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DRAFT TANZANIA STANDARD

Biotechnology - Guidance on assessment of the purity, biological activity and stability of microorganism based products (MBPs)

TANZANIA BUREAU OF STANDARDS

0 Foreword

Microorganism based products (MBPs), such as fertilizer, growth promoter, pest and weed control agents, silage additive, probiotic for feedstuff additives, bioremediation agents and biodegradation agents are used in the environment. MBPs can contain one or more micro-organism strains including genetically modified micro-organisms (GMMs). Purity, biological activity and stability of the micro-organisms present are considered to be the technical specifications for evaluating MBP quality.

A large variety of MBPs exists and the choice of methods applied to assess technical specifications depends primarily on the characteristics of the microbial component of the product such as taxonomy, genotype, metabolism, growth environment, doubling time.

During the development of the standard, reference was made to the following documents:

- The Environmental Management (Biosafety) Regulations, 2009 and amendments thereof
- The National Biosafety Guidelines for Tanzania of 2004
- BS EN 12689:1998 *Biotechnology - Guidance on assessment of the purity, biological activity and stability of micro-organism based products* published by British Standards Institute (BSI).

1 Scope

This Tanzania Standard gives guidance on the assessment of purity, biological activity and stability of the microorganism based products (MBPs) for product quality evaluation. It is also describes criteria and factors considered for the validity of the assessment of the purity, biological activity and stability of the MBPs.

This Standard only applies to the microbial components of a MBP as a whole and does not apply to any type of molecular components purified from the microorganism.

This Tanzania Standard applies to MBPs specifically manufactured to be used in agriculture and in the environment. Also, in food industry, for veterinary use or for human health.

NOTE: This Tanzania Standard can be used by the manufacturer of MBPs or anyone interested in the evaluation of product quality.

2 Normative References

The following referenced documents are indispensable in the application of this Tanzania standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies:

There are no normative reference

3 Terms and definitions

For the purposes of this Tanzania standard, the following terms and definitions should apply:

3.1 analyte

substance sought or determined

3.2 biocontaminant

undesired biological substances such as micro-organism, allergens, toxins

3.3 biological activity of product

intended performance of micro-organism based products as related to the use

3.4 control

preparation of known characteristics used to standardize an analysis

3.5 microbial component

desired micro-organism or mixture of desired micro-organisms

3.6 micro-organism

Any microscopic biological entity, cellular or non-cellular, capable of independent replication or replication within host cell,

3.7 micro-organism based product (MBP)

product whose efficacy is dependent upon its microbial component

3.8 product purity

content of the microbial component in the micro-organism based product

NOTE Depending of the type of micro-organism based product, product purity can be expressed as one or more of the following:

a) proportion of microbial component to total viable micro-organisms;

b) a specified level of biocontaminants;

c) quantity of the microbial component to strains.

3.9 product stability

preservation of biological activity and purity over time and under defined conditions

3.10 reference data

documentation of the characterization of micro-organism including any methods which are state-of-art

4 General considerations

4.1 Assessment protocol

4.1.1 The assessment of purity, biological activity and stability of the micro-organism based products (MBPs) should be consistent with the intended use of the product. This assessment refers only to the microbial component or the biological activity of the products.

4.1.2 Identification of the microbial component should be provided by the manufacturer to the user. The manufacturer can use a wide range of procedures to characterize the microbial component, depending on the characteristics of the micro-organisms). The manufacturer should process the microbial component in such a way that its identity is ensured in the end product.

4.1.3 Validity of assessment of purity, biological activity and stability of the micro-organism based products (MBPs) are assured when specific criteria and factors are considered.

The purity, biological activity and stability of the micro-organism based products (MBPs) should be assessed using appropriate methods, depending on:

i. the micro-organism contained in the MBP;
ii. the purpose and design of the assessment.

4.1.4 The method of assessment should be provided by the manufacturer. These methods should be used as the basis of the evaluation of product quality.

4.1.5 Regardless of the applied method, the following criteria should be considered to assure the validity of the assessment of product purity, biological activity of the product, and product stability (see clauses 5, 6 and 7).

4.1.6 Considerations for the assessment protocol to assess the of purity, biological activity and stability of the micro-organism based products (MBPs) should start with the definition and the statement of the objectives of the analysis, either product purity, biological activity of the product or product stability, followed by the design and documentation of an appropriate test method.

