18th meeting of the International Organization for Mycoplasmology, 11-16th July 2010, Chianciano Terme, Siena, Italy

# Molecular typing of *Mycoplasma mycoides* subspecies *mycoides* Small Colony strains by SSAP

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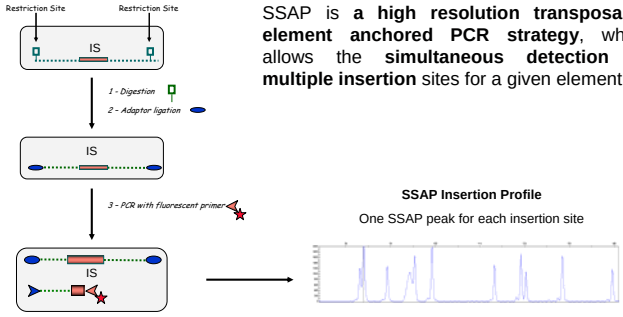
## Introduction

*Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC) is the agent of contagious bovine pleuropneumonia (CBPP), a severe notifiable disease affecting cattle in many African countries and threatening other continents. The genome sequence of the type strain, PG1, was published in 2004. This confirmed the high complexity of this genome, notably due to the presence of three types of insertion sequences (IS), some in high copy numbers, which represent 13% of the genome. IS are widespread in the bacterial world and particularly in the *Mycoplasma mycoides* cluster, where IS types and copy numbers vary from one species to another.

In the case of *Mmm*SC, it has been previously shown that strains may be differentiated by the Southern blot technique using IS1296 or IS1634 as labelled probes. Although, the low resolution of the Southern blot did not allow for the identification of all IS copies, these results showed that IS copy numbers may vary between strains and this paved the way for a new typing technique based on “Specific Sequence Amplified Polymorphism” (SSAP) (Fig 1).

## Material and Methods




SSAP is **a high resolution transposable element anchored PCR strategy**, which allows the **simultaneous detection of multiple insertion** sites for a given element.

***Fig 1: SSAP steps leading to a fluorescent PCR amplified product of various sizes***
The product size depends on the nearest enzyme cutting site at the vicinity of the IS

- Three *Mmm*SC IS types used: IS*1296*, IS*1634* and IS*Mmy1.*
- One enzyme: *Ase*I
- Two labelled primers designed at each extremity of IS.
- Amplified products detected on an ABI PRISM 3100 Genetic Analyser
- SSAP profiles analyzed with GeneMapper 3.7 software (Applied Biosystems).

## Results and Discussion

- A first validation of the technique was performed by comparing results obtained *in silico* with the PG1 sequence (Fig 2).
- The usefulness of the technique was then assessed on three different *Mmm*SC strains: PG1, T1/44 and 8740 (Fig 3) and then on 24 *Mmm*SC strains (Fig 4).

| IS copy | IS1296-22 | IS1296-09 | IS1296-27 | IS1296-03 | IS1296-08 | IS1296-11 | IS1296-06 | IS1296-23 | IS1296-14 | IS1296-20 | IS1296-10 | IS1296-19 | IS1296-04 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| in silico | 99 | 130 | 154 | 157 | 172 | 189 | 210 | 225 | 227 | 240 | 242 | 246 | 303 |
| Detected peaks | 94.4 | 126.6 | 152.3 | 155.9 | 170.5 | 181.5 | 209.9 | 222.7 | 225.1 | 236.2 | 240.8 | 243.6 | 299.9 |
| % variation | 4.6 | 2.6 | 1.1 | 0.7 | 0.9 | 1.9 | 0.0 | 1.0 | 0.8 | 1.6 | 0.5 | 1.0 | 1.0 |

***Fig 2: Analysis of SSAP profiles with PG1 strain* in silico vs. *experimental results***
Typing with the combination: *Ase*I-IS*1296*-P2. Each theoretical peak was detected with an actual size that differed by less than 5% as compared to the theoretical size. Some IS copies did not yield any detectable peak because of their size, either too big or too small.

