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DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 05-053-2]

University of Wisconsin-Madison; Availability of Environmental Assessment and a Finding of No Significant Impact for Field Tests of Genetically Engineered *Erwinia carotovora*

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public that the Animal and Plant Health Inspection Service has prepared an environmental assessment for a field trial of genetically engineered strains of a bacterium, *Erwinia carotovora*, the causal agent of tuber soft rot disease in potato. The bacteria have been genetically engineered to disrupt the disease causing pathway. This field trial will allow researchers to better understand the function of each mutated gene under field conditions. The environmental assessment provides a basis for our conclusion that these field tests will not present a risk of introducing or disseminating a plant pest and will not have a significant impact on the quality of the human environment. Based on its finding of no significant impact, the Animal and Plant Health Inspection Service has determined that an environmental impact statement need not be prepared for these field tests.

EFFECTIVE DATE: January 10, 2006.

ADDRESSES: You may read the environmental assessment, the finding of no significant impact, and any comments that we received on Docket No. 05-053-1 in our reading room. The reading room is located in room 1141 of the USDA South Building, 14th Street and Independence Avenue SW.,

Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 690-2817 before coming.

FOR FURTHER INFORMATION CONTACT: Dr. Rudaina Alrefai, Biotechnology Regulatory Services, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1236; (301) 734-4866. To obtain copies of the environmental assessment (EA), the finding of no significant impact (FONSI), or the response to comments, contact Ms. Ingrid Berlinger at (301) 734-4885; e-mail: Ingrid.E.Berlinger@aphis.usda.gov. The EA, FONSI, and response to comments are also available on the Internet at http://www.aphis.usda.gov/brs/aphisdocs/05_09701r_ea.pdf. The draft EA is available at http://www.aphis.usda.gov/brs/aphisdocs/05_09701r_pea.pdf.

SUPPLEMENTARY INFORMATION:

Background

The regulations in 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason to Believe Are Plant Pests," regulate, among other things, the introduction (importation, interstate movement, or release into the environment) of organisms and products altered or produced through genetic engineering that are plant pests or that there is reason to believe are plant pests. Such genetically engineered organisms and products are considered "regulated articles." A permit must be obtained or a notification acknowledged before a regulated article may be introduced. The regulations set forth the permit application requirements and the notification procedures for the importation, interstate movement, or release into the environment of a regulated article.

On April 7, 2005, the Animal and Plant Health Inspection Service (APHIS) received a permit application (APHIS permit number 05-097-01r) from the University of Wisconsin-Madison, Department of Plant Pathology, Madison, WI, for a permit for a field trial of *Erwinia carotovora*. These bacteria have been genetically engineered not to express specific *hrp/hrc* (hypersensitive reaction on non-host

plants and pathogenesis on host plants or conserved among plant and animal pathogens) genes resulting in the disruption of the disease-causing mechanism. These mutations are expected to make the bacterial strains avirulent or non-pathogenic. The application describes four genetically engineered strains to be used in this field trial.

The *E. carotovora* ssp. *carotovora* WPP14 strain was initially isolated from a diseased potato plant obtained from a commercial farm in Waushara County, WI. This strain was used to create four new genetically engineered strains by inserting a marker gene into genes that may be necessary for *E. carotovora* infection of potatoes. The four strains resulting from this mutagenesis that are proposed for use in this field trial are described below.

- Strain WPP40 contains an insertion of a kanamycin resistance gene (*aph*) cassette into *outD*. The *outD* gene encodes for an outer membrane porin that is required for a functional type II secretion system. This mutant is unable to secrete plant cell degrading enzymes and is avirulent. The kanamycin resistance gene cassette contains *aph*, which encodes neomycin phosphotransferase which was originally isolated from Tn5, and two FRT sites derived from *Saccharomyces cerevisiae*.

- Strain WPP60 has an insertion of a spectinomycin resistance gene (*aadA*) cassette into *hrcC*, an outer membrane porin which is required for a functional type III secretion system. This mutant is unable to secrete harpins or effector (Avr) proteins. It is hypersensitive response minus. The spectinomycin resistance gene cassette is constructed from the *aadA* gene which encodes aminoglycoside-3 adenylyltransferase, originally derived from *Shigella flexneri*, with termination sequences derived from bacteriophage T4.

- Strain WPP195 has a deletion of *hrpN* and an insertion of a chloramphenicol resistance gene (*cat*) cassette and a modified green fluorescent protein (GFPmut2) into this locus. This mutant is unable to produce or secrete the harpin, HrpN. The *gfpmut2* gene was originally cloned from *Aequorea victoria* and was modified to be brighter. Its expression is driven by the *nptII kan* promoter from Tn5. The *cat* gene encodes

cholramphenicol acetyltransferase, which was originally isolated from *Escherichia coli*. This construct also contains FRT sites.

- Strain WPP198 is an insertion of a similar chloramphenicol resistance cassette into *hrpL*, which is a sigma factor required for expression of the type III secretion system and its secreted substrates. The mutant is unable to produce or secrete harpins or effector (Avr) proteins. It is hypersensitive response minus.

