ZKBS
The Central Committee on Biological Safety

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Position statement of the ZKBS on risk assessment of bacterial environmental isolates in genetic engineering

I. Introduction

The present statement specifies criteria and test methods for the classification of wild-type isolates of bacteria and archaea from environmental samples into a risk group. They are applied when bacteria and archaea are to be used as donor or recipient organisms in genetic engineering work without being clearly identified down to species level (isolates with unclear taxonomic status). This statement does not include the evaluation of genetic engineering work with microcosms.

In principle, environmental isolates should first be reliably characterised using modern biochemical and molecular biological or taxonomic methods. However, if these taxonomic investigations, including MALDI-TOF analysis or sequencing of the 16S rRNA gene or the whole genome, do not result in a clear assignment to an already known species, the principles and procedures described below are applied.

A detailed determination of certain physiological properties and knowledge of the origin or the natural location of an isolate may, under certain circumstances, allow assignment to a risk group (see II.1. to II.3.). If this knowledge is not sufficient for risk assessment, it must be examined which of the further investigations mentioned under II.4. to II.6. are to be carried out for the assessment of a pathogenic potential.

Detailed examination of an environmental isolate may be unnecessary if the taxonomic and physiological studies allow its assignment to certain larger taxonomic units, metabolic types or the microflora of extreme sites which, due to certain characteristics, only contain organisms of risk group 1. This applies, for example, to **acidophilic, alkaliphilic, psychrophilic and thermophilic as well as autotrophic prokaryotes**.

It should be noted that for the classification of donor and recipient organisms into a risk group according to § 5 GenTSV, the formation of toxins does not automatically result in the assignment of the respective organism in risk group 2 if it is not accompanied by an infection of animals or humans. It is known, for example, that a number of cyanobacteria can form substances that are neuro-, cyto- or hepatotoxins or can act as skin irritants or have inflammatory potential. Cyanobacteria are assigned to **risk group 1**, although they can produce toxins. However, possible toxin expression must be taken into account in the hazard assessment of genetic engineering operations in order to be able to take suitable measures to protect workers (see also leaflet B006 of the Berufsgenossenschaft Rohstoffe und chemische Industrie, July 2015).

II. Risk assessment criteria

Investigations and assessment criteria that are to be included in the assignment of prokaryotic isolates with unclear taxonomic positions to a risk group are listed below:

1. Origin of the isolate

For the risk assessment, it is important whether the isolate originates from environmental samples in which predominantly saprophytic organisms occur, or whether organisms with low nutrient requirements are predominant at the site.

If a site typical for the micro-organism is characterised by extreme living conditions, e.g. exposure to toxic substances (e.g. heavy metals), high salinity, high temperatures (e.g. hot springs), very low or very high pH values or high radiation exposure, the isolate is not expected to be a pathogen for humans or animals.

2. Growth conditions

Growth conditions, especially propagation temperature and pH, provide further important information for estimating the risk potential. Both the growth optimum and the tolerance limits should be determined.

Isolates that multiply only below 25 °C or only above 42 °C are generally to be considered apathogenic for humans; the same applies if the pH value in the culture medium must be below 5.5 or above 8.5 for multiplication. From the temperature or pH tolerances, it can also be estimated whether reproduction in humans or in animal organisms is conceivable or probable.

3. Nutrient requirements

Another criterion for risk assessment are nutrient requirements. It should be checked whether an isolate only grows on mineral salt media and whether growth there is inhibited by the addition of complex, organic nutrient media additives such as yeast extract, peptone, etc.

Organisms that grow only very weakly or not at all on complex media can usually be excluded as pathogens.

The criteria mentioned under points 1 to 3 may be sufficient, especially in combination, to carry out a risk assessment of an isolate of unclear taxonomic position. For example, an isolate from a soil contaminated with toxic organic compounds, which only grows up to 30 °C, shows a strong growth inhibition by complex, organic nutrient media additives and multiplies at pH 4, is usually assigned to risk group 1 by the ZKBS.

More often, however, further investigations will be necessary to determine the pathogenic potential of a new isolate. The examinations suggested in the following are examples. The decision as to whether these and, if necessary, further investigations, such as tests for serum resistance, invasiveness, etc., are useful, is made on the basis of the taxonomic proximity of the isolate to known pathogens.

4. Animal experiments

To estimate a pathogenic potential, animal experiments can be carried out that determine e.g. the Lethal Dose 50 in a mouse model. Generally, this involves intravenous or intraperitoneal administration of the organisms. If the new isolate is related to enteropathogenic bacteria then oral tests can also be carried out.

5. Cytotoxicity investigations

Cytotoxicity of culture filtrates for eukaryotic cell lines could be tested.

A culture filtrate of the isolate should be prepared and added to one or better several common established eukaryotic cell lines to test for cytopathic effects.

6. Adhesion experiments

To determine whether an isolate can colonise eukaryotic cells, it should be tested whether it forms adhesins. For this purpose, an established cell culture can be incubated with the isolate.

The criteria listed under II.1. to II.2.3 outline the living conditions for extremophilic, halophilic or autotrophic groups of organisms. These have no hazard potential. Isolates that cannot be assigned to any known species and belong to these organism groups can be used as donor or recipient organisms of risk group 1 in genetic engineering work without having been previously classified in a risk group by the ZKBS.

The classification of an isolate that can neither be assigned to a known species nor belongs to the extremophilic, halophilic or autotrophic organism groups mentioned under II.1. and II.2. into a risk group is a case-by-case decision. This decision must be made by the ZKBS before the organisms are used as donor or recipient organisms in genetic engineering work.

Note:

When classifying prokaryotes with phytopathogenic potential, the statement of the ZKBS on criteria for the evaluation and classification of plant viruses, phytopathogenic fungi and phytopathogenic bacteria as donor and recipient organisms for genetic engineering work (April 2007, ref. 6790-10-53) must also be observed.