public protein sequence database.

## METHODS

### Query Sequence Preparation

The AAD-12 protein sequence was prepared in FASTA format for use with BLASTp search programs (Appendix 1).

### Toxicity Assessment

To assess potential toxicity of the AAD-12 protein expressed in soybean event DAS-68416-4, a search for similarity of protein sequences was conducted using the BLASTp program (3). The amino acid sequence of the AAD-12 was queried using the BLASTp (Version 2.2.22+) against a non-redundant protein dataset (update to March 18, 2010), which incorporates non-redundant entries from all GenBank and RefSeq nucleotide translations (Genpept “nr”) along with protein sequences from SWISS-PROT (<http://www.expasy.org/sprot/>), PIR (<http://pir.georgetown.edu/>), PRF (<http://www.prf.or.jp/aboutdb-e.html>), and PDB (<http://www.wwpdb.org/>). The search was done through the BLAST program installed in an internal UNIX computer with default settings (Matrix = BLOSUM62, Gap Costs = Existence: 11, Extension: 1) except that a cutoff expectation E-value of 1.0 was used to generate biologically meaningful similarity between the query sequences and proteins in the database, the low complexity filtering was turned off, and the sequence description and alignment display were set to 2000 alignments. Although a statistically significant sequence similarity generally requires a match with an expectation value less than 0.01, a cutoff of  $E < 1.0$  ensures that proteins with even limited similarity will not be overlooked in the search (2).

## RESULTS AND CONCLUSIONS

The BLASTp search returned a total of 1577 alignments ( $E() < 1$ ). By their annotations, all of the proteins associated with those alignments can be grouped into the following 10 categories (Table 1): 2,4-D/alpha-ketoglutarate dioxygenase, alkylsulfatase AtsK, alpha-ketoglutarate (dependent) dioxygenase, alpha-ketoglutarate-dependent sulfonate dioxygenase, taurine catabolism dioxygenase, taurine dioxygenase, dioxygenase, oxidoreductase, pyoverdine biosynthesis protein, and hypothetical (putative) or unnamed proteins. AAD-12 (aryloxyalkanoate dioxygenase-12) itself is an alpha-ketoglutarate dependent dioxygenase. Hypothetical and unnamed proteins are derived from conceptual translation of DNA sequences generated from massive genome sequencing projects of various fungi and bacteria. Those proteins have functional annotations such as “probable taurine catabolism dioxygenase”, “clavaminic acid synthetase (CAS) –like”, and “putative alpha-ketoglutarate dependent dioxygenase”. None of these proteins returned by the BLASTp search is associated with toxicity. In conclusion, AAD-12 protein expressed in soybean event DAS-68416-4 contains no significant sequence similarity with any known toxic protein that is harmful to humans or animals.

## REFERENCES

1. Codex Alimentarius Special Publications, FOODS DERIVED FROM MODERN BIOTECHNOLOGY (Second Edition), 2009; GUIDELINE FOR THE CONDUCT OF FOOD SAFETY ASSESSMENT OF FOODS DERIVED FROM RECOMBINANT-DNA PLANTS, pp 14.
2. Pearson, W. R. 2000. Flexible sequence similarity searching with the FASTA3 program package. *Methods Mol Biol* 132:185-219.
3. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman (1990). Basic local alignment search tool. *J. Mol. Biol.* 215:403-10.

Table 1. BLASTp Search Summary of Proteins in the Alignments with AAD-12

<b>Description</b>	<b>Number of alignments</b>	<b>E-value range</b>
2,4-D/alpha-ketoglutarate dioxygenase	91	$1.00 \times 10^{-169} - 0.002$
Alkylsulfatase AtsK	6	$8.00 \times 10^{-22} - 4.00 \times 10^{-16}$
Alpha-ketoglutarate dioxygenase	254	$1.00 \times 10^{-33} - 5.70 \times 10^{-01}$
Alpha-ketoglutarate-dependent sulfonate dioxygenase	29	$8.00 \times 10^{-18} - 0.044$
Taurine catabolism dioxygenase	155	$1.00 \times 10^{-82} - 0.33$
Taurine dioxygenase	468	$1.00 \times 10^{-32} - 0.32$
Dioxygenase	295	$2.00 \times 10^{-69} - 0.9$
Oxidoreductase	5	$1.00 \times 10^{-07} - 0.011$
Pyoverdine biosynthesis protein	29	$1.00 \times 10^{-06} - 0.18$
Hypothetical or unnamed proteins	245	$3.00 \times 10^{-31} - 0.75$

## APPENDIX

### 1. Amino Acid Sequence of AAD-12

```
1 MAQTTLQITP TGATLGATVT GVHLATLDDA GFAALHAAWL QHALLIFPGQ
51 HLSNDQQITF AKRFGAIERI GGGDIVAISN VKADGTVRQH SPAEWDDMMK
101 VIVGNMAWHA DSTYMPVMAQ GAVFSAEVVP AVGGRTCFAD MRAAYDALDE
151 ATRALVHQRS ARHSLVYSQS KLGHVQQAGS AYIGYGMDTT ATPLRPLVKV
201 HPETGRPSLL IGRHAHAIPG MDAAESERFL EGLVDWACQA PRVHAHQWAA
251 GDVVVWDNRC LLHRAEPWDF KLPRVMWHSR LAGRPETEGA ALV
```

### 2. Archive of BLASTp Search Output of AAD-12 Protein Sequence

BLASTp search of AAD-12 protein generated an output file with more than 800 pages. The file is electronically stored in a secured computer in Dow AgroSciences and available for viewing in PDF format.