



**Figure S8** : MALDI-MS analyses for  $m^3U1915$  and Cm1920 methylations. **(A)** Spectrum of RNase A fragments from this region (Fig. 4) of the *S. citri* 23S rRNA. The fragment at  $m/z$  1992 corresponds to nucleotides 1918-1923 with the sequence UAA[Cm]GGp, where 2'-*O*-methylation at C1920 renders the fragment resistant to RNase A cleavage. In the *M. agalactiae* rRNA, where there is no C1920 methylation, this sequence is cleaved to form UAAp and CGGp that run in the trinucleotide region of the spectrum (not shown). **(B)** RNase T1 digestion gives an additional analysis of this region as shown here in the expanded view of the *M. agalactiae*  $m/z$  3519 peak from Fig. 4. This sequence of nucleotides 1911-1921 contains both U1915 and C1920, and the cyclic 3'-phosphate (>p) fragment at  $m/z$  3519 has no modification. Minor peaks are evident corresponding to the same fragment with a linear 3'-phosphate (+ 18 Da) and with an adducted potassium ion (+ 38 Da), but there is no peak corresponding to modification with one or two methyl groups (theoretical  $m/z$  3533 and 3547, respectively).