NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Procedure for the safety assessment of microbial strains used in food

食品安全国家标准 食品用菌种安全性评价程序

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1 Scope

The standard lays down the procedure for assessing the safety of microbial strains used in food, covering bacteria, actinomycetes, filamentous fungi, yeasts and unicellular algae.

It applies to the safety assessment of live microbial strains used in food and in food additives, again covering bacteria, actinomycetes, filamentous fungi, yeasts and unicellular algae. It does not apply to the safety assessment of genetically modified microorganisms.

2 Terms and definitions

2.1 pathogenicity: the ability of a microorganism to infect a host, cause damage to health and give rise to disease.

2.2 toxigenicity: the ability of a microorganism to produce a substance that damages a living organism.

2.3 toxicity: damage to the health of the host caused by toxic metabolites of a microorganism.

2.4 antimicrobial resistance: the resistance of a microorganism to antimicrobial agents, divided into intrinsic resistance, also called natural resistance, and acquired resistance.

2.5 intrinsic resistance: the natural resistance of a microorganism to an antimicrobial agent, reflecting the antimicrobial pattern of all or nearly all wild type strains within one species. It

is a property determined by genes in the genome of the microorganism and handed down from generation to generation, independent of horizontal gene transfer and of spontaneous mutation of chromosomally located genes, which does not disappear or appear with the phylogenetic evolution of the species and is unconnected with the presence of the antimicrobial agent.

2.6 acquired resistance: where, after contact with an antimicrobial agent, a formerly susceptible microorganism undergoes gene mutation or acquires an exogenous resistance gene and so changes its own metabolic pathway, allowing it to avoid being inhibited or killed by the drug. It may be mediated by horizontally transferable elements such as plasmids, transposons and integrons, or arise from chromosomal mutation.

2.7 minimum inhibitory concentration: the lowest concentration of a drug at which inhibition of the growth of a microorganism can be observed, under stated in vitro test conditions and within a stated time (minimum inhibitory concentration, MIC).

2.8 whole genome sequencing: the operation of determining the DNA sequence of an organism in a single round of sequencing, including all the chromosomes, plasmids, mitochondria and, in plants, chloroplasts of that organism.

2.9 mutagenesis: the process of inducing one or more mutations in the genetic material of an organism by physical means, chemical mutagens or biological factors, the frequency of the variants arising being markedly higher than that of spontaneous mutation.

3 Basic dossier on the microbial strain to be assessed

3.1 Basic information, covering the name of the strain, being its Chinese name, Latin name and any alternative names, its source and its use.

3.2 Taxonomic material, covering standard and scientific taxonomic material for the strain at genus, species and subspecies level. Where the taxonomic position of the strain has changed, the material shall also include the name after reclassification and the former name.

3.3 Identification material, covering material identifying the strain on both phenotype and genotype.

3.4 Growth condition material, covering the media and culture conditions suited to the growth of the strain, including but not limited to culture time, culture temperature and humidity, oxygen requirement and light, together with the methods for preserving and reviving the strain.

3.5 Material on mutagenized strains. For a strain that has been mutagenized, the dossier shall include the detailed mutagenesis method, including the mutagen used, the mutagenesis conditions and the mutagenesis test procedure, and the changes in phenotype and genotype that followed mutagenesis.

3.6 Genetic stability material, covering the genetic stability of the key indices or characters of the strain over more than twice the number of generations reached by the greatest number of passages within one production cycle.

3.7 Production-related information, including but not limited to the records of raw and auxiliary materials, the product formula, the process flow, the technical requirements or the enterprise standard for the use of the strain in producing food, food additives and the like.

