IN VITRO SENSITIVITY OF *BRACHYSPIRA HYODYSENTERIAE* TO ORGANIC ACIDS AND ESSENTIAL OIL COMPONENTS

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Introduction and Objectives

Swine dysentery seems to be re-emerging in the worldwide pig industry, causing important economic losses.
Increasing antimicrobial resistance in *Brachyspira hyodysenteriae* isolates and increasing efforts to reduce the amount of antimicrobial drugs during pig production have created a growing interest in alternative control measures to ensure animal health. Various approaches such as probiotics, feed additives or the adjustment of the fermentable carbohydrate-content in feed (1) have been suggested.

The aim of the present study was to evaluate the *in vitro* antimicrobial activity of various organic acids and essential oil-components, possibly given as feed additives, against the causative agent of swine dysentery: *B. hyodysenteriae*.

Material and Methods

Based on their potential antibacterial effects, 11 organic acids (formic, acetic, propionic, butyric, citric, lactic, benzoic, caproic, caprylic, capric and lauric acid) and 4 essential oil-components (eugenol, carvacrol, thymol and cinnamaldehyde) were selected. The minimum inhibitory concentration (MIC) of these products at pH 7.2 was determined against 3 *B. hyodysenteriae* strains. The first strain, B78, was a reference strain (ATCC 27164). The two other strains, 6bI and 8dII, were field isolates, obtained from 2 farms where pigs were diagnosed with clinical swine dysentery. For isolate 8dII, the MIC values at pH 6 were also determined.

The inoculum was prepared by harvesting *Brachyspira* cells from a blood agar plate (after 4 days of incubation) and suspending these bacteria in anaerobic brain heart infusion (BHI) broth, supplemented with 10% fetal calf serum (FCS). Optical density was measured to obtain a final inoculum concentration of 1 - 5 x 10^6 bacteria/ml. For the MIC assay at pH 6, pH of the BHI was adjusted with HCl.

The susceptibility tests were performed using a broth microdilution method (2). The wells of 48 well-culture plates were filled with 100 µl of twofold serial dilutions of the tested compounds. During preparation of the twofold dilution series in BHI + FCS, the pH of each dilution was controlled and adjusted with NaOH or HCl to pH 7.2 or pH 6.

To each well, 400 µl inoculum was then added and the panels were incubated under anaerobic circumstances (84% N_2, 8% CO_2 and 8% H_2) at 37°C on a rotary shaker. Growth and sterility control wells were included.

MIC values were determined after 4 days as the lowest concentration of the compound that prevented visually observable growth of *B. hyodysenteriae*.

Results

For all compounds tested, MIC values of the 3 *B. hyodysenteriae* strains were equal (maximum difference of one twofold dilution).
B. hyodysenteriae was, at pH 7.2, most sensitive to cinnamaldehyde (MIC: 0.31 mM), lauric acid (MIC: 0.63 mM), carvacrol and thymol (MICs: 1.25 mM) and capric acid (MIC: 1.25 – 2.5 mM). The strains were least susceptible to formic, acetic and lactic acid with MIC values of 320 mM.
At pH 6, MIC values of the organic acids were 4 to 8 times lower compared to those at pH 7.2. For lauric acid and the essential oil components, only a twofold decrease of the MIC was observed.

Discussion

The *in vitro* results of the present study demonstrate a direct antibacterial effect of certain essential oil components and (medium chain) organic acids against *B. hyodysenteriae*, indicating that these products might be useful to reduce *B. hyodysenteriae* infections. However, despite the low MIC values at pH 6 (about the normal pH in the large intestine of pigs), clinical trials should be performed to evaluate the *in vivo* efficacy of these products as a control strategy for swine dysentery.

References

1. Pluske et al., 2002. Nutr Res Rev. 15: 333-371

2. Karlsson et al., 2002. Vet Mic. 84: 123-133