

Abstract

# Usutu Virus NS5: Characterization of Polymerase Activity, Protein–Protein Interaction and Cellular Localization %MCEPASTEBIN% †

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**Abstract:** Usutu virus (USUV) is a mosquito-borne arbovirus that has rapidly propagated in birds across several European countries over the last two decades, leading to substantial avian mortalities. USUV infection in humans has been associated with a growing number of cases of neurological disease in the last years, underlining the need for increased awareness and suitable treatments. Our group is working on the characterization of the NS5 protein of USUV. This protein is responsible for the replication activity of the viral genome and can be a suitable viral target to treat the infection. NS5 contains a RNA-dependent RNA polymerase (RdRpD) and a methyltransferase domains. Recombinant NS5 and RdRpD proteins expressed in bacteria were purified and biochemically characterized to determine the best conditions for their polymerase activities. Both proteins showed de novo and primer extension activities. Optimal RNA–polymerase reaction conditions included low NaCl (less than 20 mM), 2.5 mM MgCl<sub>2</sub> and 5 mM MnCl<sub>2</sub>, 30 °C, and pH 7.25. Polymerase activity was cooperative for the polymerase domain (Hill coefficient = 5.8) but not for the complete NS5 (Hill coefficient = 1.2). To study their subcellular location, suitable sequences were cloned into a pcDNA3 vector and expressed in Huh7.5 and HEK293T cells. Both proteins were preferentially located in the cytoplasmic region, although a significant amount was found in the nucleus. Preliminary results showed that the concentration of sofosbuvir (SOFTP) necessary to achieve its incorporation by NS5 in 50% of the nascent RNA is higher than 100 μM, as already observed for dengue virus DENV. In this work, we describe the main features of the full-length USUV NS5, including the polymerase activity as well as the effect of protein–protein interactions and subcellular localization. Our results will be very useful for the study of this viral enzyme as a suitable target against the infection and the effect of antiviral drugs.

**Keywords:** USUV; NS5; RdRp; MTase



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