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OUTER MEMBRANE VESICLES AS MEDIATORS OF BACTERIAL COMMUNICATION

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Gram negative bacteria are capable of producing outer membrane vesicles (OMVs). These OMVs can act as a bacterial delivery system, by providing the possibility to export DNA, RNA, lipids, proteins, and toxins, among others. The OMVs could participate in biofilm production, nutrients acquisition, and as immune protection, being the target of several antimicrobials and phages. The aim of this work was to isolate OMVs from previously characterized uropathogenic isolates *Escherichia coli* (Ec) U144 and *Proteus mirabilis* (Pm) 2921 in Luria-Bertani broth (LB) and artificial urine (AU), and to evaluate their possible function. OMVs were purified by filtering and ultracentrifugation. The OMVs were characterized on size and superficial charge by DLS (ZetasizerZS), and imaging by TEM (STEM Inspect F50). Bacterial suspensions (OD₆₀₀/0.5) of Ec_U144, Pm2921 and EcATCC were incubated for 30 min, 1, 3, and 5h with FM4-64-labeled OMVs in order to evaluate the association of OMV with bacteria. The association rate was obtained by quantification of the acquired bacterial fluorescence observed after the incubation. Finally, the effect of OMVs on biofilm formation was evaluated by the violet crystal method. The Ec_U144 OMVs have a size of 185 and 257 nm in LB and AU, respectively. Meanwhile, the Pm2921 measured 267 and 320 nm. All OMVs have a negative zeta-potential. Regarding OMV association, Ec_U144 was able to acquire its own OMVs after 30 minutes of incubation, whereas Pm2921 required over 3 hours to do so, as evidenced by the transfer of fluorescence when incubated with the OMVs. Interestingly, EcATCC was able to associate both OMV from Ec_U144 and Pm2921 in 30 min and 3 h respectively. The observed difference in association could suggest that OMVs serve distinct roles, one likely for intraspecies and the other for interspecies communication. Additionally, it is still to be determined whether the association will occur through fusion or internalization of the OMV. When evaluating biofilm formation, we observed a significant increase in biomass when Ec_U144 and Pm2921 were incubated with their own OMVs. We conclude that Ec and Pm produce OMVs that participate in communication and enhance biofilm formation.

Palabras clave: outer membrane vesicles - bacterial communication- E. coli - P. mirabilis