



US011371055B2

(12) **United States Patent**
Wright et al.(10) **Patent No.: US 11,371,055 B2**
(45) **Date of Patent:** *Jun. 28, 2022

(54) HERBICIDE RESISTANCE GENES	5,608,147 A *	3/1997	Kaphammer	C12N 9/0004 435/410
(71) Applicant: CORTEVA AGRISCIENCE LLC , Indianapolis, IN (US)	5,637,489 A	6/1997	Strauch et al.	
	5,646,024 A	7/1997	Leemans et al.	
	5,648,477 A	7/1997	Leemans et al.	
	5,656,422 A	8/1997	Crawford	
	5,879,903 A	3/1999	Strauch et al.	
	5,910,626 A	6/1999	Haselkorn	
	6,087,563 A	7/2000	DellaPenna	
	6,107,549 A	8/2000	Feng et al.	
	6,153,401 A	11/2000	Streber et al.	
	6,268,547 B1	7/2001	Weeks	
	6,518,222 B2	2/2003	Arndt et al.	
	6,645,497 B2	11/2003	Malvar et al.	
	6,664,384 B1	12/2003	Xu	
	7,112,665 B1	9/2006	Leemans et al.	
	7,205,561 B2 *	4/2007	Chelvayohanan	B41J 11/0095 250/559.16
(73) Assignee: Corteva Agriscience LLC , Indianapolis, IN (US)	7,405,074 B2	7/2008	Castle et al.	

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 217 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/468,494**(22) Filed: **Mar. 24, 2017**(65) **Prior Publication Data**

US 2017/0211087 A1 Jul. 27, 2017

Related U.S. Application Data

(63) Continuation of application No. 14/491,197, filed on Sep. 19, 2014, now Pat. No. 10,167,483, which is a continuation of application No. 13/647,081, filed on Oct. 8, 2012, now Pat. No. 8,916,752, which is a continuation of application No. 12/091,896, filed as application No. PCT/US2006/042133 on Oct. 27, 2006, now Pat. No. 8,283,522.

(60) Provisional application No. 60/731,044, filed on Oct. 28, 2005.

(51) **Int. Cl.****C12N 15/82** (2006.01)**C12N 9/02** (2006.01)(52) **U.S. Cl.**CPC **C12N 15/8274** (2013.01); **C12N 9/0069** (2013.01); **C12N 9/0071** (2013.01); **C12N 15/8275** (2013.01); **C12Y 113/11** (2013.01)(58) **Field of Classification Search**

CPC C12N 9/0071

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,683,195 A	7/1987	Mullis et al.
4,761,373 A	8/1988	Anderson et al.
5,135,867 A	8/1992	Payne et al.
5,273,894 A	12/1993	Strauch et al.
5,316,931 A	5/1994	Donson et al.
5,463,175 A	10/1995	Barry et al.
5,500,360 A	3/1996	Ahlquist et al.
5,561,236 A	10/1996	Leemans et al.
5,589,367 A	12/1996	Donson et al.

FOREIGN PATENT DOCUMENTS

CN	1471533	1/2004
EP	1025250	5/1999

(Continued)

OTHER PUBLICATIONS

Schleinitz et al , Applied and Environmental Microbiology 70(9): 5357-5365 (Year: 2004).*
Fourgoux-Nicol et al., Isolation of rapeseed genes expressed early and specifically during development of the male gametophyte, Plant Biology, 1999, vol. 40, p. 857-872.
Gressel, Jonathan, Molecular biology of weed control, Transgenic Research 2000, vol. 9, p. 355-382.
Halford et al., Weed Technology, 2001, vol. 15, p. 737-744.
Hegg, Eric L. et al., Herbicide-Degrading α -Keto Acid-Dependent Enzyme TfdA: Metal Coordination Environment and Mechanistic Insights, Biochemistry, 1999, vol. 38, p. 16714-16726.

(Continued)

Primary Examiner — David H Kruse

(74) Attorney, Agent, or Firm — Barnes & Thornburg LLP

(57)

ABSTRACT

The subject invention provides novel plants that are not only resistant to 2,4-D, but also to pyridyloxyacetate herbicides. Heretofore, there was no expectation or suggestion that a plant with both of these advantageous properties could be produced by the introduction of a single gene. The subject invention also includes plants that produce one or more enzymes of the subject invention "stacked" together with one or more other herbicide resistance genes. The subject invention enables novel combinations of herbicides to be used in new ways. Furthermore, the subject invention provides novel methods of preventing the development of, and controlling, strains of weeds that are resistant to one or more herbicides such as glyphosate. The preferred enzyme and gene for use according to the subject invention are referred to herein as AAD-12 (AryloxyAlkanoate Dioxygenase). This highly novel discovery is the basis of significant herbicide tolerant crop trait and selectable marker opportunities.

33 Claims, 3 Drawing Sheets

Specification includes a Sequence Listing.

