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

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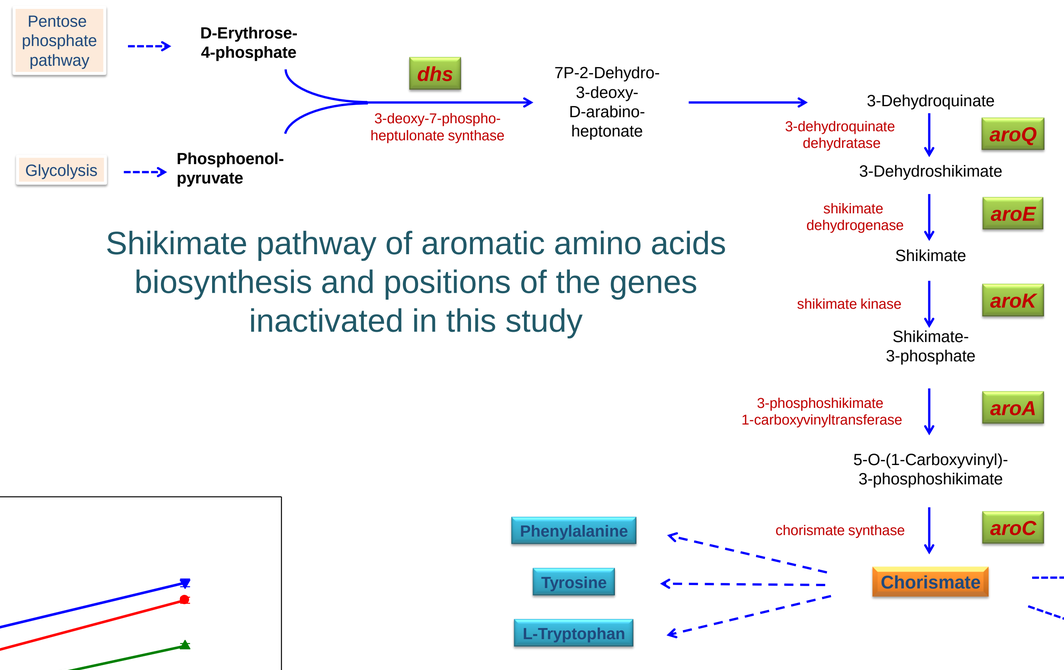
BACKGROUND

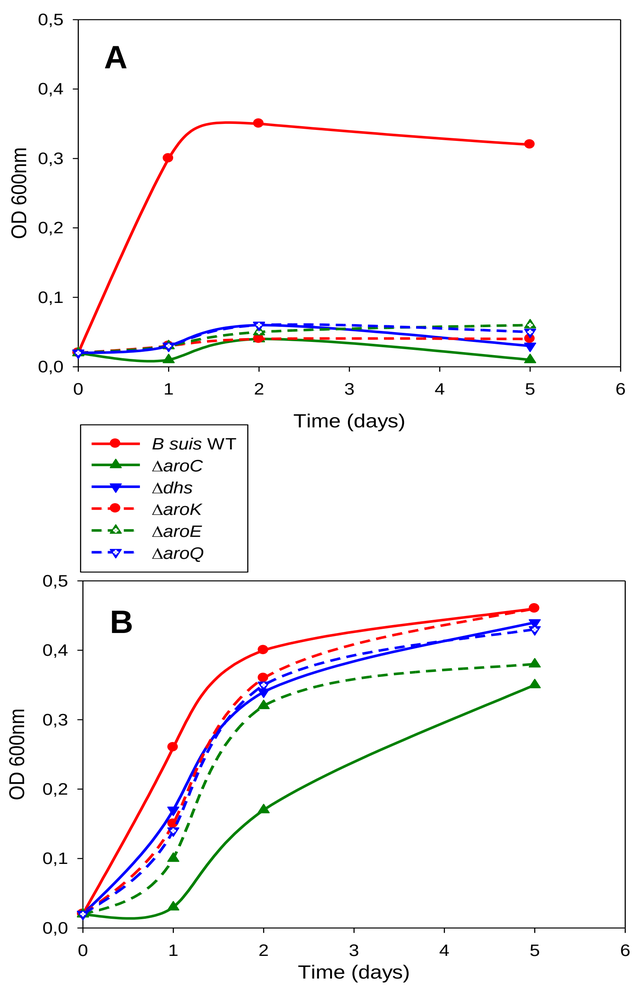
A major characteristic of the intracellular pathogen *Brucella suis* is its ability to replicate in the macrophages of the host. Previous work using Signature-Tagged Mutagenesis (STM) on the genome of *B. suis* allowed isolation of an *aroC* (BR0428) mini-Tn5-mutant that was highly attenuated in cellular and murine models of infection, and characterization of the gene[1,2]. 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[2]. 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[3]. In *Salmonella* sp., *aroA* 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 Foulongne *et al.* with *B. suis*, 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* (BR1013), 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.

