

Project Title:	Effects of Cattle Feeding Location and use of Tylosin on Expression of Antimicrobial Resistance (AMR) and Liver Abscesses
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Completion Date:	September 2017

Background

In recent years, the use of antibiotics (especially antibiotics used as growth promotants) in food animals has come under great scrutiny. Therefore, as the beef industry looks ahead to feed the increasing world population with a high-quality protein source, ensuring “antibiotic stewardship” is critical to the industry's future. Studies have demonstrated that continuous use of antibiotics can result in collections of antibiotic resistance genes within bacteria. Therefore, the need to develop novel approaches to reduce antimicrobial use while improving animal health and efficiency is critically needed. Such strategies that are developed to reduce antibiotic use need to be supported by science-based evidence.

With the increase of food animal production, the need for better animal health and well-being, disease control, and “health management” (Mathew et al., 2007) has increased. As a result, the use of antimicrobial compounds in the industry has increased. Currently in the beef industry, antibiotics are used for many purposes including: 1) to treat animals when they are sick, 2) to treat animals when they are stressed or at risk “prophylactic use” (Mathew et al., 2007), and 3) as a growth promotant to increase gain and efficiency (McEwen and Fedorka--Cray, 2002; Viola and DeVincent, 2006; Mathew et al., 2007). However, as an industry, we need to “ensure antibiotic stewardship.” To this end, a recent paper published by McEwen and Fedorka-Cray reported that substitutes to growth promoting and “prophylactic” antimicrobials are needed and such alternatives will incorporate better management practices, greater use of vaccines, and novel probiotics (McEwen and Fedorka--Cray, 2002). As such, identifying alternative technologies and management strategies to reduce the use of antimicrobial agents in the beef industry is critically needed.

These circumstances have led investigators to consider direct fed microbials (DFMs) as a means to control liver abscesses as they may reduce pathogenic organisms through competitive exclusion. However, to date, the identification of DFMs that are effective in controlling liver abscesses remains elusive (Luebke et al., 2013; Scott et al., 2017). As a consequence, opportunities are available to develop a novel DFM to control and reduce the incidence of liver abscesses. In this study, a library of rumen bacterial isolates was developed and screened to identify microbial species in the rumen that reduce the population of *Streptococcus bovis* (the major organism associated with acidosis) and *Fusobacterium necrophorum* subsp. *Necrophorum* (the major organism associated with liver abscesses). The results presented in this preliminary study provide compelling evidence that DFMs can be used as an alternative to control acidosis and liver abscesses.

The objectives of the study were to 1) isolate microbial species from the rumen using different media compositions and incubation methods to develop a library of rumen microbial isolates and 2) screen the isolates for microbial species that could inhibit the growth of



Streptococcus bovis, *Fusobacterium necrophorum*, *Arcanobacterium pyogenes*, and *Pasteurella multocida*.

Methodology

Isolation of microbial species from the rumen of finishing cattle using different isolation media and methods: Using previously described methods we isolated microbial species that produce bioactive compounds (Satoshi, 1992; Sultan, 2010) from the rumen. We have isolated over 1000 microbial isolates from the rumen using different culture media including ISP1, ISP2, ISP4 media formulated by the international Streptomyces species project (ISP) and Casein starch agar medium. The isolation strategy was focused on isolating spore formers and therefore we used heat to kill vegetative cells by heating the samples to 70 °C for 1-2 hrs before culturing. Screen the isolates for microbial species that could inhibit the growth of *Streptococcus bovis*, *Fusobacterium necrophorum*, *Arcanobacterium pyogenes*, and *Pasteurella multocida*: The screening for isolates with antimicrobial activity against the test species noted above were performed as described previously using the agar overlay method (Kang and Fung, 1998). The isolates that show inhibition of the test microbial species were grown and characterized by sequencing the 16S gene. Following characterization and identification of the species, additional tests were performed based on published literature to identify the antimicrobial activity displayed by the organism. Finally, cell extracts of the isolates identified were used to further test the applicability of the strains isolated as direct fed microbial species in beef cattle.

Findings

We have isolated 5 different bacterial isolates each with the capability to inhibit the growth of *Streptococcus bovis* and *Fusobacterium necrophorum*. Additionally, some of the strains isolated are also capable of inhibiting the growth of *Arcanobacterium pyogenes* and *Pasteurella multocida*. This study clearly demonstrates the ability of naturally occurring microbes in the rumen to produce inhibitory metabolites against pathogenic or opportunistic pathogens in the rumen.

Implications

This new approach of identifying naturally occurring probiotic isolates from the rumen as an alternative to current antibiotics is a viable strategy to control pathogens and opportunistic pathogens in the rumen while reducing antibiotic use in the beef cattle industry. As the industry moves forward to feed the growing world population with a rich protein source, strategies developed in this project will be critical to improve animal health and maintain “antibiotic stewardship.”



References

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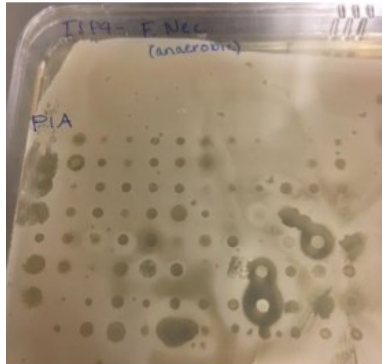


Figure 1. A representative plate with many potential candidates that was used for further screening. The clear zones around the culture shows a zone of inhibition by the isolate inhibiting the growth of *Fusobacterium necrophorum subsp. necrophorum*. See methods for further details

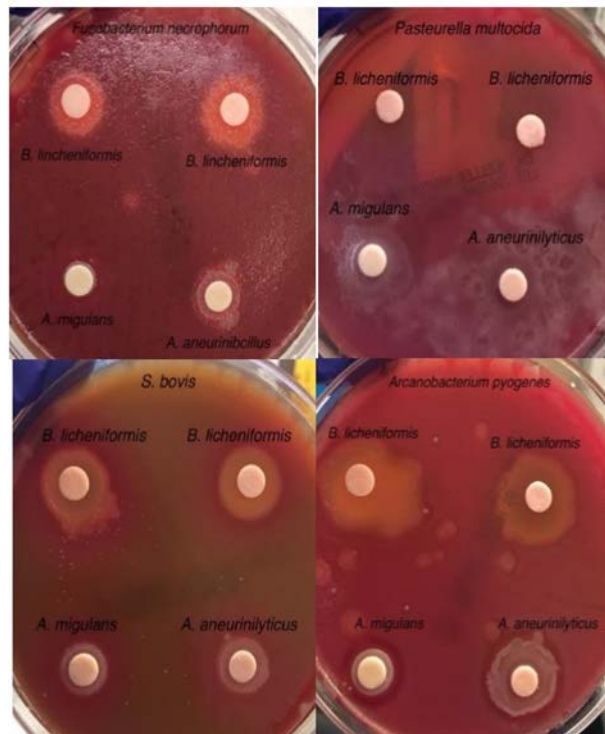


Figure 2. Antimicrobial activity of isolated probiotic strains against *Fusobacterium necrophorum subsp. necrophorum*, *Streptococcus bovis*, *Arcanobacterium pyogenes*, and *Pasteurella multocida* using Kirby-Bauer Disk Diffusion Susceptibility test