(56)

References Cited**U.S. PATENT DOCUMENTS**

7,462,481	B2	12/2008	Castle et al.
7,659,448	B2	2/2010	Ahrens et al.
7,838,733	B2	11/2010	Wright et al.
7,863,503	B2	1/2011	Castle et al.
7,998,703	B2	8/2011	Castle et al.
8,222,489	B2	7/2012	Castle et al.
8,278,505	B2	10/2012	Lira
8,283,522	B2 *	10/2012	Wright
			C12N 9/0071 800/300
8,598,413	B2	12/2013	Cui et al.
8,916,752	B2 *	12/2014	Wright
			C12N 9/0071 800/300
9,062,284	B2	6/2015	Lira
9,074,007	B2	7/2015	Danilevskaya et al.
9,127,289	B2	9/2015	Wright
9,944,944	B2 *	4/2018	Cui
10,167,483	B2 *	1/2019	Wright
10,174,337	B2	1/2019	Wright
2002/0059659	A1	5/2002	Stemmer
2003/0041357	A1	2/2003	Jepson
2003/0056245	A1	3/2003	Chatterjee et al.
2003/0135879	A1	7/2003	Weeks et al.
2009/0069182	A1	3/2009	Castle et al.
2012/0245339	A1	9/2012	Castle et al.
2014/0325713	A1	10/2014	Kovalic et al.
2016/0108422	A1	4/2016	Ellis

FOREIGN PATENT DOCUMENTS

EP	1167531	1/2002
EP	1695983	B1
EP	1740039	8/2006
JP	2005/287415	6/2012
WO	87/05629	10/2005
WO	96/33270	9/1987
WO	96/33270	10/1996
WO	97/13402	4/1997
WO	98/02562	1/1998
WO	9808963	3/1998
WO	98/20144	5/1998
WO	9820144	5/1998
WO	9838294	9/1998
WO	9838336	9/1998
WO	9844139	10/1998
WO	9910513	3/1999
WO	1999/063092	12/1999
WO	2000/006757	2/2000
WO	0009727	2/2000
WO	0066748	11/2000
WO	2001/038513	5/2001
WO	03/013224	2/2003
WO	WO 2003/034813	5/2003
WO	2003/056904	7/2003
WO	WO 2005/107437	11/2005
WO	WO 2007/053482	5/2007

OTHER PUBLICATIONS

- Iowa State University Extension 2005 Herbicide manual for Agricultural Professionals, p. 50-72.
- Kasuga, Mie, Improving Plant Drought, Salt, and Freezing Tolerance by Gene Transfer of a Single Stress-Inducible Transcription Factor, *Nature Biotechnology*, Mar. 1999, vol. 17, p. 287-291.
- Maliga, Current Opinion in Plant Biology, 5:164-172 (2002).
- Perlack, Frederick J., Modification of the Coding Sequence Enhances Plant Expression of Insect Control Protein Genes, *Proc. Natl. Acad. Sci.*, USA, Biochemistry, Apr. 1991, vol. 88, p. 3324-3328.
- Schleinitz, et al., Localization and Characterization of two Novel Genes Encoding Stereospecific Dioxygenases Catalyzing 2(2,4-Dichlorophenoxy)propionate Cleavage in *Delftia acidovorans* MC1,

