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Standard Classification for Industrial Microorganisms[1]

This standard is issued under the fixed designation E3214; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This classification applies to all industrial microorganisms, both classically derived and those produced through genetic engineering methods.

1.2 The scope of this classification does not include plants and animals. This classification would not be applied to any downstream products derived from industrial microorganisms unless they contain microbial deoxyribonucleic acid (DNA).

1.3 This classification includes fields for genotype class, biosafety, mode/intent of use, and the extent of DNA sequence information for a given industrial microbial strain.

1.4 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E3072 Terminology for Industrial Biotechnology

3. Terminology

3.1 *Definitions*—See Terminology E3072 for industrial biotechnology terms.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *genotype, n*—portion of genetic material within an organism that determines the expression of a specific characteristic (phenotype).

3.2.2 *mutagenesis, n*—addition, deletion, or substitution of at least one base within an organism's genome.

3.2.2.1 *Discussion*—Mutagenesis can occur under either natural or directed means.

3.2.3 *native deoxyribonucleic acid (DNA), n*—DNA sequences derived from within the same microbial species

3.2.4 *non-native DNA, n*—DNA sequences originating from other microbial species or designed sequences with three or more intentional and predesignated DNA base-pair alterations across a genome, relative to native DNA.

3.2.4.1 *Discussion*—Non-native DNA shall fall outside the observed genomic diversity within a given microbial species. DNA base-pair alterations are defined as either base-pair insertions, or substitutions, but not deletions.

3.2.5 *transgenic, adj*—organism that contains non-native DNA sequences.

3.2.5.1 *Discussion*—Non-native DNA sequences can be derived from another species or designed de novo.

4. Significance and Use

4.1 The technology to engineer industrial microorganisms (IMs) is evolving rapidly and the public, regulatory bodies, and industrial sectors require new tools to help evaluate the products of biotechnology (**1**)[3]. In particular, there is a need to clarify the nature and intent of genetic alterations present in many industrial microbial strains (**2, 3**).

4.2 Currently, there is no systematic classification system to help differentiate among the many subtypes of engineered industrial microorganisms (**4, 5**). In response, a classification system for industrial microorganisms has been developed with the intent of facilitating the commercial use and development of industrial microorganisms and the biotechnology sector in general.

4.3 This classification will be applied to all microorganisms for which there is an intended use, broadly referred to as

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The boldface numbers in parentheses refer to a list of references at the end of this standard.

"industrial microorganisms." This classification covers both viable and non-viable microorganisms, in addition to any product that contains microbial DNA.

4.4 This classification is not intended to apply to downstream products of industrial microorganisms that do not contain microbial DNA, for example, highly purified proteins or small molecules produced by industrial microorganisms.

5. 5. Basis of Classification

5.1 This classification consists of four fields that represent genotype class, biosafety risk grouping, mode/intent of use, and the extent of genome sequence information.

5.2 Field 1 refers to genotype class and is assigned to one of four categories (A-D). Classes A-C are organisms that only contain or produce naturally observed biochemicals regardless of the origin of DNA sequences contained within the strain. Classes A-C are also referred to as Type 1 microorganisms. In contrast, Class D organisms contain or produce chemical entities not previously observed in nature, for example, non-natural amino acids or pharmaceuticals produced through engineered biochemical pathways (**6**). Class D organisms are also referred to as Type 2 microorganisms.

5.2.1 Class A organisms are defined as native strains in which no intentional selection or engineering was applied with the intent of creating a new phenotype. Class A strains include microorganisms present in the environment, as well as natural isolates and laboratory propagated strains derived from natural isolates. Class A organisms may be subject to unintentional selection pressures that may lead to spontaneous genetic changes, for example, those driven by changes to the natural environment or through propagation under laboratory conditions. Examples of Class A strains include wild yeasts used in brewing, the human microbiome, microorganisms present in the soil, and naturally-occurring pathogens.

5.2.2 Class B organisms are defined as strains that have been subjected to deliberate alteration of the organism's native genome without the introduction of any non-native DNA, with the intent of producing an altered phenotype. Any form of selective pressure or genetic engineering can be applied provided the genomic sequence of the resulting strains falls within the bounds of the known genetic diversity of the parent microbial species. The definition of microbial species is adopted from Parker, Tindall, and Garrity (**7**). The origin of native DNA can be natural or synthetic provided the sequence is within the observed native genomic diversity as determined by DNA sequencing.

5.2.3 Class C organisms are defined as strains that have been subjected to intentional selection or engineering with addition of non-native DNA. For the purposes of this classification, non-native DNA is defined as DNA sequences originating from other species or designed DNA sequences with three or more intentional base-pair alterations relative to an otherwise homologous native DNA sequence. By definition, non-native DNA shall fall outside of the observed genomic diversity of a given microbial species as determined by DNA sequencing. Class C organisms are typically produced through modern genetic techniques involving recombinant or synthetic DNA sequences. Class C organisms can also be produced by classical approaches, for example, forced lateral gene transfer between similar, but distinct, microbial species under directed conditions. This class also includes strains in which a native gene is modified by noncoding regulatory elements [promotors, repressors, ribosome binding site (RBS) regions, and so forth] originating from other species or synthetically derived. Class C organisms are comprised of otherwise native biochemical building blocks and are only distinct from Classes A and B in that they contain one or more non-native DNA sequences.

