Biochemical characterization of giant viruses

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In 1992 researchers believed they had isolated a new species of gram-positive coccus, 650nm in diameter. It was then classified and named as Bradford coccus. In 2003 this 'bacterium' was discovered to be the first species of giant virus (GV), called Acanthamoeba polyphaga mimivirus (APMV). The discovery of the APMV triggered several prospecting studies, seeking to explore this unknown viral biodiversity. Besides APMV, two members of the Mimiviridae family, both found in Brazil, standout among the isolated species: Samba virus (SMBV) and Tupã virus (TPV). This viroses have have a capsid with icosahedral symmetry, a stargate in one of its vertices, and a covering fibrils, that are believed to be associated with important aspects of the virus infection cycle such as cell adhesion and invasion. TPV has a tail connected to the apex opposite to the stargate, by mechanisms that have not yet been elucidated but might be associated with the fibrils. Fibrils composition presents a high carbohydrate content that has yet to be fully characterized, with this aspect being extremely important for our goals. This study aims to evaluate the differences in the biochemical composition of giant viruses, seeking to understand more about their biology and develop new strategies for studies with these models. Thus, our first objective is to determine and compare the saccharide composition of mimiviruses, in order to elucidate how the sugars present in the fibrils and surface structures influence the different steps in virus-cell interaction. In addition, this work's second objective is to propose the use of MALDI-TOF as a rapid methodology for identifying giant viruses. This will follow from the fingerprint approach that has been widely used for microorganisms, including in clinical analysis. Based on our previous data indicating the accumulation of sulfur in the TPV capsid (element microanalysis), a colorimetric test was performed to detect sulfated sugar by DMB. It was observed that TPV has a sulfated carbohydrate, different from APMV and SBMV. This result may explain the sulfur accumulation mentioned above. GC/MS analyzes were performed to trace and compare the monosaccharide profile of the 3 viruses, in order to identify the regions of difference and subsequently associate the composition of the fibrils sugars. Our chromatograms confirmed the existence of a different total monoscaridic profile for the 3 viruses, so that no peak pattern is exactly the same. Both have regions that differ them from each other. The next step is to identify all the monosaccharides present in each virus and trace the differences between them. Finally, our MALDI-TOF results

show the potential of this technique for differentiating GVs. In this approach the spectra form the 'fingerprint' of each species. The results indicated that intensity spectra by mass/charge ratio are unique for each virus. As much as they have similar regions, the total pattern is never identical. Seven viruses were tested, including 3 different large groups. Here we show two mass spectrometry techniques for biochemical characterization of GVs, indicating their potential as approaches to species differentiation, based on differences in their biochemical compositions.

Key words: Giant virus, mimivirus, biochemical characterization, carbohydrate composition, MALDI-TOF.

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