- Verdaguer, Bertrand, Isolation and Expression in Transgenic Tobacco and Rice Plants, of the Cassava Vein Mosaic Virus (CVMV) Promoter, *Plant Molecular Biology*, 1996, vol. 31, p. 1129-1139.
- Westendorf et al., Purification and Characterisation of the Enantiospecific Dioxygenases from *Delftia acidovorans* MC1 Initiating the Degradation of Phenoxypropionate and Phenoxyacetate Herbicides, *Acta Biotechnol.* 2003, vol. 23, p. 3-17.
- Hotopp et al, *Biochemistry* 2002, 41, 9787-9794.
- Kohler, Genbank Accession No. Q8KSC8 first published on Oct. 1, 2002 (Year: 2002).
- Hogan et al, *The Journal of Biological Chemistry*, 275(17): 12400-12409 (Year: 2000).
- Genbank Accession No. M16730, Apr. 1993.
- Horvath, M. et al., Isolation and characterization of a 2-(2,4-dichlorophenoxy) propionic acid-degrading . . . , *Appl. Microbiol Biotechnol.*, 1990, pp. 33, 213-216, (Abstract only).
- Kohler, *Sphingomonas herbicidovorans* tnpA gene for transposase and rdpA gene for (R)-2-(2,4-dichlorophenoxy) propionate, 2-oxoglutarate dioxygenase, Database EMBI, Accesion No. AJ628859, Mar. 3, 2004, pp. 1-2.
- Kohler, "Sphingomonas herbicidovorans MH: A versatile phenoxyalkanoic acid herbicide degrader," *J. Industrial Microbiology & Biotechnology*, vol. 23, No. 4-5: 336-340 (Oct. 1999).
- Lyon, B.R., et al., "Cotton plants transformed with a bacterial degradation gene are protected from accidental spray drift damage by the herbicide 2,4-dichlorophenoxyacetic acid," *Transgenic Res.*, 1993, pp. 2: 166-169.
- Lyon, B.R., et al., Expression of a bacterial gene in transgenic tobacco confers resistance to the herbicide 2,4-dichlorophenoxyacetic acid, *Transgenic Res.*, 1989, pp. 33: 166-169.
- Schleinitz, K.M., Localization and Characterization of Two Novel Genes Encoding Stereospecific Dioxygenases Catalyzing 2(2,4-Dichlorophenoxy) propionate cleavage in *Delftia acidovorans* MC1, *Applied and Environmental Microbiology*, Sep. 2004, pp. 5357-5365, vol. 70, No. 9.
- Smejkal, C.W. et al., "Substrate specificity of chlorophenoxyalkanoic acid-degrading bacteria is not dependent upon phylogenetically related tfdA gene types," *Biol. Fertil.*, 2001, pp. 33:507-513.
- Spencer et al., Segregation of transgenes in maize, *Plant Molecular Biology*, Jan. 1992, pp. 201-210, vol. 18, Issue 2.
- Streber et al., Analysis, cloning, and high-level expression of 2,4-dichlorophenoxyacetic monoxygenase gene tfdA of *Alcaligenes eutrophus* JMP134, *J. Bacteriol.*, 1987, pp. 169: 2950-2955.
- Streber et al., Transgenic tobacco plants expressing a bacterial detoxifying enzyme are resistant to 2,4-D, *Bio/Technology*, 1989, pp. 7:811-816 (Abstract only).
- Van der Meer, Genbank Accession No. AJ628859, NCBI—National Library of Medicine USA, Bethesda, Maryland (Mar. 3, 2004).
- Westendorf et al., The two enantiospecific dichlorprop/.alpha.-ketoglutarate-dioxygenases from *Delftia acidovorans* MC1—protein and sequence data of RdpA and SdpA, *Microbiol. Res.*, 2002, pp. 157:317-322 (Abstract only).
- Muller et al, *Protein Science* (2006), 15:1356-1368.
- Bayley et al., Engineering 2,4-D resistance into cotton, *Theoretical and Applied Genetics* (1992), 83(5), 645-9.
- Bhat et al., Purification of 3,5-dichlorocatechol 1,2-dioxygenase, a nonheme iron dioxygenase and a key enzyme in the biodegradation of a herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), from *Pseudomonas cepacia* CSV90, *Archives of Biochemistry and Biophysics* (1993), 300(2), 738-46.
- Chaudhry and Huang, Isolation and characterization of a new plasmid from a *Flavobacterium* sp. which carries the genes for degradation of 2,4-dichlorophenoxyacetate, *Journal of Bacteriology* (1988), 170(9), 3897-902.
- Chen and Martin, Reasons for the acclimation for 2,4-D biodegradation in lake water, *Journal of Environmental Quality* (1989), 18(2), 153-6.
- Cosper et al., X-ray absorption spectroscopic analysis of Fe(II) and Cu(II) forms of a herbicide-degrading ketoglutarate dioxygenase,

(56)