4.1.7 The major steps of the assessment protocol are:

i. design and review of the assessment protocol (see 4.2);
ii. execution of the assessment protocol (see 4.3);
iii. record keeping (see 4.4).

4.2 Design and review of the assessment protocol

4.2.1 The design of the assessment protocol should be documented and reviewed for validity according the criteria in clauses 5, 6 and 7. The assessment protocol should include procedures for sampling and pre-treatment of samples.

4.2.2 The assessment of purity, biological activity and stability of the micro-organism based products (MBPs) is carried out on product samples as prepared for use.

4.2.3 The sampling should be consistent with the type of applied statistical analysis. The type of applied statistical analysis should provide representative data concerning production.

NOTE Sampling methods and sample pre-treatment methods described by national and international standards for the type of product to be tested should be used.

4.2.4 Degradation and contamination of the MBP microbial component should be minimized during storage and transportation of samples. Conditions of storage and transportation of MBP samples, such as temperature, humidity and light, should be as close as possible to those of the storage of the whole product. Pre-treatment of samples is usually necessary for the isolation of the microbial component of the MBP.

4.2.5 Pre-treatment of samples is carried out after collection and storage if required. The choice of a pre-treatment procedure depends on:

i. the nature and composition of the MBPs (e.g. liquid or solid samples);
ii. the applied method to assess product purity, biological activity of the product and product stability.

4.2.6 Pre-treatment can alter the number and activity of the microbial component of the MBPs. Therefore, it is essential that the pre-treatment procedure should be validated before being incorporated into assessment protocols.

4.3 Execution of assessment protocol

The assessment protocol should be carried out in accordance with the design as follows:

i. collect samples and assay;
ii. identify and label samples;
iii. store the samples under conditions which minimize degradation of the analyte;
iv. pre-treat the samples;
v. collect raw data and obtain results;
vi. sample disposal as per relevant Tanzania Standards
vii. record any deviation from the assessment protocol.

4.4 Record keeping

A record should be kept in all aspects of the assessment protocol. This should not be considered an exhaustive list and should include:

i. identification of the test as foreseen by the assessment protocol;
ii. name and designation of person performing the test;
iii. date of the test;
iv. method chosen for of purity, biological activity and stability of the micro-organism based products (MBPs) assessment;
v. origin, storage and transportation of samples;
vi. number and size (weight and/or volume) of samples tested;
vii. controls used;
viii. deviation from the protocol;
ix. interpretation of the results.

5 Validity of purity assessment

5.1 General

Purity assessment provides preliminary data on biological activity and dosage of the product. A large variety of MBPs exists and many methods are available to assess purity. For any type of product the validity of purity assessment depends on the criteria and factors given in 5.2 to 5.5.

5.2 Identification of the microbial component

5.2.1 Purity is expressed by quantitative data based on the evidence of the identity of the microbial component. Therefore, identity is the primary factor to be confirmed to validate purity assessment.

NOTE Identification methods described by national and international standards for the type(s) of micro-organism to be identified should be used.

5.2.2 Identity can be expressed as the degree of morphological or genetic similarity of the micro-organism contained in the MBP to the reference strain(s). Identity can be determined from the analysis of factors related to the activity of the microorganisms.

5.2.3 Methods applied to identify the microbial component such as serological, genetic and physiological analysis or isolation by use of selective media or other appropriate substrates should have been previously calibrated or validated.

5.3 Quantitative data

Quantitative data on purity refers to a method of calculation and to the precision of results which should both be reported in the analysis of the results.

5.4 Availability of controls and/or reference data

5.4.1 Availability of controls and/or reference data improves the assessment of the applied methods). MBP sample results should be compared with control results or with reference data provided by the manufacturer.

5.4.2 Negative controls consist of a MBP sample without its microbial component. Negative controls are processed under the same conditions as the MBP sample containing the microbial component. Negative controls are used to check for any contamination occurring during execution of the assessment protocol.