3.8 Other material: any further technical material that needs to be stated.

4 Assessment methods

On the basis of the material listed in 3.1 to 3.8, the safety of a microbial strain is assessed by the following methods.

a) Analysis of domestic and foreign safety assessment material. The history of use of the strain at home and abroad and its safety, including but not limited to reports on its pathogenicity and toxigenicity, clinical trial material, scientific literature and reviews, are analysed together. Where no such material exists, the history of use and safety assessment material of other strains within the same species, or of species and genera closely related to it, are analysed instead, including but not limited to a statement of the relationship to the strain in question and an analysis of gene sequence match.

b) Whole genome sequencing. The strain is subjected to whole genome sequencing to obtain both a draft genome and a finished genome, algae excepted, and the sequencing data analysed together for virulence genes, resistance genes, genes related to toxin production and the like.

c) Animal pathogenicity testing. Animal pathogenicity tests are carried out by the methods of Annexes A to D or by other equivalent methods and the results evaluated.

d) Resistance testing. Bacterial strains are tested for resistance by the method of Annex E or another equivalent method, and evaluated together with the resistance genes carried, as shown by the finished genome obtained from whole genome sequencing.

e) Toxigenicity testing. For bacterial strains, the presence and expression of toxin-producing genes in the genome are analysed and evaluated together. Where such genes are carried and can be expressed, toxigenicity testing is carried out with reference to the production conditions in several substrates, including the production medium, covering single solid substrates, composite solid substrates of several kinds and combinations of liquids of different composition. The culture time of the toxigenicity test shall be not shorter than one product production cycle, and the content or activity of the toxin, or of the antimicrobial substance, determined by the method of examination laid down in a national food safety standard or by another equivalent method. For fungal strains, toxigenicity testing follows the method of Annex F, the mycotoxin content being determined by the method of examination

laid down in a national food safety standard or another equivalent method. Antimicrobial substances produced by actinomycetes and algal toxins produced by algae shall be examined by the method of examination laid down in the corresponding national food safety standard or another equivalent method. Where the toxigenicity test shows that the strain under test produces a toxin or an antimicrobial substance, the effect on the health of the population shall be assessed from the level produced together with the intake by the population of food produced with the strain.

f) Toxicological testing. A strain with no history of dietary use either at home or abroad shall undergo an acute oral toxicity test or pathogenicity test, three genotoxicity tests, a 90 day oral toxicity test, a teratogenicity test and a reproductive toxicity test. A strain with a history of dietary use only in individual foreign countries or in particular parts of China shall undergo an acute oral toxicity test or pathogenicity test, three genotoxicity tests and a 90 day oral toxicity test. A strain already approved for dietary use in several countries may undergo an acute oral toxicity test or pathogenicity test and two genotoxicity tests. The two genotoxicity tests are the bacterial reverse mutation test and the mammalian erythrocyte micronucleus test, and the three genotoxicity tests follow the genotoxicity test combination laid down in GB 15193.1, National food safety standard, Procedures for toxicological assessment of food safety.

g) Determination of other active substances. A strain added directly to food for infants under one year of age shall also be tested for D-lactic acid by the method of examination laid down in a national food safety standard or another equivalent method. A strain with haemolytic activity or able to produce other metabolites shall also be tested for the haemolytic substance or the metabolites concerned by the method of examination laid down in a national food safety standard or another equivalent method.

5 Judgement of the results

5.1 Pathogenicity and toxigenicity. A strain is held to be safe where all three of the following are met: whole genome sequence analysis shows no known virulence or pathogenicity related gene and no key gene of toxin synthesis, or shows a gene possibly related to pathogenicity which is ruled out as related to pathogenicity in the host on the strength of the functional characters of the strain and the literature; animal testing shows no pathogenicity; and the toxigenicity test shows that no known active metabolite harmful to human health is produced in any of the substrates tested.

5.2 Where the whole genome sequence is found to contain a known virulence or pathogenicity gene or a key gene of toxin synthesis, but animal testing shows no pathogenicity, or the toxigenicity test shows that a known toxic active metabolite is produced in the medium tested but at a low level and assessment shows that long term intake has no effect on human health, the strain may be held to be safe on the strength of that assessment together with the history of safe use at home and abroad.