References Cited**OTHER PUBLICATIONS**

- De Lipthay et al., Enhanced degradation of phenoxyacetic acid in soil by horizontal transfer of the *tfdA* gene encoding a 2,4-dichlorophenoxyacetic acid dioxygenase, *FEMS Microbiology Ecology* (2001), 35(1), 75-84.
- De Lipthay et al., Expression of *tfdA* genes in aquatic microbial communities during acclimation to 2,4-dichlorophenoxyacetic acid, *FEMS Microbiology Ecology* (2002), 40(3), 205-214.
- De Lipthay et al., In situ exposure to low herbicide concentrations affects microbial population composition and catabolic gene frequency in an aerobic shallow aquifer, *Applied and Environmental Microbiology* (2003), 69(1), 461-467.
- Feng and Kennedy, Biodegradation and plant protection from the herbicide 2,4-D by plant-microbial associations in cotton production systems, *Biotechnology and Bioengineering* (1997), 54(6), 513-519.
- Fuechslin et al., Effect of integration of a GFP reporter gene on fitness of *Ralstonia eutropha* during growth with 2,4-dichlorophenoxyacetic acid, *Environmental Microbiology* (2003), 5(10), 878-887.
- Harker et al., Phenoxyacetic acid degradation by the 2,4-dichlorophenoxyacetic acid (TFD) pathway of plasmid pJP4: mapping and characterization of the TFD regulatory gene, *tfdR*, *Journal of Bacteriology* (1989), 171(1), 314-20.
- Hawkins and Harwood, Chemotaxis of *Ralstonia eutropha* JMP134(pJP4) to the herbicide 2,4-dichlorophenoxyacetate, *Applied and Environmental Microbiology* (2002), 68(2), 968-972.
- Hoffmann et al., A transposon encoding the complete 2,4-dichlorophenoxyacetic acid degradation pathway in the alkalitolerant strain *Delftia acidovorans* P4a, *Microbiology* (Reading, United Kingdom) (2003), 149(9), 2545-2556.
- Hogan et al., Distribution of the *tfdA* gene in soil bacteria that do not degrade 2,4-dichlorophenoxyacetic acid (2,4-D), *Microbial Ecology* (1997), 34(2), 90-96.
- Hogan et al., Site-directed mutagenesis of 2,4-dichlorophenoxyacetic acid/ketoglutarate dioxygenase. Identification of residues involved in metallocenter formation and substrate binding, *Journal of Biological Chemistry* (2000), 275(17), 12400-12409.
- Jackman et al., Electrokinetic movement and biodegradation of 2,4-dichlorophenoxyacetic acid in silt soil, *Biotechnology and Bioengineering* (2001), 74(1), 40-48.
- Ka and Tiedje, Integration and excision of a 2,4-dichlorophenoxyacetic acid-degradative plasmid in *Alcaligenes paradoxus* and evidence of its natural intergeneric transfer, *Journal of Bacteriology* (1994), 176(17), 5284-9.
- Ka et al., Use of gene probes to aid in recovery and identification of functionally dominant 2,4-dichlorophenoxyacetic acid-degrading populations in soil, *Applied and Environmental Microbiology* (1994), 60(4), 1116-20.
- Kitagawa et al., Novel 2,4-dichlorophenoxyacetic acid degradation genes from oligotrophic *Bradyrhizobium* sp. strain HW13 isolated from a pristine environment, *Journal of Bacteriology* (2002), 184(2), 509-518.
- Kleinsteuber et al., Expression of the 2,4-D degradative pathway of pJP4 in an alkaliphilic, moderately halophilic soda lake isolate, *Halomonas* sp. EF43, *Extremophiles* (2001), 5(6), 375-384.
- Laemml et al., Mutation analysis of the different *tfd* genes for degradation of chloroaromatic compounds in *Ralstonia eutropha* JMP134, *Archives of Microbiology* (2004), 181(2), 112-121. (Published online: Dec. 16, 2003).
- Laurent et al., 2,4-Dichlorophenoxyacetic Acid Metabolism in Transgenic Tolerant Cotton (*Gossypium hirsutum*), *Journal of Agricultural and Food Chemistry* (2000), 48(11), 5307-5311.
- Loos et al., Phenoxyacetate herbicide detoxication by bacterial enzymes, *Journal of Agricultural and Food Chemistry* (1967), 15(5), 858-60.