5.4.3 Positive controls can consist of a sample of the reference strain whose characteristics are known. When such a positive control is not available, the results of the assessment should be confirmed by comparison with reference data from the manufacturer. In the case of a lack of a positive control, accuracy of the applied method of assessment should be carefully determined

5.5 Presence of biocontaminants

5.5.1 The presence of biological entity (s), other than those intended in the product, can interfere with the purity assessment. Biocontaminants, whose activity can cause a reduction in the quantity of the microbial component or interfere with the biological activity of the product, should be considered. In this regard the effects of, for example, predators or phage on the microbial component should be considered.

5.5.2 Biocontaminations can occur at any step of the methods used for the assessment protocol. The choice of the method of assessment should consider the practicality and feasibility of the operations under aseptic versus septic conditions for the reduction of biocontamination.

5.5.3 Purity can be assessed through quantification of biocontaminants in the product according to different objectives such as safety and efficacy of the product. In this case, the test conditions used for the assessment of biocontaminants should be stated in the assessment protocol.

6 Validity of biological activity assessment of product

6.1 General

6.1.1 Biological activity assessment of the product provides data on the efficiency of the microbial component of the product. Biological activity assessment of the product is carried out by processing the

MBP samples under conditions appropriate for the intended use. The biological activity of the product can be assessed by direct analysis of the analyte and/or by comparison with the activity of a similar product.

6.1.2 A large variety of MBPs exists, and many methods are available to assess the biological activity of the product. For any type of product the validity of the biological activity assessment of the product depends on the criteria and factors given in 6.2 to 6.6.

6.1.3 An assessment protocol should consider the following specific factors:

i. the intended use of the MBP;
ii. the target for the microbial component such as living organisms, substances, plant tissue on which the MBP produces the desired effects;
iii. the mechanism of interaction of the microbial component with its target.

6.2 Quantification of the biological activity of the product

The quantification of the biological activity of the product by direct or comparative measurement refers to a method of calculation and to the precision of results which should both be reported in the analysis of results.

6.3 Availability of controls and/or reference data

6.3.1 The availability of controls and/or reference data improves the assessment of the applied methods). MBP sample results should be compared with control results or with reference data provided by the manufacturer.

6.3.2 Negative controls consist of a MBP sample without its microbial component. Negative controls are processed under the same conditions as the MBP sample containing the microbial component. Negative controls are used to check for any contamination occurring during the execution of the assessment protocol.

6.3.3 A positive control can consist of a sample of material that contains a comparable activity, biological or chemical, of known characteristics. When such a positive control is not available, the results of the assessment should be confirmed by comparison with reference data.

6.4 Strategies for application of the MBPs

Application conditions can affect microbial performance, especially when the product is based on a mixture of micro-organisms. A careful consideration of product application conditions is recommended.

6.5 Biotic and abiotic factors

Preliminary studies on environmental variables, which directly affect the biological activity of the product, improve the reliability of the designed assessment protocol. A careful consideration of such factors is recommended.

6.6 Mechanism involved in the product-environment interaction

For the purpose of a product quality evaluation, the level of understanding for the mechanism involved in the product-environment interaction should be consistent with the intended use of the product.

7 Validity of stability assessment

7.1 Product stability data indicate product purity and the biological activity of the product as defined in clauses 5 and 6 over a period of time and under defined conditions.

7.2 The aim of the stability assessment for product quality evaluation is to confirm, within acceptable limits, the MBP purity, biological activity and stability over a period of time and under defined conditions such as the product storage period.

7.3 Stability studies should take it into consideration that microbial degradation or inactivation varies according to storage period and storage conditions.

7.4 The following criteria and factors are important to validate stability assessment.

a) Stability should be expressed as the confirmation of change or lack of change of the biological activity of product and/or product purity at specific point of time. Environmental factors and storage conditions should be defined and controlled.

Wherever significant changes or product degradation occur, acceptable limits should be defined with respect to the intended use of the product.

b) The stability assessment should be carried out with respect to:

i. a period of time or the proposed shelf life;
ii. established environmental factors or the proposed storage conditions.

NOTE: The temperature of the product can be the most crucial factor, while factors such as humidity and light can be less important when it has been demonstrated that the container protects the product.

c) When shelf life is proposed, the stability assessment should be conducted more frequently during the beginning of the shelf life period followed by longer testing intervals towards the end of the period.

d) Stability data of freeze-dried products can be obtained after their reconstitution.