BIOCHEMICAL TESTS USED FOR IDENTIFICATION OF BORDETELLE BRONCHISEPTICA

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Abstract: 18 isolates from pigs suspected to be B. bronchiseptica were submitted to citrate, oxidase, catalase, production of H₂S and indole, acid production from glucose, lactose, manose, inositol, sorbitol, rhamnose, sucrose, melobiose and arabinose biochemical tests. Urease and motility tests were performed on MIU culture media in both modulating and non-modulating conditions. The obtained biochemical profile (positive reactions for oxidase, catalase production; urease production and motility in modulating conditions; utilisation of the sodium citrate as a single carbon source; negative reactions for acid production in the slant or butt of TSI agar; H₂S production in the TSI agar; acid production from manose, inositol, sorbitol, rhamnose, sucrose, melobiose and arabinose in the column of OF culture media; urease and mobility in MIU culture media in non-modulating conditions) matched 100% the biochemical profile of B. bronchiseptica with combined with the fact that all strains had typical B. bronchiseptica colonial morphology on MacConkey agar allowed us to identify the studied strains as B. bronchiseptica.

INTRODUCTION

The natural habitat of B. bronchiseptica is the upper respiratory tract of healthy and diseased humans, pigs, dogs, rabbits, guinea pigs, rats, horses and cats. The most frequently affected species are pigs, dogs and cats, B. bronchiseptica being involved in the development of the athrophic rhinitis in pigs (3), the Upper Respiratory Tract Disease (URTD) in cats (4) and it also invades dogs suffering of canine infectious tracheobronchitis (“kennel cough”) and canine distemper (3).

Isolation of B. bronchiseptica can be performed on various culture medias such as MacConkey agar, Bordet-Gengou agar or Smith-Baskerville culture media. These culture medias allows the growth of numerous contaminants that often alters the colonial morphology of B. bronchiseptica, making necessary the performance of numerous biochemical tests for identification of B. bronchiseptica.

B. bronchiseptica is a strict aerobe bacteria. Cultivated in modulating conditions (in the presence of 20 mM of MgSO₄ in 1000 ml culture media) at 37°C B. bronchiseptica is motile and urease positive. In the absence of MgSO₄ in the culture media B. bronchiseptica is non-motile and is urease negative (2).

B. bronchiseptica is positive to the oxidase, catalase and citrate tests (3) and is negative for indole and H₂S production. It does not produce acid from glucose, lactose, manose, inositol, sorbitol, rhamnose, sucrose, melobiose and arabinose (1).

The aim of this study was to perform the oxidase, catalase, citrate, urease and motility tests, the indole and H₂S production tests and also the acid production tests from glucose, lactose, manose, inositol, sorbitol, rhamnose, sucrose, melobiose and arabinose on 18 isolates
from pigs suspected to be *B. bronchiseptica* and to compare the obtained biochemical profiles with those of *B. bronchiseptica* for a positive identification based on these biochemical tests.

**MATERIAL AND METHOD**

The biochemical tests were performed during August 2006 within the Microbiology Laboratory of Veterinary Medicine Faculty, Cluj-Napoca.

For the urease and motility tests for each isolate we used two glass tubes with MIU (motility, indole, urease) culture media, one tube with 20 mM of MgSO$_4$ for 1000 ml culture media and another tube without MgSO$_4$ mixed in the MIU (motility, indole, urease) culture media. The tubes were stab inoculated and incubated at 37°C and examined after 48 hours.

For acid production from glucose and lactose and for H$_2$S production we used TSI (tripple sugar iron) agar cooled in glass tubes in inclined position so that we obtained a 2 cm high column in the bottom of the tubes and an approximately 5 cm long slant above the column. The column portions of the TSI culture medias were stab inoculated and the slants were surface-inseminated. The tubes were aerobically incubated at 37°C and examined after 24 and 48 hours.

All isolates were surface-inseminated on Simmons-citrate agar slants to establish if they use or not the sodium citrate as a single carbon source. For this test the tubes were aerobically incubated at 37°C for 24 hours.

To determine the oxidase activity of the isolates each of them was cultured on a plate containing MacConkey agar. They were aerobically incubated at 37°C for 48 hours. One isolated colony with specific *B. bronchiseptica* colonial morphology from every plate was mixed with a droplet of oxidase reagent on a piece of filter paper. Two another isolated colonies with specific *B. bronchiseptica* colonial morphology from every plate were inseminated in two tubes, one containing 5 ml liquid media and an another tube containing 1 ml liquid media and the tubes were aerobically incubated at 37°C for 24 hours. After incubation we added 3 ml of H$_2$O$_2$ to the tubes with 5 ml of liquid media to determine the presence of the catalase enzime. The tubes with 1 ml of liquid media were used for the establishment of indole production. To each tube we added a sufficient quantity of Erlich-Kovács reagent (5-6 droplets) to form a ring above the liquid media.

