



GMM assessment: experiences from the evaluation of food enzymes applications

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GUIDANCE DOCUMENT

- Updated GD:
(EFSA Journal 2011; 9(6):2193)
- Two trial enzyme descriptions were assessed and a technical report is available as additional guidance to the applicants

(EFSA Journal 2011; 9(6):2284)



ASSESSMENT OF TRIAL DESCRIPTIONS

ASSESSMENT

- In concordance with the updated GMM GD
- According to current practices of EFSA
- Considering individual aspects of the trial descriptions

TECHNICAL REPORT

- Structured according to the outline of an opinion
- For each point, missing information (required for the RA) is stated
- Conceived as further guidance for the preparation of applications

Technical report: EFSA Journal 2011;9(6):2284

FOOD ENZYMES: MAINLY CATEGORY 2

Category 1: Chemically defined purified compounds and their mixtures; both GMMs and newly introduced genes are absent

ERA not needed according to the GMM GD

Category 2: Complex products; both GMMs and newly introduced genes are absent

Most enzymes

Category 3: Products derived from GMMs; GMMs capable of multiplication or of transferring genes are not present; newly introduced genes are still present

ERA: possible transfer of recombinant DNA into other organisms

Category 4: Products consisting of or containing GMMs capable of multiplication or of transferring genes

1. SAFETY EVALUATION PARENTAL/RECEPTOR AND DONOR STRAIN

- Evaluation of the safety of the parental/recipient and donor strains, e.g. in relation to their capability to produce undesirable metabolites that remain in the enzyme product
 - If QPS
 - identification of the strain is required
 - Information related to the assumptions for QPS status (e.g. for some *Bacillus* spp. the absence of potential toxigenic potential) have to be checked
 - If not QPS, safety evaluation of the strain is required



EXPERIENCE & FURTHER GUIDANCE

Issue	Further guidance
Section 1.1.1. of the GMM GD requests deposition number for the parental strain	Strain deposition number in a culture collection is required only for the final production strain, and is not necessary for the recipient strain.
Taxonomic identification of the recipient or parental strain	A proper taxonomic identification of the recipient or parental strain has to be provided, also in the case of QPS
Relationship between the strain that is safety evaluated and the parental/recipient strain	Strains, which are evaluated for safety, should have a clear genealogical linkage to the parental/recipient strain
Genetic modifications used to develop the recipient strain.	Genetic modifications made during the development of the recipient strain should be fully described (e.g. vectors used, including a map and a description of the different genetic elements; transformation tool used; method used for integration and selection)



2. ASSESSMENT OF THE GM PRODUCTION STRAIN

- Assessment of the genetic modification, especially the introduction of genes of concern (e.g. antibiotic resistance genes), which could remain in the enzyme product
- Assessment of the genetic stability to verify that no undesirable effects due to unintended genetic alterations would emerge in the production strain and, consequently, in the enzyme product
- The production strain should be deposited in an internationally recognized culture collection

EXPERIENCE & FURTHER GUIDANCE

Issue	Further guidance
<p>Confirmation of the absence of antibiotic resistance (AR) genes in case a gel-purified fragment expected to lack these genes was used for transformation</p>	<p>Gel purification of a plasmid fragment is insufficient to fully eliminate other fragments and thus their integration. Other purification methods (e.g. HPLC) can give more confidence about the purity. Therefore, in the case gel purification is used, confirmation of the absence of any AR gene is requested, e.g., by Southern analysis or by analysis of the total genome sequence.</p>
<p>Use of PCR instead of Southern analysis to demonstrate the absence of AR genes used during the development of the production strain</p>	<p>Acceptable as long as positive controls include the same gene inserted in an intermediate strain. Otherwise PCR is not accepted because of uncertainty regarding the validity of the selected primers for testing the absence of the complete gene.</p>

EXPERIENCE & FURTHER GUIDANCE

Issue	Further guidance
<p>Whole genome sequence analysis of the production strain to demonstrate the absence of AR genes</p>	<ul style="list-style-type: none"> • In case the production strain contains extra- chromosomal DNA (e.g. plasmids), the analysis should include those elements • Sufficient details should be provided, including the sequencing method used (Illumina etc.), read lengths and coverage, and bioinformatic analysis (e.g. identification of specific AR genes)