- Ludwig et al., Chromatographic separation of the enantiomers of marine pollutants. Part 5: enantioselective degradation of Mae et al., Characterization of a new 2,4-dichlorophenoxyacetic acid degrading plasmid pEST4011: physical map and localization of catabolic genes, *Journal of General Microbiology* (1993), 139(12), 3165-70.
- Maltseva et al., Degradation of 2,4-dichlorophenoxyacetic acid by haloalkaliphilic bacteria, *Microbiology* (Reading, United Kingdom) (1996), 142(5), 1115-1122.
- Marriott et al., Biodegradation of mixtures of chlorophenoxyalkanoic acid herbicides by *Alcaligenes denitrificans*, *Journal of Industrial Microbiology & Biotechnology* (2000), 25(5), 255-259.
- Matheson et al., Evidence for acquisition in nature of a chromosomal 2,4-dichlorophenoxyacetic acid/ketoglutarate dioxygenase gene by different *Burkholderia* spp., *Applied and Environmental Microbiology* (1996), 62(7), 2457-2463.
- McGowan et al., Evidence for interspecies gene transfer in the evolution of 2,4-dichlorophenoxyacetic acid degraders, *Applied and Environmental Microbiology* (1998), 64(10), 4089-4092.
- Muller and Babel, Pseudo-recalcitrance of chlorophenoxyalkanoate herbicides—correlation to the availability of ketoglutarate, *Acta Biotechnologica* (2001), 21(3), 227-242.
- Muller and Babel, Separation of two dichlorprop/ketoglutarate dioxygenases with enantiospecific properties from *Comamonas acidovorans* MC1, *Acta Biotechnologica* (1999), 19(4), 349-355.
- Muller et al., *Comamonas acidovorans* strain MC1. A new isolate capable of degrading the chiral herbicides dichlorprop and mecoprop and the herbicides 2,4-D and MCPA, *Microbiological Research* (1999), 154(3), 241-246.
- Nickel et al., Involvement of two ketoglutarate-dependent dioxygenases in enantioselective degradation of (R)- and (S)-mecoprop by *Sphingomonas herbicivorans* MH, *Journal of Bacteriology* (1997), 179(21), 6674-6679.
- Ohkouchi et al., Cloning and expression of DL-2-haloacid dehalogenase gene from *Burkholderia cepacia*, *Water Science and Technology* (2000), 42(7-8, Hazard Assessment and Control of Environmental Contaminants (Ecohazard 99)), 261-268.
- Parker and Doxatader, Kinetics of microbial decomposition of 2,4-D in soil: effects of herbicide concentration, *Journal of Environmental Quality* (1982), 11(4), 679-84.
- Perkins et al., Use of *Alcaligenes eutrophus* as a source of genes for 2,4-D resistance in plants, *Weed Science* (1987), 35(Suppl. 1), 12-18.
- Plumeier et al., Importance of different *tfd* genes for degradation of chloroaromatics by *Ralstonia eutropha* JMP134, *Journal of Bacteriology* (2002), 184(15), 4054-4064.
- Poh et al., Complete characterization of Tn5530 from *Burkholderia cepacia* strain 2a (pJB1) and studies of 2,4-dichlorophenoxyacetate uptake by the organism, *Plasmid* (2002), 48(1), 1-12.
- Preston et al., Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes, *Pesticide Biochemistry and Physiology* (1996), 54(2), 123-134.
- Saari et al., Stereospecific degradation of the phenoxypropionate herbicide dichlorprop, *Journal of Molecular Catalysis B: Enzymatic* (1999), 6(4), 421-428.
- Schneiderheinze et al., Plant and soil enantioselective biodegradation of racemic phenoxyalkanoic herbicides, *Chirality* (1999), 11(4), 330-337.
- Shaw and Burns, Enhanced mineralization of [U-14C]2,4-dichlorophenoxyacetic acid in soil from the rhizosphere of *Trifolium pratense*, *Applied and Environmental Microbiology* (2004), 70(8), 4766-4774.
- Tett et al., Enantioselective degradation of the herbicide mecoprop [2-(2-methyl-4-chlorophenoxy)propionic acid] by mixed and pure bacterial cultures, *FEMS Microbiology Ecology* (1994), 14(3), 191-200.
- Top et al., Capture of a catabolic plasmid that encodes only 2,4-dichlorophenoxyacetic acid:ketoglutaric acid dioxygenase (*TfdA*) by genetic complementation, *Applied and Environmental Microbiology* (1996), 62(7), 2470-2476.
- Top et al., Methane oxidation as a method to evaluate the removal of 2,4-dichlorophenoxyacetic acid (2,4-D) from soil by plasmid-

