CONCLUSIONS

In this study we focused on the process of bilirubin reduction catalyzed by an anaerobic intestinal bacterium *C. perfringens*. We aimed to undertake analysis of bile pigments metabolized by *C. perfringens* and their respective reduction products and to identify gene(s) encoding protein(s) involved in metabolism of bilirubin.

Analysis of bile pigments metabolized by *C. perfringens* and their respective reduction products

1) The *C. perfringens* strain BR1 isolated from neonatal stools reduces a variety of different bile pigments indicating that this broad substrate specificity could be an effective tool for disposal of electrons produced in catabolic processes within these bacteria.
2) The examined strain reduces UCB only to the level of urobilinogen. Other bacterial strains and species, absent in neonates, are presumed to be essential for catabolism to the level of stercobilinogen.

Identification of gene(s) involved in bilirubin metabolism

1) The *C. perfringens* strain BR1 is resistant to the transformation of plasmid DNA mediated by electroporation and therefore it is not a candidate suitable for transposon mutagenesis.
2) A transformable *C. perfringens* P90.2.2. strain was found to reduce bilirubin. Rapid and simple method suitable for electroporation of this strain was developed providing transformation efficiency up to 1.37 x 104.
3) Transposon mutagnenesis of the strain P90.2.2. failed to identify gene(s) involved in metabolism of bilirubin. We were not able either to introduce transposons Tn*916* and Tn*917* into this strain or to isolate clones incapable to reduce bilirubin.
4) The attempt, based on construction of *C. perfringens* P90.2.2. genomic library in *E. coli* and its screening for clones able to reduce bilirubin, failed to isolate gene(s) responsible for the reduction of bilirubin as well.