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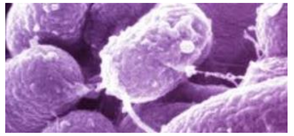
Genome wide DNA methylation analysis reveals role of DNA methylation in cow's ileal response to *Mycobacterium avium* subsp. *paratuberculosis*



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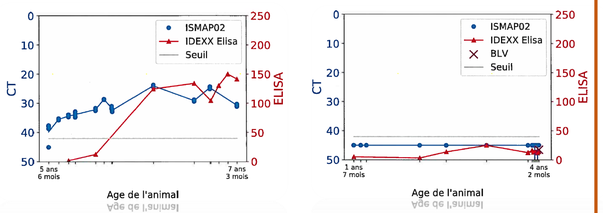
INTRODUCTION

- Several investigations on disease progression of Johne's disease (JD) in dairy cows have revealed molecular mechanisms implicated in *Mycobacteria avium* ssp. paratuberculosis (MAP) pathogenesis[1, 2, 3]
- Epigenetic processes regulate the expression of genes and many biological processes [4].
- Limited studies have examined the role of DNA methylation in the pathogenesis of JD.

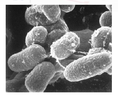
OBJECTIVES

This study examined the impact of subclinical MAP infection on DNA methylation profile in the ileum of cows, the site of initial interaction between MAP and host.

METHODS



MAP +/+ (N=5) **MAP -/- (N=5)**



DNA isolation from IL tissues

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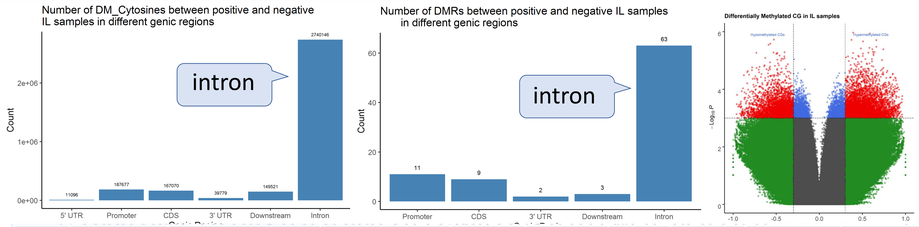
Library preparation and whole genome bisulfite sequencing (WGBS)

↓

Bioinformatics analysis

RESULTS AND DISCUSSION

- 2000 DMCs (FDR< 0.05) and 205 DMRs (p< 0.01) were detected.
- Majority of DMCs and DMRs are located in **intergenic regions** (87.2% and 57.1%) followed **by intronic regions** (12.8% and 30.7%) of genes, respectively.
- Some DMCs are located on **250** genes including genes that were previously identified to be associated with JD (Table 1).



	*Total DMCs/DMRs	DMCs/DMRs in intergenic regions	DMCs/DMRs in genic regions	5' UTR	Promoter	CDS	3' UTR	Downstream	Intron
DMC	2125	1774	351	1	34	25	0	26	265
DMR	1180	112	88	0	11	9	2	3	63

Table 1. Some DMC between MAP+ve and MAP-ve cows and their annotated genes

Chr	Position	Strand	Pvalue	FDR	Gene Symbol	Genic Region	Meth Diff*	Meth Status**
3	95344349	-	0.003	0.001	CDKN2C	Promoter	0.286	Hyper
5	55720776	-	0.006	0.012	TSPAN31	Promoter	0.256	Hyper
13	54461185	-	0.010	0.011	SLC17A9	Promoter	0.361	Hyper
13	54461479	-	0.003	0.009	SLC17A9	Promoter	0.667	Hyper
15	37810679	-	0.009	0.017	CALCB	Promoter	0.274	Hyper
19	42828645	-	0.007	0.003	CCDC56	Promoter	0.590	Hyper
14	21016234	-	0.007	0.046	PCMTD1	CDS	-0.391	Hypo
14	21016249	-	0.006	0.046	PCMTD1	CDS	-0.327	Hypo
1	1.26E+08	+	0.005	0.003	SLC9A9	Intron	0.361	Hyper
1	1.26E+08	+	0.000	0.013	SLC9A9	Intron	0.424	Hyper
2	79554358	-	0.007	0.027	STAT1	Intron	-0.476	Hypo
2	79554015	-	0.006	0.011	STAT1	Intron	-0.514	Hypo
3	78014291	-	0.004	0.049	IL-12RB2	Intron	0.645	Hyper
3	78014301	-	0.002	0.049	IL-12RB2	Intron	0.636	Hyper
4	44007508	-	0.006	0.001	CCDC146	Intron	-0.292	Hypo
6	1.11E+08	+	0.006	0.035	CD38	Intron	-0.164	None
6	1.11E+08	+	0.010	0.003	CD38	Intron	-0.185	None
12	75652512	-	0.005	0.032	SLC15A1	Intron	0.210	None
12	75652535	-	0.002	0.021	SLC15A1	Intron	0.227	None
13	61031998	-	0.000	0.008	DEFB122	Intron	-0.766	Hypo
13	75284322	-	0.002	0.046	SLC13A3	Intron	-0.391	Hypo
18	4586603	-	0.004	0.025	ADAMTS18	Intron	-0.692	Hypo
18	55892714	-	0.004	0.022	SLC17A7	Intron	0.358	Hyper
20	40026050	+	0.003	0.040	ADAMTS12	Intron	-0.301	Hypo
21	15209172	-	0.006	0.019	SLCO3A1	Intron	0.664	Hyper
21	47145883	-	0.000	0.028	SLC25A21	Intron	-0.777	Hypo
22	12648666	+	0.006	0.040	SLC25A38	Intron	0.321	Hyper