(56)

References Cited**OTHER PUBLICATIONS**

- Travkin et al., Characterization of an intradiol dioxygenase involved in the biodegradation of the chlorophenoxy herbicides 2,4-D and 2,4,5-T, FEBS Letters (1997), 407(1), 69-72.
- Vallaey et al., PCR-RFLP analysis of 16S rRNA, tfdA and tfdB genes reveals a diversity of 2,4-D degraders in soil aggregates, FEMS Microbiology Ecology (1997), 24(3), 269-278.
- Vallaey et al., The metabolic pathway of 2,4-dichlorophenoxyacetic acid degradation involves different families of tfdA and tfdB genes according to PCR-RFLP analysis, FEMS Microbiology Ecology (1996), 20(3), 163-172.
- Vedler et al., Analysis of the 2,4-dichlorophenoxyacetic acid-degradative plasmid pEST4011 of *Achromobacter xylosoxidans* subsp. *denitrificans* strain EST4002, Gene (2000), 255(2), 281-288.
- Vedler et al., TfdR, the LysR-type transcriptional activator, is responsible for the activation of the tfdCB operon of *Pseudomonas putida* 2,4-dichlorophenoxyacetic acid degradative plasmid pEST4011, Gene (2000), 245(1), 161-168.
- Zhang et al., In vitro assay for 2,4-D resistance in transgenic cotton, in Vitro Cellular & Developmental Biology: Plant (2001), 37(2), 300-304.
- Zipper et al., Complete microbial degradation of both enantiomers of the chiral herbicide mecoprop [(RS)-2-(4-chloro-2-methylphenoxy)propionic acid] in an enantioselective manner by *Sphingomonas herbicidovorans* sp. Nov, Applied and Environmental Microbiology (1996), 62(12), 4318-4322.
- Zipper et al., Enantioselective uptake and degradation of the chiral herbicide dichlorprop [(RS)-2-(2,4-dichlorophenoxy)propanoic acid] by *Sphingomonas herbicidovorans* MH, Journal of Bacteriology (1998), 180(13), 3368-3374.
- Cho et al., Agricultural Chemistry and Biotechnology (English Edition) (1999), 42(2), 57-61 , Isolation and characterization of 2-methyl-4-chlorophenoxyacetic acid-degrading bacteria from agricultural soils.
- Lim et al., Journal of Microbiology (Seoul, Republic of Korea) (2004), 42(2), 87-93 Genetic and phenotypic diversity of (R/S)-mecoprop [2-(2-methyl-4-chlorophenoxy)propionic acid]-degrading bacteria isolated from soils.
- Tamura et al., Nippon Noyaku Gakkaishi (2001), 26(3), 309-314 , Microbial pesticide degradations and evolutionary analysis of degrading enzymes.
- Vallaey et al., Biotechnology Letters (1998), 20(11), 1073-1076 , Isolation and characterization of a stable 2,4-dichlorophenoxyacetic acid degrading bacterium, *Variovorax paradoxus*, using chemostat culture.
- Fukamori and Hausinger, JBC (268)24311(1993), Purification and characterization of 2,4-Dichlorophenoxyacetate/a-ketoglutarate dioxygenase.
- Fukamori and Hausinger, JBacteriology (175)2083(1993), Alcaligenes eutrophus JMP134 "2,4-Dichlorophenoxyacetate monooxygenase" is an a-Ketoglutarate-dependent dioxygenase.
- Ehrig et al., Acta Biologica (17)351-356(1997), Isolation of phenoxyherbicide-degrading *rholoferax* species from contaminated building material.
- Hausinger, Critical Reviews in Biochemistry (39)21-68(2004), Fe(II)/.alpha.-Ketoglutarate-Dependent Hydroxylases and Related Enzymes.
- Hausinger and Fukamori, Env Health Perspectives (103)37-39(1995), Characterization of the first enzyme in 2,4-Dichlorophenoxyacetic acid metabolism.
- Don et al., J Bacteriol (161)85(1985), Transposon Mutagenesis and Cloning Analysis of the Pathways for Degradation of 2,4-Dichlorophenoxyacetic Acid and 3-Chlorobenzoate in Alcaligenes eutrophus JMP134(pJP4).
- Don et al., J Bacteriol (161)466(1985), Genetic and Physical Map of the 2,4-Dichlorophenoxyacetic Acid-Degradative Plasmid pJP4.
- Hotopp et al., J. Molec. Catalysis B: Enzymatic (15)155-162(2001), Park et al, Journal of Microbiology and Biotechnology (2003), 13(2), 243-250 , Isolation and characterization of 4-(2,4-dichlorophenoxy)butyric acid-degrading bacteria from agricultural soils.
