Aromatic amino acid biosynthesis is dispensable for intramacrophagic growth of *Brucella suis*, despite attenuation of an *aroC* mutant

**BACKGROUND**

A major characteristic of the intracellular pathogen *Brucella suis* is its ability to replicate in the macrophages of the host. Previous work using Signature-Tagged Mutagenesis (STM) on the genome of *B. suis* allowed isolation of an *aroC* (BRO428) mini-Tn5-mutant that was highly attenuated in cellular and murine models of infection, and characterization of the gene. The authors concluded that in *B. suis*, the aromatic amino acids biosynthesis (shikimate) pathway encoded by the *aro* genes was necessary for intracellular and intramurine replication and that *aro*-mutants may be good candidates for vaccine development. However, no other genes of the pathway could be obtained by STM approach, and our group did not isolate any attenuated *B. suis* *aro* mutants by a large-scale Tn5-mutagenesis screen in macrophages. In *Salmonella sp.*, *aroD* mutation had been described to result in attenuation, and mutant strains have been used as vaccine and vaccine carrier strains. In our search for new targets of original anti-bacterial agents against *Brucella* sp., and based on the above-cited results obtained by Foulounge et al., we therefore focused on defined genes of the shikimate pathway to abolish intramacrophagic replication of *Brucella* sp. First, allelic exchange mutants of *B. suis* 1330 were constructed in *aroC*, encoding chorismate synthase, and in *dhs* (BRI013), the first gene of the pathway, encoding 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase.

**OBJECTIVE**

The aim of this study is to analyze the potential importance of aromatic amino acids biosynthesis via the shikimate pathway for intramacrophagic and intramurine replication of *B. suis*. The expected results will allow to evaluate the usefulness of enzymes encoding the different steps of this pathway as targets for novel anti-infective molecules.

**CONCLUSIONS**

1. The shikimate pathway, leading to aromatic amino acids, folate and ubiquinone biosynthesis, is dispensable for intramacrophagic growth of *B. suis* and for its replication in the murine Balb/c model of infection: all tested mutants, except *aroC*, are not attenuated and replicate as the wild-type strain. These observations are consistent with the lack of isolation of attenuated mutants in this pathway during large-scale Tn5-mutagenesis screen.

2. The enzymes encoded by the shikimate pathway are not of interest as potential targets for novel anti-bacterial agents against *Brucella* sp.

3. The observed attenuation of the *aroC* mutant is possibly due to toxic/growth inhibitory effect of the accumulated intermediate product 5-O-(1-carboxyvinyl)-3-phosphoshikimate.

**References:**