EXPERIENCE & FURTHER GUIDANCE

Issue	Further guidance
Southern analysis to demonstrate the insertion of the genetic construct and the number of copies inserted	Sufficient details on the methodology needs to be provided to allow proper interpretation of the result
Whole genome sequence analysis to demonstrate the insertion of the construct and the number of copies inserted	See previous slide

3. ASSESSMENT OF THE PRODUCT

- Absence of the production strain from the product
 - Description of the fermentation and downstream processes, including the method used to remove the cells
 - Demonstration of the absence of the GM production strain in the product, paying attention to the resuscitation of stressed cells



3. ASSESSMENT OF THE PRODUCT (CONT.)

- Absence of recombinant DNA
 - PCR-based method with documented reliability, efficacy and sensitivity; controls including addition of DNA and cells to the product before DNA extraction
 - At least one recombinant genetic element, and all functional genes of possible concern (e.g. AR genes, virulence genes, genes encoding toxic compounds) should be targeted. This is because DNA degradation is sequence-dependent and can differ between genes.

EXPERIENCE & FURTHER GUIDANCE

Issue	Further guidance
<p>The GMM guidance requests information about the presence/absence of the production strain in the final product. This should be experimentally verified. Argumentation without experimental evidence is not sufficient. If not provided, environmental risk assessment has to be performed according to the guidance for category 3 products.</p>	<p>Information should be provided on:</p> <ul style="list-style-type: none"> •cultivation media and growth conditions and why these conditions are optimal to support growth of the production strain; detection of stressed cells should be ensured by including a resuscitation step. Resuscitation should be made in cultivation media with minimal selective pressure and/or using longer incubation time compared to normal culture of viable organisms. •how the detection sensitivity has been determined; •how the production strain could be differentiated from possible contaminating micro-organisms; •sampling; at least three independent batches of product preparations should be sampled, each analysed in triplicate. A proper sampling method should be chosen and documented

EXPERIENCE & FURTHER GUIDANCE

Issue	Further guidance
<p>PCR analysis demonstrating the presence/absence of DNA was provided with an high limit of detection (μg), however, evidence for the presence of DNase in the final product was demonstrated</p>	<p>Absence of recombinant DNA in the product was accepted due to the presence of DNase, which was sufficiently demonstrated</p>

CHOICE OF THE SAMPLE

Question from AMFEP

- The unformulated food enzyme is considered as a representative sample.
 - A: Will it be acceptable to submit the additional data after submission of the dossier, with a timeline for expected availability of such data? To be answered by EFSA staff
 - B: Will it be acceptable to submit data on final formulated commercial product? (final product retain samples are more easily available, but are food enzyme preparations as opposed to food enzyme as defined under EU Reg 1332/2008)

CHOICE OF THE SAMPLE

Answer to question B

- The confirmation of the absence of recombinant DNA and of the production strain can be performed on samples of the final formulated commercial products because these samples are released in the environment and are therefore representative.
- Samples of the unformulated enzyme are acceptable if they are equally or higher concentrated, because absence of the recombinant DNA and the production strain also implies absence in the final formulated commercial product.
- The unformulated enzyme could be a more suitable sample than the final formulated commercial product in the case PCR analysis (necessary to demonstrate the absence of recombinant DNA) would be inhibited by components present due to the formulation of the product.

4. ENVIRONMENTAL RISK ASSESSMENT

- If no production organism or recombinant DNA are released in the product, no extensive environmental risk assessment of the enzyme product is required
- Experience is that companies adapt their product purification process to meet this criterion.



5. GENERAL COMMENT

Issue	Further guidance
<p>Not all data requested by the GMM guidance were provided</p>	<p>Be as complete as possible! Questions will be asked if limited amount of data are missing; if a considerable amount of data are missing, a general question will be asked, requesting to provide data as indicated in the GMM guidance</p>