For establishing the acid formation from different sugars (manose, inositol, sorbitol, rhamnose, sucrose, melobiose and arabinose) we used OF basal medium according to Hugh and Leifson. We prepared 625 ml of OF basal medium according to Hugh and Leifson. We prepared 625 ml of OF basal medium and we sterilised it by autoclaving for 15 minutes at 121°C. After the distribution of the sterile OF basal media in 126 sterile glass tubes (approximately 5 ml/ tube) we separated 7 groups of 18 tubes. To obtain a concentration of 1% sugars in these tubes, in the first group of tubes we added 0.25 ml of 20% filter-sterilised manose for each tube, in the second group of tubes we added 0.25 ml of 20% filter-sterilised inositol for each tube, in the third group of tubes we added 0.25 ml of 20% filter-sterilised sorbitol for each tube, in the fourth group of tubes we added 0.25 ml of 20% filter-sterilised rhamnose for each tube, in the fifth group of tubes we added 0.25 ml of 20% filter-sterilised sucrose for each tube, in the sixth group of tubes we added 0.25 ml of 20% filter-sterilised melobiose for each tube and in the seventh group of tubes we added 0.25 ml of 20% filter-sterilised arabinose for each tube. Each isolate was stab inoculated in one of the tubes of each groups and the tubes were incubated for 24 hours at 37°C in aerobic conditions.
RESULTS AND DISCUSSIONS

The isolates inseminated in MIU culture media with 20 mM of MgSO₄ for 1000 ml culture media proved to be urease positive (the pinkish colour of the culture media turned to violet after 48 hours of incubation) and motile (star-shaped colonies were present in the whole volume of the culture media). The isolates inseminated in MIU culture media without MgSO₄ were urease negative (they did not changed the pinkish colour of the culture media) and non-motile (bacterial growth was present only on the insemination line).

On TSI slants all isolates produced an alkaline reaction (they did not produced acid from lactose and the TSI slants turned to red from oranges after 24 hour of incubation, without any modifications of the TSI columns at this time. After 48 hour of incubation all isolates produced an alkaline butt reaction with no gas or H₂S (they did not produced acid from glucose and the TSI column become red).

All isolates proved to be citrate positive, turning the green colour of the Simmons-citrate medium to blue.

The oxidase reaction was positive in all cases, the mixture of colonies with oxidase reagent on filter-paper was dark blue for all the 18 isolates.

The tubes with 5 ml of liquid media mixed with 3 ml of H₂O₂ showed massive gas and foam production in all cases, so all tested isolated proved to be catalase positive.

All isolates taken in study were indole negative, the Erlich-Kovács reagent did not turned to red, instead it remained brown after the stirring of the tubes.

All the 126 tests for acid productions were negative, all tested strains proved to be inactive for all tested sugars (the culture media formed in all cases a blue ring on the surface and the column below remained green).

The biochemical profile of all strains was identical. All strains taken in study were negative for the following tests: acid production in the slant or butt of TSI agar; H₂S production in the TSI agar; acid production from manose, inositol, sorbitol, rhamnose, sucrose, melibiose and arabinose in the column of OF culture media; urease and mobility in MIU culture media. All strains used the sodium citrate as a single carbon source; they produced oxidase and catalase; in MIU (motility, indole, urease) culture media with 20 mM of MgSO₄ for 1000 ml they were motile and urease positive. This biochemical profile matches 100% the biochemical profile of *B. bronchiseptica*, and combined with the fact that all strains had typical *B. bronchiseptica* colonial morphology on MacConkey agar allowed us to identify the studied strains as *B. bronchiseptica*.

CONCLUSIONS

- All strains taken in study were negative for the following tests: acid production in the slant or butt of TSI agar; H₂S production in the TSI agar; acid production from manose, inositol, sorbitol, rhamnose, sucrose, melibiose and arabinose in the column of OF culture media; urease and mobility in MIU culture media in non-modulating conditions;
- All strains used the sodium citrate as a single carbon source; they produced oxidase and catalase; in MIU (motility, indole, urease) culture media with 20 mM of MgSO₄ for 1000 ml they were motile and urease positive;
- The biochemical profile of the 18 strains taken in study was identical and these biochemical profiles matches 100% the biochemical profile of *B. bronchiseptica*. 
• The obtained biochemical profiles witch matches 100% the biochemical profile of B. bronchiseptica combined with the fact that all strains had tipical B. bronchiseptica colonial morphology on MacConkey agar allowed us to identify the studied strains as B. bronchiseptica.

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