- Mueller RH, Babel W. Degradability and recalcitrance of phenoxyalkanoic acid herbicides—traits of the microbial metabolism. InSecond International Conference on Remediation of Chlorinated and Racalcitrant Compounds 2000 (pp. 229-236).
- Tett, Biodegradation (1997), 8(1), 43-52 , Biodegradation of the chlorophenoxy herbicide (R)-(+) -mecoprop by Alcaligenes denitrificans. Llewellyn et al, Herbicide-Resistant Crops (1996), 159-74, Genetic engineering of crops for tolerance to 2,4-D.
- Baelum et al., Appl. Environ. Microbiol. (72)1476-1486 (2006), Degradation of 4-Chloro-2-Methylphenoxyacetic Acid in Top- and Subsoil is Quantitatively Linked to the Class III tfdA Gene.
- Baelum et al., Appl. Environ. Microbiol. (75)2969-2972 (2009), TaqMan Probe-Based Real-Time PCR Assay for Detection and Discrimination of Class I, II, and III tfdA Genes in Soils Treated with Phenoxy Acid Herbicides.
- Griffin et al., J Agric Food Chem (61)6589-6596(2013), Characterization of Aryloxyalkanoate Dioxygenase-12, a Nonheme Fe(II)/.alpha.-Ketoglutarate-Dependent Dioxygenase, Expressed in Transgenic Soybean and *Pseudomonas fluorescens*.
- Hausinger, RSC Metallobiology (2015) Chapter 1 of 2-Oxoglutarate-dependent oxygenases, Biochemical diversity of 2-oxoglyutarate-dependent oxygenases.
- Leibeling et al, Eng Life Sci (13)278(2013), Posttranslational oxidative modification of (R)-2-(2,4-dichlorophenoxy) propionate/.alpha.-ketoglutarate-dependent dioxygenases (RdpA) leads to improved degradation of 2,4-dichlorophenoxyacetate (2,4-D).
- Muller et al., Applied and Environmental Microbiology (72)4853(2006), Purification and Characterization of Two Enantioselective a-Ketoglutarate-Dependent Dioxygenases, RdpA and SdpA, from *Sphingomonas herbicidovorans* MH.
- Zaprasis et al, Appl. Environ. Microbiol. (76)119(2010), Abundance of Novel and Diverse tfdA-Like Genes, Encoding Putative Phenoxyalkanoic Acid Herbicide-Degrading Dioxygenases, in Soil.
- Zhang et al. Journal of Agricultural and Food Chemistry (2020), 68(26), 6967-6976 CODEN: JAFCAU; ISSN: 0021-8561 Enantioselective Catabolism of the Two Enantiomers of the Phenoxyalkanoic Acid Herbicide Dichlorprop by *Sphingopyxis* sp. DBS4.
- Krausse et al., 4th biotechnology symposium of the University of Leipzig (abstract) p. 60 (2005), Crystal structure of the 2-oxoglutarate dependent dioxygenase RdpA.
- Mueller et al., Un Mich ICBIC 2005 abstract, Insights into the enantiospecificities of (R)- and (S)-dichlorprop/a-ketoglutarate dioxygenases.
- Krausse et al., Freiberg Un Deutschen Gesellschaft fur Kristallographie (2006), (Poster title 72L) Structure of the 2-Oxoglutarate Dependent Dioxygenase RdpA by X-ray Crystallography and Small Angle X-ray Scattering.
- Gazitua et al., Environ Microbiol (12)2411(2010), Novel .alpha.-ketoglutarate dioxygenase tfdA-related genes are found in soil DNA after exposure to phenoxyalkanoic herbicides.
- Chekan et al., Proceedings of the National Academy of Sciences (116)13299-13304(2019), Molecular basis for enantioselective herbicide degradation imparted by aryloxyalkanoate dioxygenases in transgenic plants.
- Larue et al, Pest Manag Sci (75)2086(2019), Development of enzymes for robust aryloxyphenoxypropionate and synthetic auxin herbicide tolerance traits in maize and soybean crops.
- Liu et al., FEMS Microbiol Ecol (86)114-129(2013), Consumers of 4-chloro-2-methylphenoxyacetic acid from agricultural soil and drilosphere harbor cadA, r/sdpA, and tfdA-like gene encoding oxygenases.
- Muller et al., Protein Science (15)prot1356(2006), Structural basis for the enantiospecificities of R- and S-specific phenoxypropionate/a-ketoglutarate dioxygenases.
- Muller, Eng Life Sci (7)311-321 (2007), Activity and Reaction

