ANALYSIS OF SAFETY BASED ON PARIZA/JOHNSON DECISION TREE

Pariza and Johnson have published updated guidelines for the safety assessment of microbial enzyme preparations (2001)[1] from the 1991 IFBC Decision Tree[2]. The safety assessment of a given enzyme preparation is based upon an evaluation of the toxigenic potential of the production organism. The responses below follow the pathway indicated in the decision tree as outlined in Pariza and Johnson, 2001. The outcome of this inquiry is that lysophospholipase enzyme preparation from *Trichoderma reesei (T.reesei)* strain RF7206 expressing the gene encoding lysophospholipase from *Aspergillus nishimurae* is "ACCEPTED" as safe for its intended use.

Decision Tree:

1. **Is the production strain genetically modified?** *Trichoderma reesei* strain RF7206 was genetically modified to express lysophospholipase from *Aspergillus nishimurae*. Yes go to #2;

2. **Is the production strain modified using rDNA techniques?** Yes go to #3a;

3.

3a. Does the expressed enzyme product which is encoded by the introduced DNA have a history of safe use in food? Yes, Go to 3c;

3c. Is the test article free of transferable antibiotic resistance gene DNA? Yes, transferable DNA was not detected in the lysophospholipase enzyme preparation manufactured using *T. reesei* and production process described herein. Additionally, no antibiotic resistance gene has been integrated. Go to 3e;

3e. Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products? Yes, inserted DNA is well characterized. Go to 4;

4. **Is the introduced DNA randomly integrated into the chromosome?** Yes, go to #5;

5. **Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects which may result in the synthesis of**

[1] Pariza M.W. and Johnson E.A. Reg. Toxicol. Pharmacol. Vol. **33** (2001) 173-186

[2] IFBC (International Food Biotechnology Committee), Chapter 4: Safety Evaluation of Foods and Food Ingredients Derived from Microorganisms in Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification, Regulatory Toxicology and Pharmacology. Vol. **12**:S1-S196 (1990).

toxins or other unsafe metabolites will not arise due to the genetic modification method that was employed? Yes, there is no concern for pleiotropic effects. Go to #6;

6. **Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?** Yes, *T. reesei* has been demonstrated as a safe production host and methods of modification have been well documented. Safety of this organism has been evaluated and confirmed through toxicological testing as described herein.

ACCEPTED