ABSTRACT

Title:

Identification and Enrichment of Extrachromosomal Circular DNA (eccDNA) in *Saccharomyces cerevisiae* Extracellular Vesicles

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Presenting:

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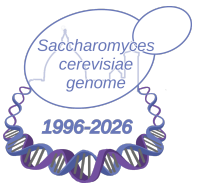
Abstract content:

Extrachromosomal circular DNAs (eccDNAs) are independent, double-stranded DNA molecules derived from chromosomal loci that play a fundamental role in gene regulation, environmental adaptation, and genome plasticity by enabling rapid gene copy number variations. While extracellular vesicles (EVs) are established mediators of intercellular communication through the transfer of proteins and RNAs, their role as carriers of eccDNA in fungi is still unknown. In this study, we investigated the eccDNA cargo of EVs from four *Saccharomyces cerevisiae* strains isolated from diverse ecological niches: winery, fermented milk beverage, social insect gut, and human intestine. The cells were cultivated and harvested during the early stationary phase. EVs were purified at the same stage from culture supernatants using differential centrifugation and ultracentrifugation, with their size and concentration validated via Nanoparticle Tracking Analysis (NTA). We employed the Circle-Seq method to specifically enrich circular DNA by enzymatically digesting linear fragments, followed by rolling-circle amplification (RCA) using phi29 DNA polymerase and high-throughput Illumina sequencing. Our results provide the first evidence of eccDNA presence within yeast-derived EVs. Comparative bioinformatic analysis between cellular and vesicular DNA, performed using the Circle-Map++ pipeline, reveals that specific eccDNA species are significantly enriched in EVs rather than being a reflection of the internal cellular pool. These findings suggest the existence of a potential selective packaging mechanism for circular DNA into EVs. This discovery highlights a novel pathway for the horizontal exchange of bioactive genetic elements, potentially facilitating rapid adaptation and genomic plasticity across yeast populations.


Saccharomyces
cerevisiae
genome
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