Lactococcal extracellular membrane vesicles deliver bioactive vitamin K2 to human cells

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Vitamin K2

Vitamin K2 (menaquinone, MK-n) is a lipophilic vitamin located the cell membranes of many species of bacteria and is essential for human health as a carboxylation co-factor. Beneficial effects in relation to human cardiovascular and bone health have also been associated with vitamin K2 intake.

The long-chain forms of vitamin K2 show high bioavailability for target tissues in the human body. However, the strong lipophilicity of long-chain vitamin K2 forms poses challenges to their uptake by target cells of the human host to achieve desired biological function.

Extracellular vesicles

Extracellular membrane vesicles (EVs) are nano-sized, lipid bilayer-enclosed spheres secreted by members of all domains of life. Bacterial EVs carry a diversity of cargo molecules, pointing to various roles of bacterial EVs in microbial ecophysiology, cellular signaling and communication, and interactions with the human host.

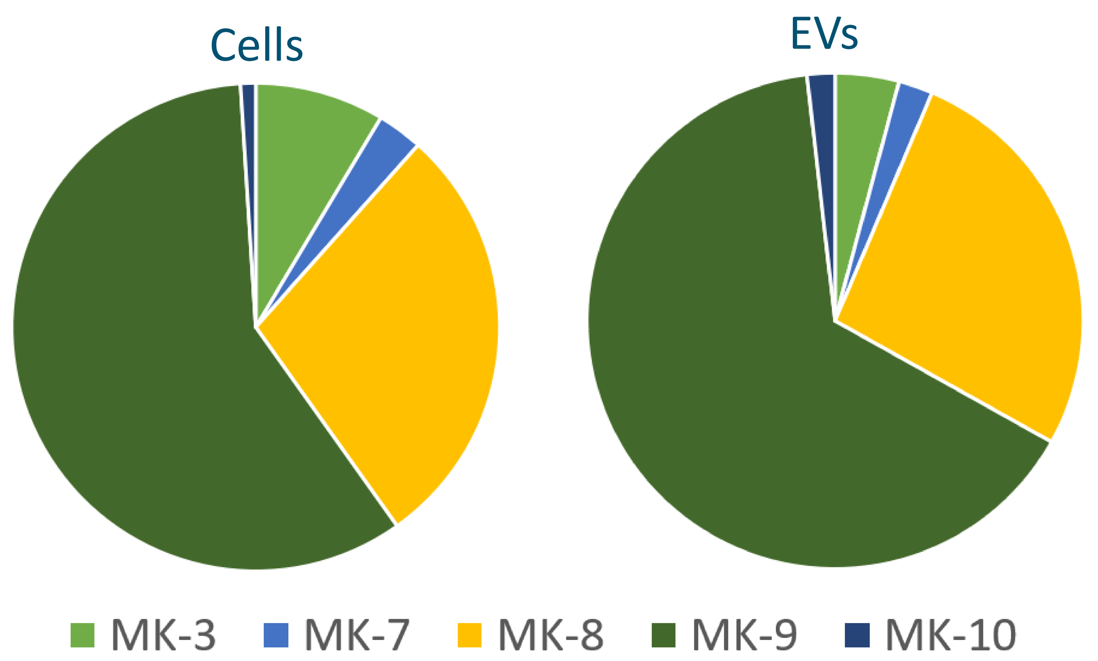
A wide range of species well-known as beneficial commensal bacteria produce EVs. The potential of applying these bacterial EVs in contribution to human health is drawing attention.

Lactococcus lactis has a long history of safe use in fermented foods and produces mainly long-chain vitamin K2 (in the forms of MK-9 and MK-8), providing opportunities for vitamin K2 enrichment of food. Notably, we demonstrated that *L. lactis* produces EVs, which are potentially ideal vehicles for efficient delivery of this hydrophobic, membrane-bounded vitamin to the human host.

Do lactococcal EVs carry vitamin K2?

Yes they do!