| Sample | Allele 1 | Allele 2 | Allele 3 | Allele 4 | Allele 5 | Allele 6 | Allele 7 | Allele 8 | Allele 9 | Allele 10 | Allele 11 | Allele 12 | Allele 13 | Allele 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PG1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 8740 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| T1/44 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

| Sample | Size 1 | Size 2 | Size 3 | Size 4 | Size 5 | Size 6 | Size 7 | Size 8 | Size 9 | Size 10 | Size 11 | Size 12 | Size 13 | Size 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PG1 | 52.5 | 61.14 | 67.83 | 83.12 | 0 | 89.24 | 94.31 | 95.37 | 99.29 | 113.3 | 119.17 | 120.26 | 128.35 | 0 |
| 8740 | 52.39 | 61.15 | 67.88 | 83.13 | 83.98 | 89.24 | 94.22 | 95.37 | 99.29 | 113.24 | 119.13 | 120.3 | 128.44 | 0 |
| T1/44 | 52.46 | 61.14 | 67.74 | 83.12 | 0 | 89.24 | 94.37 | 95.35 | 99.25 | 113.24 | 119.05 | 120.3 | 128.44 | 129.48 |

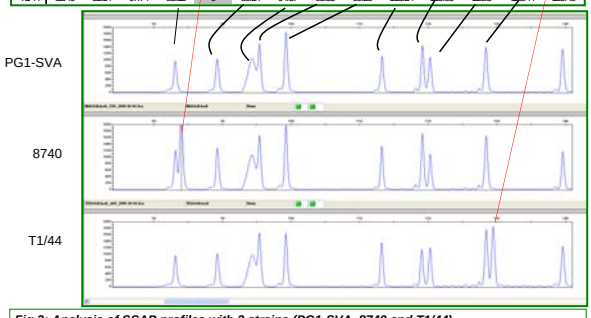



***Fig 3: Analysis of SSAP profiles with 3 strains (PG1-SVA, 8740 and T1/44)***
Typing with the combination: *Ase*I-IS*1634*-P1. The two strains 8740 and T1/44 are differenciated from PG1 by an additional band with fragment sizes of 84 and 130 respectively

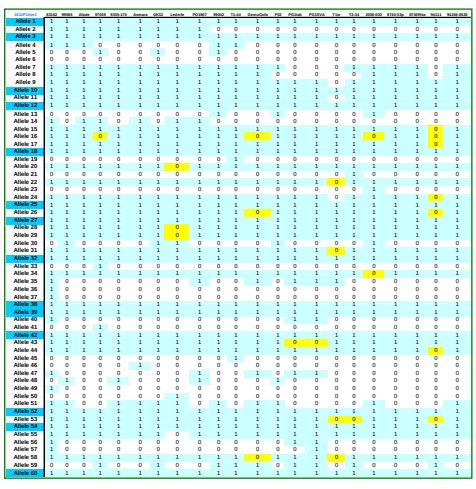


***Fig 4: SSAP allelic profiles with 22* MmmSC *strains of various origins***
Typing with the combination: *Ase*I-IS*1634*-P1. Each allele correspond to one band
Invariant alleles are marked in blue. Lacking bands are marked in yellow

The validation with 22 *Mmm*SC strains of various origins confirmed the suitability of the technique (Fig 3). The stability of the marker was assessed with two strains that differed by 53 *in vitro* passages (8740 and 8740-53P). These two strains displayed the same allelic profile. However, when comparing two PG1 stocks, one from Cirad (France) and one from SVA (Sweden), the profiles differed on one allele (N°57). Additional analysis by whole DNA restriction analysis confirmed that the two stocks differed (data not shown). This finding confirmed that strain stocks that have a different history might have diverged significantly over time. Finally, all the other strains had specific allelic profiles, which shows that IS-based SSAP is a powerful discriminatory tool for *Mmm*SC strains.

This technique has however some limitations that are inherent to the various steps involved and can be responsible for a certain difficulty to get reproducible results. It is in fact quite cumbersome to obtain peaks of similar sizes from one experiment to another.

## Acknowledgements

This study was part of an INRA-Cirad project « Pathomyco »

Centre de coopération internationale en recherche agronomique pour le développement

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