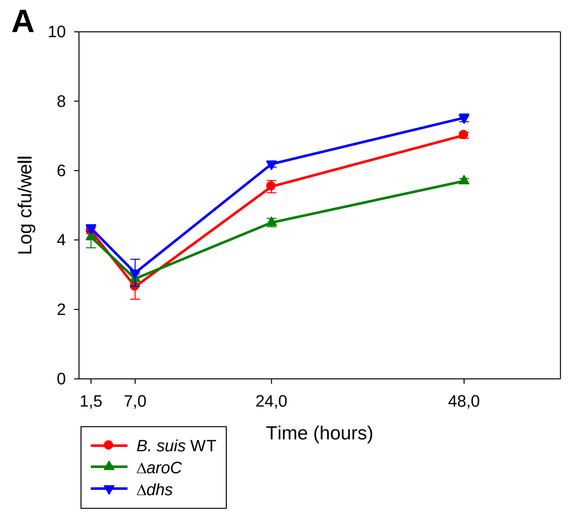


Shikimate pathway of aromatic amino acids biosynthesis and positions of the genes inactivated in this study



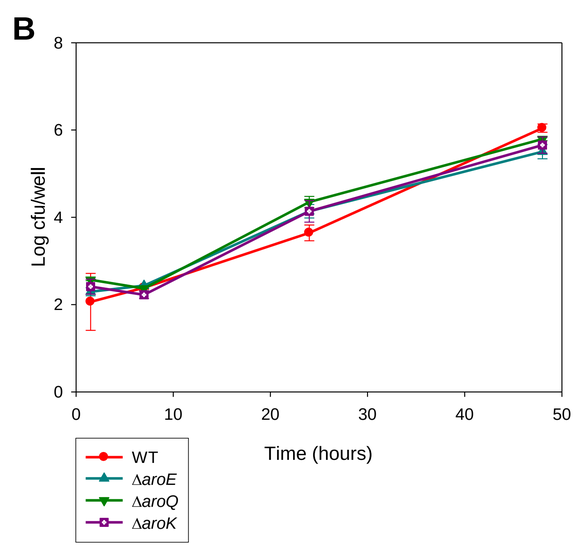
Culture of *B. suis* WT and of shikimate pathway mutants in minimal medium MM (A), and in MM supplemented with aromatic amino acids Phe, Trp, Tyr (B)

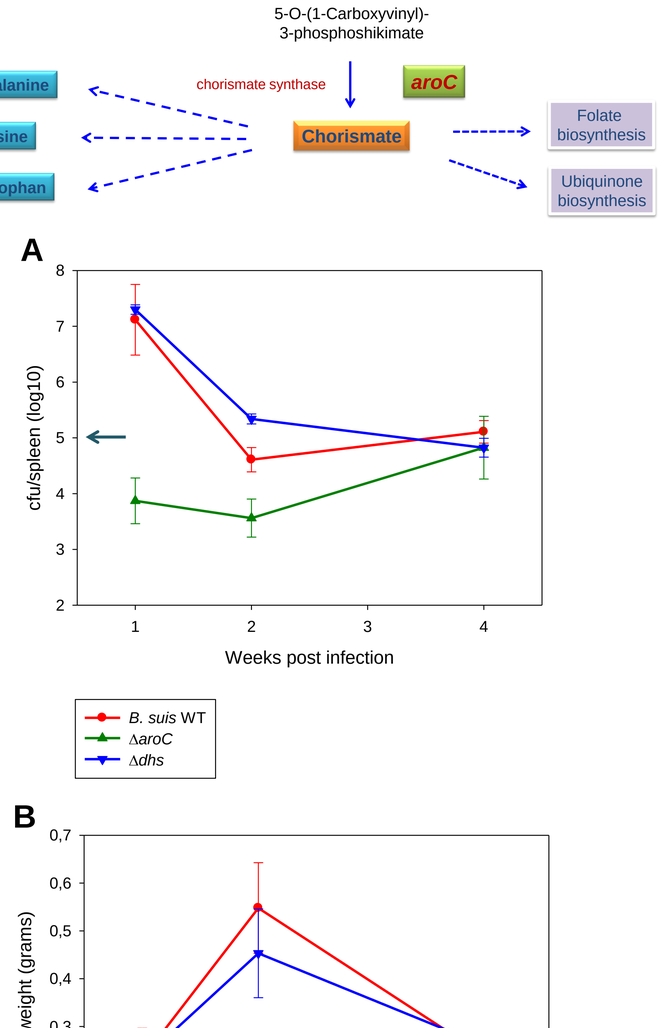
➢ **Addition of aromatic amino acids restores growth of all mutants, despite reduced growth rate of Δ*aroC***



Intracellular replication of *B suis* WT and (A) Δ*aroC*, Δ*dhs*, or (B) Δ*aroQ*, Δ*aroE*, Δ*aroK* mutant strains in murine J774A.1 macrophage-like cells

➢ **Only the Δ*aroC* mutant shows a 10-20-fold reduction**





(A) Growth and survival of *B. suis* WT and of Δ*aroC* and Δ*dhs* in Balb/c spleens; arrow indicates i.p.-injected bacterial dosis. (B) Spleen weights of infected mice

➢ **Mutation of *dhs* does not affect intra-murine growth**

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 Δ*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 our 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: [1] Foulongne *et al.*, Infect. Immun. 68 : 1297-1303 (2000); [2] Foulongne *et al.*, Infect. Immun. 69 : 547-550 (2001); [3]Köhler *et al.*, Proc. Natl Acad. Sci. USA 99 : 15711-15716 (2002)