The genetically engineered *E. carotovora* are considered regulated articles under the regulations in 7 CFR part 340 because they may be plant pests. The purpose of the field trial is to use genetically engineered *E. carotovora* strains with mutations in homologs of the well-characterized *Pseudomonas syringae* *hrp* genes as tools to:

- Understand the effects of specific genes on the fitness of *E. carotovora*,
- Use the results from these experiments to better understand the function of these genes in plant-bacterial interactions, and
- Compare the results obtained with *E. carotovora* mutants with those found for *P. syringae* to determine if homologous genes play similar roles in fitness in different environments.

In a notice published in the **Federal Register** on August 12, 2005 (70 FR 47170-47171, Docket No. 05-053-1), APHIS announced that it had prepared an environmental assessment (EA) for a field trial of the genetically engineered strains of *E. carotovora* and made the EA available for public review and comment. During the 30 day comment period for the draft EA, which ended on September 12, 2005, APHIS received six comments. Responses to the issues raised in the comments are provided as an attachment to the finding of no significant impact (FONSI). Three of the comments were from private individuals, one was from a public interest group, and the remaining two comments were from the same person, writing first as a private individual and then representing a public interest group. All six comments opposed the field test. One individual was generally opposed to field tests of genetically engineered organisms. However the comment did not provide support for the opposition. The remaining comments raised two issues. One issue is the concern that horizontal gene transfer of the antibiotic resistance gene in these bacterial strains might result in transfer of this trait to soil or enteric bacteria. This issue was addressed in the EA and is again addressed in the response to comments. The second issue is that the experiment is conducted with

bacterial strains that may be as virulent as the native bacteria strains. APHIS disagrees with the comment that this field trial “provides high risk with little or no benefit.” This issue is also addressed in the response to comments.

APHIS has edited the EA to include specific contact information in Appendix I and to add a new section IX, “Consultations,” in the final EA. The changes are not substantive and do not impact the analysis in the EA. Copies of both the draft EA and the final EA are available (see **FOR FURTHER INFORMATION CONTACT**).

Pursuant to its regulations (7 CFR part 340) promulgated under the Plant Protection Act, APHIS has determined that this field trial will not pose a risk of the introduction or dissemination of a plant pest for the following reasons:

- (1) *Erwinia carotovora* is widely spread in the environment and commonly present on plant roots of numerous species as well as in lakes, streams, rain, and ground water.
- (2) Screening weeds for the past year around potato fields did not reveal any naturally-occurring “hypersensitive reaction on non-host plants and pathogenesis on host plants or conserved among plant and animal pathogens” (*hrp/hrc*) mutants of *E. carotovora* even though these mutants have been found on potato. Therefore, it is not likely that the host range of the bacterium will change because of the modification.

- (3) Reversion of the genetically engineered strains would not pose any additional environmental risk because reverted mutants will be similar to the other *E. carotovora* strains that are commonly present on these plants. The risks associated with the introduction of genetically engineered organisms generally are the same kind as those associated with the introduction into the environment of unmodified organisms and organisms modified by other genetic techniques.

- (4) The field trial is a confined release and would have no significant impact on the environment. The field release conditions and measures described in the permit should be sufficient to prevent any unplanned release of the transgenic bacteria or the inoculated plant material; or the persistence of the transgenic material in the environment.

- (5) This small field test of 0.2 acre should not have any significant impact on existing agricultural practices because this test is solely for research purposes. The antibiotic resistance genes themselves should not cause these mutant strains to have any competitive advantage in the environment and would not interfere with current

agricultural practices to control the soft rot disease in potato. Although spraying with streptomycin is used to control *Erwinia amylovora* on fruit trees, it is not normally used to control the soft rot disease in potatoes on this field station.

- (6) Resistance to antibiotics is already widely prevalent in enteric bacteria and soil-borne bacteria. Gene transfer from *E. carotovora* to animals and plants is highly unlikely under the conditions of this field test.

- (7) *Erwinia* species are not known as animal or human pathogens and there are no references that associate it with human or animal disease even though farm workers have been exposed to *Erwinia* spp. for decades. There should be no risk to university personnel handling the inoculated potatoes since they hand-inoculate potatoes while wearing gloves and all diseased plants are removed from the field. No potential impact of this experiment on people living in the area of the field trial test plot or any other human population can be identified.

- (8) An examination of threatened and endangered species for Wisconsin listed in the U.S. Fish and Wildlife Service’s Threatened and Endangered Species System¹ showed that 6 threatened or endangered plant species and 12 animal species exist or once existed in the State. Only one plant species has been reported in Waushara County but is not a host for *E. carotovora*. None of the listed threatened or endangered plant and animal species would be impacted by this test.

The EA was prepared in accordance with (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS’ NEPA Implementing Procedures (7 CFR part 372). Copies of the EA and FONSI are available from the individual listed under **FOR FURTHER INFORMATION CONTACT**.

Authority: 7 U.S.C. 7701-7772 and 7781-7786; 31 U.S.C. 9701; 7 CFR 2.22, 2.80, and 371.3.

Done in Washington, DC, this 3rd day of January 2006.

Kevin Shea,
Acting Administrator, Animal and Plant Health Inspection Service.
[FR Doc. E6-76 Filed 1-9-06; 8:45 am]

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¹ http://ecos.fws.gov/tess_public/servlet/gov.doi.tess_public.servlets.RegionLists?lead_region=3#WI.