Genomic profile of Bacillus pumilus 3-19 strain extracellular membrane vesicles

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Extracellular membrane vesicle (EV) secretion is a widespread intercellular communication process in prokaryotes. However, there is not a lot of information about the DNA content and functions of vesicles. So we performed genomic characterization of vesicles of Bacillus pumilus 3-19, chemical mutant overproducing extracellular ribonuclease. EVs were isolated from an exponential-phase culture with ultracentrifugation and ultrafiltration method and were visualized by Hitachi HT7700 Exalens transmission electron microscope. The fragment library obtained after multiple displacement amplification (MDA) of DNA extracted after DNase treatment of purified vesicles was sequenced on the Miseq (Illumina) platform. Reads mapped on the B. pumilus 3-19 genome assembly with Bowtie2 aligner were summarized with featureCount program. In this study, we found that vesicular DNA fragment library covered over 20% of the entire B. pumilus 3-19 assembly sequence. An overabundance of reads was revealed within the region containing 34 genes (about 40 kb). It is interesting that 50% of this region represented genes of hypothetical proteins with unknown function. The remaining genes were phage related ones, N-acetylmuramoyl-L-alanine amidase and genes annotated as transcription regulators. This overabundant region also was found in vesicles by sequencing the fragment library prepared with commercial kit NEBNext Ultra II for small amount of DNA without applying MDA technique. Although functional role of the revealed overabundant region is unclear to date, the obtained data can be useful for further investigations of the principles of DNA fragment generation and the mechanism of packaging into vesicles.