(56)

References Cited**OTHER PUBLICATIONS**

- Westendorf, Eng Life Sci (6)552-559(2006), Kinetic Traits and Enzyme Form Patterns of (R)-2-(2,4-Dichlorophenoxy) propionate/a-Ketoglutarate Dioxygenase (RdpA) after Expression in Different Bacterial Strains.
- Wright et al., Proc Natl Acad Sci (107)20240(2010), Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes.
- GenBank accession No. AAA21983.1 tfdA Cupriavidus necator (replaced *Ralstonia eutropha*), Apr. 23, 1993.
- GenBank accession No. AAK81681.1 tfdA Burkholderia cepacia, Aug. 24, 2020, Jul. 23, 2016.
- UniProtKB accession No. Q45423.1 tfdA *Burkholderia* sp. RASC, Nov. 1, 1996.
- GenBank accession No. AAS49436 tfdA Achromobacter denitrificans, Apr. 3, 2020.
- GenBank accession No. AAB47567.1 tfdA *Burkholderia* cepacia pJJB, Jul. 26, 2016.
- GenBank accession No. AAM76772.1 tfdA *Delftia acidovorans* _-287 aaProtein_--NCBI Jul. 26, 2016.
- GenBank accession No. BAB92965.1 tfdA alpha proteobacterium HWK12, Jul. 2, 2002.
- GenBank accession No. BAB92966.1 tfdA alpha proteobacterium HW13, Jul. 2, 2002.
- NCBI accession No. WP_011084309.1 tfdA *Bradyrhizobium diazoefficiens* (replaced *B. japonicum*), Jul. 27, 2019.
- UniProtKB accession No. P83309.2 sdpA MC1 *Delftia acidovorans*, Sep. 18, 2019.
- UniProtKB accession No. Q700X4.1 sdpA MH Shingobium herbicidovorans, Nov. 1, 1996.
- GenBank accession No. ABD67501.1 sdpA P230 *Rhodoflex* sp. P230, Mar. 14, 2006.
- Muller, Swiss Federal Institute of Technology thesis (Nov. 2004), Metabolism of phenoxyalkanoic acid herbicides in *Sphingomonas herbicidovorans* MH cloning and characterization of two enantiospecific .alpha.-ketoglutarate-dependent dioxygenases and degradation pathway analysis.
- Westendorf Universitat Leipzig thesis—German (2005), Enantioselektive Verwertung von Phenoxyalkanoat-Herbiziden durch Bakterien—Eine Charakterisierung der Dioxygenasen des initialen Abbau-Schrittes.
- Baelum et al., System Appl Microbiol. (33)67(2010), Supplemental Material to Comparison of 16S rRNA gene phylogeny and functional tfdA gene distribution in thirty-one different 2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid degraders.
- Han et al., World Journal of Microbiology & Biotechnology (2014), 30(10)2567-2576 , 16S rRNA gene phylogeny and tfdA gene analysis of 2,4-D-degrading bacteria isolated in China.
- Zhang et al., J. Agric. Food Chem. (58)12878(2010), Enantioselective Environmental Behavior of the Chiral Herbicide Fenoxaprop-ethyl and Its Chiral Metabolite Fenoxaprop in Soil.
- Wu et al., J. Agric. Food Chem. (65)3711(2017), Rapid Biodegradation of the Herbicide 2,4-Dichlorophenoxyacetic Acid by Cupriavidus gilardii T-1.
- Zabaloy et al., Ann Microbiol (64)969(2014), Isolation and characterization of indigenous 2,4-D herbicide degrading bacteria from an agricultural soil in proximity of Sauce Grande River, Argentina.
- Nielsen et al., PLOS One 8(12)e83346(2013), Novel Insight into the Genetic Context of the cadAB Genes from a 4-chloro-2-methylphenoxyacetic Acid-Degrading Sphingomonas.
- Mierzejewska et al., Bulletin Env Contam Tox (104)200(2020), Biodegradation Potential and Ecotoxicity Assessment in Soil Extracts Amended with Phenoxy Acid Herbicide (2,4-D) and a Structurally-Similar Plant Secondary Metabolite (Ferulic Acid).
- Shimojo et al., J Biosc Bioengin. (108)56(2009), Analysis of genes Han et al, World Journal of Microbiology & Biotechnology (2015), 31(7), 1021-1030 , Cloning, expression, characterization and mutational analysis of the tfdA gene from *Cupriavidus campinensis* BJ71.
- Paulin et al., Environ Microbiol (13)1513-1523(2011), (R,S)-dichlorprop herbicide in agricultural soil induces proliferation and expression of multiple dioxygenase-encoding genes in the indigenous.
- Muller et al., Protein Science (15)prot1356(2006), Supplemental Table and Figures to Structural basis for the enantiospecificities of R- and S-specific phenoxypropionate/a-ketoglutarate dioxygenases. GenBank accession No. BAB92964.1 tfdAa alpha proteobacterium RD5-C2—NCBI. Jul. 2, 2002.
- Poh et al., 2,4-Dichlorophenoxyacetate / .alpha.-ketoglutarate dioxygenases from *Burkholderia cepacia* 2a and *Ralstonia eutropha* JMP134, Microbios 105 43-63 2001.
- Suwa et al., Characterization of a chromosomally encoded 2,4-dichlorophenoxyacetic acid/ketoglutarate dioxygenase from *Burkholderia* sp. strain RASC, Applied and Environmental Microbiology (1996), 62(7), 2464-2469.
- Itoh et al., tfdA-like genes in 2,4-dichlorophenoxyacetic acid-degrading bacteria belonging to the *Bradyrhizobium-Agromonas-Nitrobacter-Afipia* cluster in Proteobacteria, Applied and Environmental Microbiology (2002), 68(7), 3449-3454.
- Vedler et al. The Completely Sequenced Plasmid pEST4011 Contains a Novel IncP1 Backbone and a Catabolic Transposon Harboring tfd Genes for 2,4-Dichlorophenoxyacetic Acid Degradation, Journal of Bacteriology, vol. 186, No. 21, Nov. 2004, p. 7161-7174.
- Hoffmann et al., Development and application of PCR primers for the detection of the lid genes in *Delftia acidovorans* P4a involved in the degradation of 2,4-D, Acta Biotechnologica (2001), 21(4), 321-331.
- Itoh et al., Root nodule *Bradyrhizobium* spp. harbor tfdA and cadA, homologous with genes encoding 2,4-dichlorophenoxyacetic acid-degrading proteins, Applied and Environmental Microbiology (2004), 70(4), 2110-2118.
- Kaneko et al., Complete Genomic Sequence of Nitrogen-fixing Symbiotic Bacterium *Bradyrhizobium japonicum* USDA110 (Supplement). DNA Research 9,—Supplement, 225-256 (2002).
- Muller et al., Genetic Analysis of Phenoxyalkanoic Acid Degradation in *Sphingomonas herbicidovorans* MH; Applied and Environmental Microbiology, Oct. 2004, vol. 70, No. 10, p. 6066-6075.
- Muller et al., Physiological and genetic characteristics of two bacterial strains utilizing phenoxypropionate and phenoxyacetate herbicides, Microbiological Research (2001), 156(2), 121-131.
- Eichhorn Eric et al: "Characterization of alpha-ketoglutarate-dependent taurine dioxygenase from *Escherichia coli*", Journal of Biological Chemistry, vol. 272, No. 37, 1997, pp. 23031-23036, ISSN: 0021-9258.
- Bish Na Veena Chandra et al: "Development of 2,4-O-resistant transgenics in Indian oilseed mustard (*Brassica juncea*)", Current Science (Bangalore), vol. 87, No. 3, Aug. 10, 2004 (Aug. 10, 2004), pp. 367-370, ISSN: 0011-3891.
- Opposition filed against EP 10012200.1, filed on Dec. 20, 2013.
- Opposition filed against EP 10012201.9, filed on Dec. 6, 2013.
- Opposition filed against EP 10012202.7, filed on Dec. 20, 2013.
- Opposition filed against EP 10012199.5, filed on Jun. 18, 2014.
- Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Second Edition., 1989, vol. 2, p. 11.45.
- Applicant Response to Opposition filed against EP 10012200.1, filed on Aug. 8, 2014.
- Applicant Response to Opposition filed against EP 10012201.9, filed on Jul. 28, 2014.
- Applicant Response to Opposition filed against EP 10012202.7, filed on Jul. 28, 2014.
- Applicant Response to Opposition filed against EP 10012199.5, filed on Nov. 22, 2014.
- Final Decision in Opposition filed against EP 10012200.1, filed on Aug. 1, 2016.
- Final Decision in Opposition filed against EP 10012201.9, filed on Aug. 1, 2016.

Explore Litigation Insights



Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.