5.2.4 Class D organisms are defined as strains subjected to intentional selection or engineering with addition of non-native DNA enabling the production of non-natural biochemical building blocks (for example, non-natural amino acids, chemically modified nucleic acids and so forth). Class D includes organisms with metabolic pathways engineered to produce new chemical entities from native precursors (for example, sugars, protein, and lipids). Class D organisms typically display phenotypes not previously observed in nature. Examples include organisms with express proteins containing non-natural amino acids and strains engineered to produce non-natural pharmaceuticals as an alternative to chemical synthesis. Class D organisms contain non-native DNA that may be composed of the natural four nucleotide bases or chemical analogs thereof.

5.3 Field 2 is biosafety risk grouping (1 to 4). Biosafety risk groupings (**8**) take into account the extended impact of a microorganism and the availability of countermeasures such as vaccines and antibiotics. A risk grouping level for a microorganism is assigned on the following basis:

5.3.1 *Risk Group 1:* Not associated with disease in healthy adult humans;

5.3.2 *Risk Group 2:* Associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available;

5.3.3 *Risk Group 3:* Associated with serious or lethal human disease for which preventive or therapeutic interventions may be available; and

5.3.4 *Risk Group 4:* Microbial agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

5.3.5 The great majority of industrial microorganisms fall within Risk Group 1 and a sub-classification of this category will likely be required in a future revision of this standard that also takes into account the extended impact of industrial microbial strains beyond any direct effects on human health.

5.4 Field 3 represents the mode of use and primary application of an industrial microbial strain expressed as a code containing two alphabetical symbols separated by a period where the first letter is uppercase (for example, X.y). These codes are listed in Annex A1 and reflect the current modes of use and application areas for industrial microorganisms currently in commerce. In cases in which a strain has more than one end use, only the primary application is cited.

5.5 Field 4 describes genomic sequence information and reflects the extent to which genome of a microbial strain or culture has been characterized by genetic analysis (that is, DNA sequencing) on a scale of 1 to 4. Genomic sequencing

can confirm whether the genome of a microbial strain altered by modern biotechnological methods (for example, gene editing or other targeted techniques) falls within observed natural genetic variation (that is, native or non-native DNA). The four categories within this field are:

5.5.1 *Unsequenced*—Little or no DNA sequence information,

5.5.2 *Partially Sequenced*—Incomplete DNA sequence information,

5.5.3 *Fully Sequenced*—Complete sequence of at least one genome, and

5.5.4 *Population Sequenced*—Strain genome diversity characterized.

6. Implementation and Test Methods

6.1 This classification should be applied to IMs in all forms, including solid and liquid formulations of both viable and non-viable cells, regardless of morphology or cell type. This includes spores, cells, biofilms, protoplasts, active dry preparations, and any other format in which industrial microorganisms are present.

6.2 This classification should be applied to products containing either viable or non-viable IMs, for example, fermented, brewed, or baked products. Other examples include cosmetic products containing microbial cells or enzyme products comprising whole, homogenized fermentation broth.

6.3 Clarified products derived from IMs should not be subject to this classification unless the product contains detectable genetic material (typically DNA) upon analysis using polymerase chain reaction (PCR) or equivalent analytical techniques for the sequencing and identification of genetic material.

6.4 This classification should be applied by the producer of the industrial microorganism using it as a guide. Full or even partial disclosure of an industrial microorganism's genetic sequence is not required to apply this classification beyond declaring whether non-native DNA was incorporated into a given strain.

6.5 Verification of whether this classification has been applied in a manner consistent with it can in some cases be determined by genetic sequencing of DNA derived from an IM or IM-containing product by comparison to known sequences available in public databases.

6.6 The onus is upon the producer of the IM, or IM-containing product, to verify that non-native DNA, when used to construct or otherwise engineer a Class A or B organism, is entirely absent from the final strain. In the absence of this information (that is, <3 for Field 4), the strain shall be designated as Class C or D.

7. Keywords

7.1 biosafety; biotechnology; classification; deoxyribonucleic acid; DNA; genotype; industrial microorganism

ANNEX

(Mandatory Information)

A1. SUMMARY OF THE CLASSIFICATION FOR INDUSTRIAL MICROORGANISMS

A1.1 Field 1—Genotype Class

A1.1.1 *A*—Native strain, no intentional selection or engineering applied. Isolated from nature, without selection pressure or intentional mutagenesis.

A1.1.2 *B*—Intentional selection/engineering involving native DNA sequences. Includes strains developed through random or targeted mutagenesis, and self-cloning.

A1.1.3 *C*—Intentional selection/engineering with addition of non-native DNA. Non-native DNA can be isolated from other species or synthetic in origin.

A1.1.4 *D*—Strains altered with non-native DNA enabling the production of chemicals not previously observed in nature (for example, non-natural amino acids, non-natural pharmaceutical drugs, chemically-modified nucleic acids, and so forth).

A1.2 Field 2—Biosafety Risk Group

A1.2.1 Biosafety risk grouping is assigned on a scale of 1 to 4 per the NIH 2016 guidelines (**8**).

A1.2.1.1 *Risk Group One (1)*—Not associated with disease in healthy adult humans.

A1.2.1.2 *Risk Group Two (2)*—Associated with human disease that is rarely serious and for which preventive or therapeutic intervent

A1.2.1.3 *Risk Group Three (3)*—Associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

A1.2.1.4 *Risk Group Four (4)*—Microbial agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

A1.3 Field 3—List of Modes and Applications for Industrial Microorganisms

A1.3.1 *Mode of Use*—See Table A1.1

A1.3.2 *Primary Application*—See Table A1.2.

A1.4 Field 4—Extent of Genomic Sequence Information

A1.4.1 Field 4 reflects the extent to which genome of a microbial strain or culture has been characterized by DNA sequencing. DNA sequencing can confirm whether the genome of a microbial strain altered by modern biotechnological