* The difference in methylation level between positive and negative samples (Methylation level in positive samples - Methylation level in negative samples)

** If MethDiff >= 0.25: Hypermethylated; If MethDiff <= -0.25: Hypomethylated; otherwise None

- Some genes with hypomethylated or hypermethylated promoters are known to impact innate immunity related to many animal diseases[1].
  - **Hypo-**: *HS6ST1, CCDC106, SLC17A9* and *CCSMST1*
  - **hyper-**: *CHRNG* and *RGS14*
- ***CD38*** is known to play roles in the effective containment of mycobacteria within granulomata in cows [5].
- Genetic polymorphisms in ***IL-12RB2*** are associated with JD and human Crohn's disease [6].
- Several genes of **the solute carrier family**, including *SLC13A3*, *SLC15A1*, *SLC17A7*, *SLC25A21*, *SLC25A38* and *SLC9A9*, harbored DMCs. Some members of this gene family participate in pathogen clearance and have associations with JD [7].
- A total of **162** GO terms and **51** KEGG pathways were enriched for IL DMCs genes.
- Most of the enriched IL BP GO terms are related to cellular processes, transport and system development while very few enriched terms (less than 1%) are related to disease and the immune process.
- HIF-1 signaling pathway, a regulator of oxygen homeostasis was enriched by DMR genes (Figure 1).

Table 2 Select genes harboring DMRS and their mRNA expression patterns in the ileum

	Differentially methylated region genes				mRNA expression				
Gene Symbol	CHR	DMRs	CpGs	Met status	Genic region	baseMean	log2Fold Change	pvalue	padj
IL2RA	13	1	7	-	Intron	151.2055	0.9952	0.00249	0.0896
NOD1	4	1	3	-	Promoter	140.7861	2.1691	0.00042	0.0313
SLC8A3	10	1	3	Hypo	Intron	27.08057	-1.43	0.00022	0.0214
PLAC8	6	1	10	Hypo	Intron	1265.874	1.9459	0.00014	0.0168
ELK3	5	1	4	Hyper	Intron	232.6613	0.6412	2.04E-06	0.001
ASAP1	14	3	16	Hyper, +	CDS, intron	643.3811	0.427	0.00035	0.0286
NRN1	23	1	4	+	Intron	53.42144	1.1934	0.00077	0.0461
TIFA	6	1	7	+	Intron	314.23	1.3081	0.0005	0.035

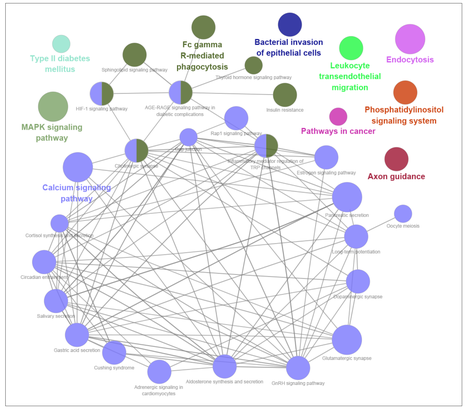


Figure 1:KEGG (FDR<0.01) enriched by DMR genes

- Some enriched disease and immune pathways included bacterial invasion of epithelial cells, pathways in cancer and inflammatory mediator regulation of TRP channels, etc.

CONCLUSION

- ✓ DNA methylation changes are involved in ileum response to MAP infection.
- ✓ DNA methylation changes contribute to the regulation of host response to MAP pathogenesis and may be one of the mechanisms that MAP uses to subvert host immune responses for its survival.

ACKNOWLEDGEMENTS

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