Characterization and possible probiotic influence of Bacillus smithii strain TBMI12 in vivo

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Abstract
The objective of our study was to investigate the possibilities to use sporogenic lactic acid producing bacilli (Hong, et al 2005) as preventive agent to avoid Clostridium difficile associated diseases in murine and hamster model. Bacillus smithii TBMI12 (sporogenic, lactic acid producing) was isolated from human gut and so we try this train probiotic ability against Clostridium difficile associated diseases. Clostridium difficile (CD) is a common nosocomical pathogen that is the causative agent of pseudomembraneous colitis and a major cause of antibiotic-associated diarrhoea (Naaber, et al 1998). Usual therapy in these cases is metronidazol or vancomyine oral treatment. But in this way there’s also a chance of recurrences of therapy, estimably up to 50% of cases (Aslam, et al 2005).

Two models were used for in vivo CD infection: cefoxitine treated mice model and ampicilline treated hamster model.

Results in CD mice model do not indicate good effectivity of B. smithii TBMI12 spores. Significant positive influence was visible only when metronidazol or metronidazol+ TBMI12 groups were compared. This indicates that mouse model is complicated for such investigation. Bacillus smithii TBMI12 influence in antibiotic-compromised hamster CD infection model was more promising. As hamsters are extremely sensitive to CD toxins, the in vivo experiments with hamsters are more trustable than experiments with mice. B. smithii TBMI12 was maintained in hamsters during a month and all hamsters colonized with spores (3 days, 10⁴), survived the infection with 10⁶ CD cells. Hamsters in control group who received only 10⁴ CD cells, died within 48 hours. Current research demonstrates, that Bacillus smithii TBMI12 spores might have probiotic effect against CD infection, especially in hamster model.

Introduction and Purpose
The objective of our study is to investigate the possibilities to use sporogenic lactic acid producing bacilli as preventive agent to avoid Clostridium difficile associated diseases in murine and hamster model. Since Bacillus smithii TBMI12 (sporogenic, lactic acid producing bacterium) was isolated from human gut, we decided to examine this strain’s probiotic ability against Clostridium difficile associated diseases.

Methods
Two in vivo models were used for Clostridium difficile infection: cefoxitine treated mouse model and ampicilline treated hamster model. In mouse model experimental animals were treated with cefoxitine during 5 days and then inoculated intragastrically with C. difficile strain VPI 10463 (10⁴ cells). Next day four groups were formed and treated as follows: 1) with B. smithii TBMI12 (10⁴ spores); 2) with B. smithii TBMI12 (10⁴ spores)+metronidazol; 3) with metronidazol and 4) control group. In hamster model experimental animals were treated with single dose ampicilline, inoculated with B. smithii TBMI12 (10⁴ spores) and after 4 hours were group infected with CD strain VPI 10463 (10⁶ cells). After CD infection hamsters received again 2 doses of spores in next 2 days. Control group was infected and left without probiotic treatment (without B. smithii TBMI12 12 spores).

Figure 1. C. difficile VPI 10463 (CD) counts from infected mice after treatment with B. smithii TBMI12 (10⁴ spores) (n=5) and without treatment.

Figure 2. C. difficile VPI 10463 counts from infected mice faeces after treatment with metronidazol and B. smithii TBMI12 (n=5), or only with metronidazol (n=5).

Figure 3. Bacillus smithii TBMI12 survival (mean n=5) in hamster, after antibiotic (ampicilline), probiotic (B. smithii TBMI12 3 x 10⁸ spores; 2,3,4 day) and C. difficile VPI 10463 (10⁶ cells; 2 day) treatment and in mice(B. smithii TBMI12 3 x 10⁸ spores; 0 and 6 day), after cefoxitine 5-day treatment.

Results
Results in CD mouse model do not indicate good effectivity of B. smithii TBMI12 spores. Significant positive influence was visible only when metronidazol or metronidazol+ TBMI12 groups were compared. This indicates that mouse model is complicated for such investigation. Bacillus smithii TBMI12 influence in antibiotic-compromised hamster CD infection model was more promising. As hamsters are extremely sensitive to CD toxins, the in vivo experiments with hamsters are more trustable than experiments with mice. B. smithii TBMI12 was maintained in hamsters during a month and all hamsters colonized with spores (3 days, 10⁴), survived the infection with 10⁶ CD cells. Control group hamsters who received only 10⁴ CD cells died within 48 hours.

Conclusion
Current research demonstrates, that Bacillus smithii TBMI12 spores might have probiotic effect against Clostridium difficile infection, especially in hamster model.

References