16S rRNA MILK MICROBIOTA PROFILES ON HOLSTEIN COWS HIGHLIGHT QTL AND PROVIDE A NOVEL TRAIT TO ASSESS THE GENETIC REGULATION OF MASTITIS

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PROJECT OBJECTIVE



- Previous discriminant analysis of bacterial genera from pyrosequencing of I6SrRNA gene from Holstein milk samples showed variation between healthy and clinical mastitis samples
 - (Oikonomou et al., 2012)
- Assess the use of I6SrRNA milk microbiota sequencing profiles for cows as a novel variable to identify QTL for mastitis susceptibility/ resistance.

Rodrigo C. Bicalho

CURRENT PROJECT (PAPER #508) – CLINICAL MASTITIS





 Step1.
 Veterinary diagnosis of clinical mastitis or healthy within 0-30 DIM

 Clinical Mastitis (CM)
 Healthy

 Fealthy
 Healthy

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MATERIALS & METHODS (PAPER #508) – 16SrRNA

Step 2. Colostrum samples were collected from mammary gland quarters at 1st milking after parturition

Colostrum





<u>Step 3a.</u> Pyrosequencing of I6SrRNA gene hypervariable region 4 (V4)

<u>Step 3b.</u> Sequences binned into Operational Taxonomic Units (OTU)

<u>Step 3c.</u> General linear model was used to compare the relative abundance of microbial phyla & taxa

Step 3d. Mammary gland quarter statistics were merged and averaged to generate a single composite milk microbiota profile per cow

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(Oikonomou et al., 2012; Lima et al., 2017; Oultram et al., 2017)

MATERIALS & METHODS (PAPER #508) – COW GENOTYPING

<u>Step 4.</u> Whole-genome characterization using the Illumina 777K beadchip

100 cows, 777K SNPs



SNP filtering: •> 90% call rate

•< 5% minor allele frequency

Sample filtering: •> 90% genotyping call rate

100 cows, 578,497 SNPs



5

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MATERIALS & METHODS (PAPER #508) – GWAS





GWAS RESULTS – CLINICAL MASTITIS

Significantly associated regions on BTA 5, 16, and 29
 Suggestive results on BTA 26

Horizontal line denotes FDR ≤ 0.05 .



GWAS RESULTS – COW MILK MICROBIOTA PROFILES

Significantly associated regions on BTA 5, 26, and 29
 Suggestive results on BTA 9 & 16

Horizontal line denotes FDR ≤ 0.05 .



GWAS COMPARISON

Figure 1. Manhattan plots for the genome-wide association analysis respective of A) clinical mastitis or B) cow milk microbiota profile. Blue boxes highlight regions showing variation and red boxes highlight regions showing similarity between trait GWAS. Horizontal black line denotes FDR ≤ 0.05 .



DOWNFALLS & CONTROVERSY

- Small dataset (100 Holstein, single farm)
- The origin of milk microbiota is currently controversial, pitting long-time beliefs that the mammary gland and the milk therein is sterile thereby microbiota found are specifically related to infection and comparing this belief with the theory of commensal microbial communities within the mammary gland

The comparison of milk microbiota, irrespective of origin from mammary, teat, or even udder, provides an opportunity to compare individual cow microbiota profiles with their genome.

CONCLUSIONS

- Over 15 potential genes were found in the associated regions of the two GWA studies. TBK1 on BTA 5 and RARRES3 and CTSE on BTA 29 appear the most promising candidate genes affecting immune response.
- Previous QTL for clinical mastitis have been reported on BTA 5, 9, and 26 with similar levels of significance, providing validation towards the potential of our preliminary results
- Both of our analyses, CM and microbiota profile, demonstrate novel QTL on BTA 16 and 29. This could be due to variation in study cohort including breed, increased phenotyping consistency by using cows as opposed to indirect measures on bulls, and the utility of the high-density 777K SNP array.

Both the similarity and difference between the two GWAS

- Hypothesize that the microbiota analysis will generate novel QTL potentially related to host predisposition to certain microbiota profiles as well as QTL reflected in the CM GWAS as reflected by QTL on BTA 6, 26, and 29.
- Expect some QTL to overlap, showing similar association relating milk microbiota profiles to CM as seen on BTA 5, 9, 16, 26, and 29

FUTURE DIRECTIONS: UNDERSTANDING MASTITIS ETIOLOGY USING A LONGITUDINAL APPROACH

Asha Miles PhD Candidate



524 cows, 2 commercial farms

12

FUTURE DIRECTIONS

<u>Mastitis:</u> Identification of QTL related to milk microbiota profiles correlated to clinical mastitis and elevated somatic cell count



Host - Microbiome Interactions

- •Longitudinal characterization of microbiota over lactation
- •Cross-sectional characterization at specific physiological changes
- •Relationship of microbiota profiles to cow genotype





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