Position statement of the ZKBS on classifying
Salmonella typhimurium LT2 strains and Salmonella typhimurium strains
with stable mutations in aroA, galE or cya and crp genes
as recipient organisms in genetic engineering operations

I. Introduction

Salmonella typhimurium belongs to the family of Enterobacteriaceae organisms and as wild type is an
obligate pathogen for humans and various animals. According to § 5 para. 2 and 6 in con-
nection with Appendix I Part B of the GenTSV it is assigned to risk group 2.

For genetic engineering operations many strains (e.g. mutants) of S. typhimurium are used
that have greatly weakened virulence. This therefore presents the question of whether these
strains should be allocated to risk group 2.

After ingesting at least \(10^5 - 10^6\) bacteria in food, wild type strains of S. typhimurium cause
gastroenteritis in healthy human adults. The bacteria penetrate the epithelial cells of the
lower small intestine, are transported to the underlying connective tissue (lamina propria) and
replicate, in part, inside macrophages. The inflammatory reaction involving the bacterial en-
dotoxins and exotoxins results in disruption of fluid and electrolyte transport and in diarrhea,
vomiting and fever. The symptoms last for several days. Salmonella are frequently detected
in stool samples 4-6 weeks after the initial infection. However, long-term excretion is seldom
the case.

The pathogenic principle of S. typhimurium comprises a number of factors, whose signifi-
cance is currently not completely understood. They include the lipopolysaccharide (LPS) of
the cell wall with the O antigen and the endotoxin activity of lipid A, an endotoxin with ADP
ribosyl-transferase activity (Chopra et al., 1987), a cytolysine with inhibitory action on protein
synthesis (Koo et al., 1984) and a range of plasmid encoded factors that are thought to be
involved in serum resistance (Hackett et al., 1987; Vandenbosch et al., 1987). In addition,
S. typhimurium possesses factors that confer invasive properties. Switching off individual
factors of the pathogenic principle, for example the complete O antigen of the cell wall, usu-
ally leads to a considerable decrease or even complete loss of the bacteria’s virulence (Gro-

1. S. typhimurium LT2 strains

The strains of S. typhimurium used in genetic engineering operations mostly belong to the
lysotype LT2. In the scientific literature S. typhimurium LT2 wild type stain is described as a
weak pathogen (Hoiseth & Stocker, 1981). The S. typhimurium LT2 lab strain used in labora-
tory settings has to some extent, like E. coli K12, also been used for many years for genetic
engineering operations, without resulting (when adhering to “good microbiology practices”) in
an infection of personnel (Sanderson & Stocker, 1987). In the USA, S. typhimurium LT2
strains are not under the directives of the NIH for genetic engineering operations and can be
handled like E. coli K12 under laboratory safety measures of the lowest level. However, there
do not seem to be generally valid, safety-relevant markers for strains of lysotype LT2. In
clinical areas lysotype LT2 frequently appears in epidemiological investigations, so with reference to its hazard potential it is not assessed any differently from the usual lysotypes of *S. typhimurium* (Kühn & Tschäpe, Robert Koch-Institute, pers. comm.).

2. *S. typhimurium* mutants

In the last few years a range of stable mutants (reversion rate < $10^{-9}$ per bacterium and generation) have been derived from *S. typhimurium* strains, with the goal of developing non-virulent live vaccine strains (Cardenas & Clements, 1992). The mutations introduced into salmonella include auxotrophies for certain amino acids and purine (Fields et al., 1986) or disruption of bacterial metabolism, e.g. in gene transcription (Curtiss et al., 1989). The effect of the mutations on virulence was mainly tested in mice, where *S. typhimurium* causes disease symptoms similar to the pathogen for abdominal typhoid, *Salmonella typhi*, in humans. Whereas with *S. typhimurium* wild type strains an intraperitoneal administration of only about 10 bacteria can lead to the death of a mouse, the LD for the attenuated mutants, depending on the means of application, increases to between $10^5$ to over $10^9$ bacteria. Thus these strains can be considered weakly virulent.

a) *S. typhimurium* mutants with a defect *aroA* gene

Mutants that show a defect in the *aroA* gene are no longer able to synthesize the enzyme 3-enolpyruvylshikimate-5-phosphate-synthetase, which catalyses the conversion of shikimate-3-phosphate und phosphoenolpyruvate into 5-enolpyruvylshikimate-3-phosphate. Inactivation of this gene blocks the synthesis pathway of several aromatic compounds, including the aromatic amino acids and the metabolite p-aminobenzoate and 2,3-dihydroxybenzoate, precursors for the vitamin folic acid and the enterochelins (iron binding siderophor), respectively (Hoiseth & Stocker, 1981). Since the latter metabolite is not present in mammalian tissues, the mutants cannot replicate there very well and thus their virulence is weakened (Dougan et al., 1988).

b) *S. typhimurium* mutants with a defect *galE* gene

The *galE* gene codes for the enzyme galactose epimerase, which catalyses the conversion of UDP-glucose into UDP-galactose in the synthesis of the bacterial cell wall. Inactivation of this gene results in disrupting the synthesis of lipopolysaccharides (LPS). The mutants no longer possess a complete O-antigen, with the corresponding smooth-form LPS, and are weakened in their virulence, since they are eliminated by the immune system (complement, macrophages) (Hone et al., 1987; Jimenez-Lucho & Leive, 1990).

c) *S. typhimurium* mutants with defect *cyA* and *crP* genes

Mutants with a defect in the genes for *cyA* and *crP* can no longer synthesize adenylate cyclase (*cyA*) or the cAMP-receptor protein (*crP*) (Curtiss & Kelly, 1987). Adenylate cyclase catalyses the conversion of ATP into cAMP in the cell. cAMP can bind to the cAMP-receptor protein and form a complex that can activate transcription of a number of genes (Pastan & Adhya, 1976). This particularly affects genes that are essential for the transport and breakdown of carbohydrates and amino acids. Through the introduced mutations the bacteria are no longer able to use a range of carbohydrates and have a generation time almost twice as long as wild type strains of *S. typhimurium*. Investigations with revertants that have regained the ability to utilize certain carbohydrates have shown that the revertants were also attenuated. It can be concluded that the attenuation of the generated mutants is due to other, yet unidentified factors.
II. Recommendations of the ZKBS for classifying *Salmonella typhimurium* LT2 strains and *Salmonella typhimurium* strains with stable mutations in the genes *aroA*, *gaE* or *cya* and *crp* as recipient organisms in genetic engineering operations

*Salmonella typhimurium* LT2 strains are basically assigned to **risk group 2**. Down-grading to **risk group 1** according to § 5 para. 2 in connection with Appendix I Part B of the GenTSV can only be undertaken after individual case examination, if it can be guaranteed that the organisms to be used are not pathogenic.

*Salmonella typhimurium* strains that carry stable mutations in the genes *aroA*, *gaE* or *cya* and *crp* are, according to § 5 para. 2 in connection with Appendix I Part B of the GenTSV, assigned to **risk group 1**, if they are used as recipient organisms in genetic engineering operations. It is however to be noted that the mutants can be complemented back to wild type through the cloning of foreign DNA, particularly the genes *aroA*, *gaE* or *cya* and *crp*, which can also be present in a mixture of DNA sequences (e.g. gene libraries). A higher allocation of the GMOs to **risk group 2** is necessary then in special cases. Genetic engineering operations where bacterial nucleic acids are introduced into the mutants, which can increase the survival capacity of the bacteria or that code for virulence factors of other pathogenic bacteria are to be submitted to the ZKBS for classification.

**References**


