Development of Multilocus Sequence Typing (MLST) for *Mycoplasma synoviae*

Mohamed El-Gazzar[1A], Mostafa Ghanem[1], Kristina McDonald[1], Naola Ferguson-Noel[2], Ziv Raviv[1*] and Richard D. Slemons[1]

[1]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210.[2]Poultry Diagnostic and Research Center, 953 College Station Road, The University of Georgia, Athens, Georgia 30602[*]Current address: Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel

Summary

Mycoplasma synoviae (MS) is a poultry pathogen has had an increasing incidence and economic impact over the past few years. Strain identification is necessary for outbreak investigation, infection source identification and establishing prevention and control plans. Currently, a segment of the variable Lipoprotein Hemagglutinin A (*vlhA*) gene (420 bps) is the only target that is used for MS strain identification. A major limitation of this assay is that colonality of typed samples can only be inferred if their *vlhA* sequences are identical; however, if their sequences are different, the degree of relatedness is uncertain. In this study we propose a multilocus sequence typing (MLST) assay to further refine MS strain identification. After initial screening of 24 housekeeping genes as potential targets, 7 genes were selected for the MLST assay. An internal segment (450 bps - 711 pb) from each of the 7 genes was successfully amplified and sequenced from 58 different MS strains and field isolates (N=30) or positive clinical samples (N=28). The collective sequence of all 7 gene segments (3960 bps total) was used for MS sequence typing. The 58 tested MS samples were typed into 30 different sequence types using the MLST assay, and coincidentally all the samples were typed into 30 sequence types using *vlhA* assay. However, the phylogenetic tree generated using the MLST data was more congruent to the epidemiological information than the tree generated by *vlhA* assay. We suggest that the newly developed MLST assay and the *vlhA* assay could be used in tandem for MS typing. The MLST assay will be a valuable and more reliable tool for MS sequence typing, providing better understanding of the epidemiology of MS infection. This in turn will aid disease prevention, control and eradication efforts.

[A]Corresponding author.
Telephone: 614-688-1074, E-mail: el-gazzar.1@osu.edu

Table 2: Final 7 Genes for the MLST assay forward and a reverse primers, amplicon size, final segment used for MLST after sequencing, and the point the final segments starts and ends.

Gene	*Forward primer sequence (5' to 3')*	*Reverse primer sequence (5' to 3')*	*Amplicon Size*	*Final Segment Size*	*Final [A] Segment Start*	*Final [A] Segment End*
Adk	GCTTTGGATTAGATTCWGAGCTA[B]	TTCTTATGGGAATGCCAGGTT	585	498	73	570
atpG	GCTACAATTTCGGTTATTTCTTGAG	ATGCTATGCAGYTGGTTTCTACTT[B]	784	669	82	750
Efp	CGACATATTTACCGGTTTCAGTT	CTGGAATTACATTTCAAGATTCAGGAA	522	450	52	501
Gmk	TCAATGTCTAACTCCTTGTGAAGA	TTTACAGGTCCATCAGGTGTT	563	470	49	519
nagC	CCGATTATTCCGGCGTTATT	ATYGGGGGCACTTCTATTAAAT[B]	803	708	73	780
Ppa	AGTAATTGAAATTCCAAAAGGCTCA	AACTATATTTCTCTGGATGTTTTTCTT	530	453	58	510
recA	CTTTACCTTGCGCTACGTTATT	TTCGGAAAAGAATCTATTATGGTTC	847	711	130	840

A Number of nucleotide from the start codon where the final segment begins and ends

B Underlined letters represent International Union of Pure and Applied Chemistry/International Union

PCR amplification conditions:

PCRs in this study were carried out using the Roche FastStart High Fidelity kit (Roche Diagnostics, Indianapolis, IN) according to manufacturer's recommendations with some modifications. The PCR was performed using the MJ-Mini thermocycler (BioRad Laboratories, Hercules, CA) in a total volume of 25 µl containing 0.5 µl of 10 mM deoxynucleotides, 2.5 µl of 10X FastStart High Fidelity reaction buffer (1.8 mM $MgCl_2$), 2 µl of 5 mM of each primer, 0.25 ml of 5 U/ml FastStart High Fidelity enzyme, and 2.0 µl of DNA template.

To compensate for the limited amount of DNA template used, the number of cycles was increased from 40 to 45 cycles. All reactions were performed using a thermocycler program of a hot start at 95 ˚C for 3 min, 45 cycles of denaturing at 94 ˚C for 30 sec, annealing at 54 ˚C for 30 sec, extension at 72 ˚C for 90 sec, and a final extension at 72 ˚C for 5 minutes. Electrophoresis was performed on PCR products on a 1% agarose gel with 0.53 Tris-borate-ethylenediaminetetraacetic acid buffer and 0.5 mg ethidium bromide per ml, at 90V for 45 minutes. The PCR products were visualized by ultraviolet trans-illumination to confirm the success of amplification (Figure 1s).