Chemical analysis reveals similar profiles of vitamin K2 in *L. lactis* strain FM-YL11 cells and EVs:



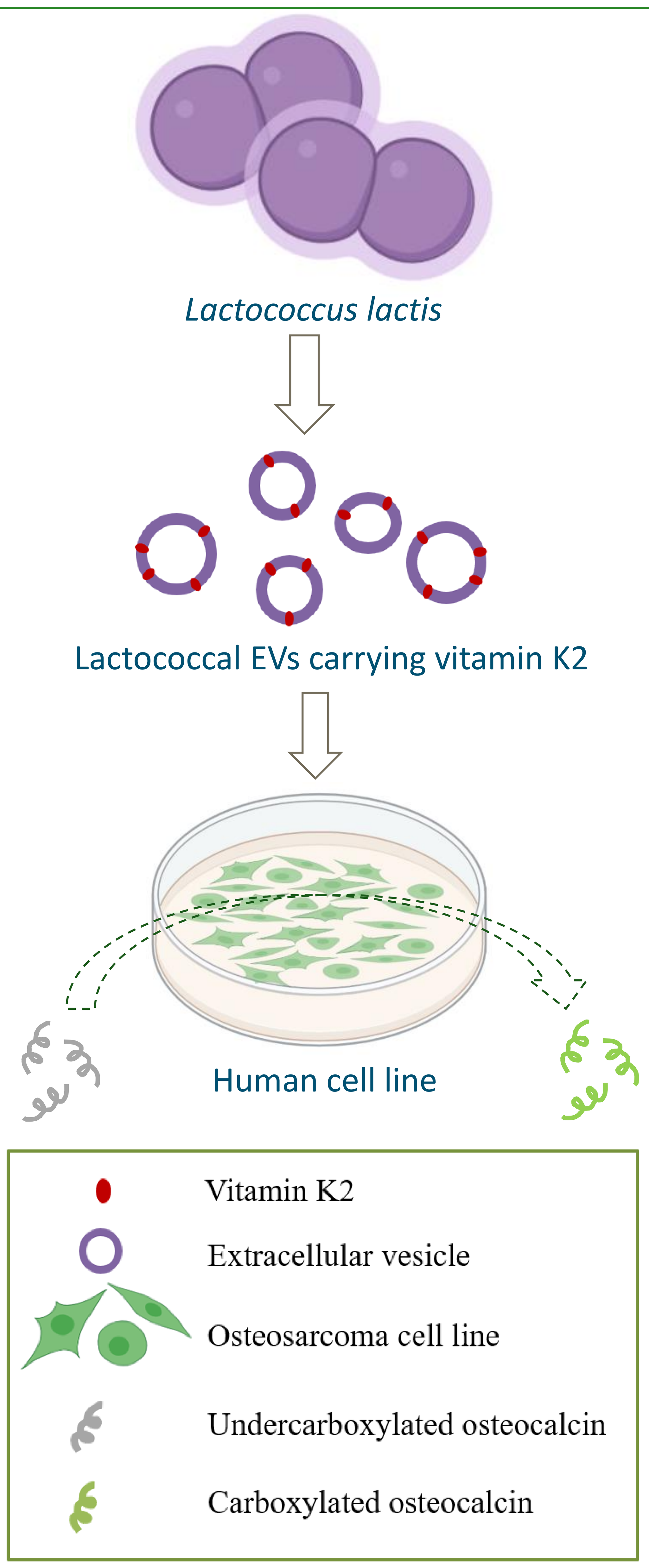
Can lactococcal EVs deliver vitamin K2 to human cells?

Yes they can!

Vitamin K2 functions as a carboxylation cofactor and one of the target tissues is the osteoblasts (bone cells). Using the human osteosarcoma cell line, and the carboxylation status of the protein osteocalcin (OC) as an indicator, we observed upon receiving lactococcal EVs:

$$\text{Ratio } \frac{\text{Carboxylated OC}}{\text{Undercarboxylated OC}} \uparrow$$

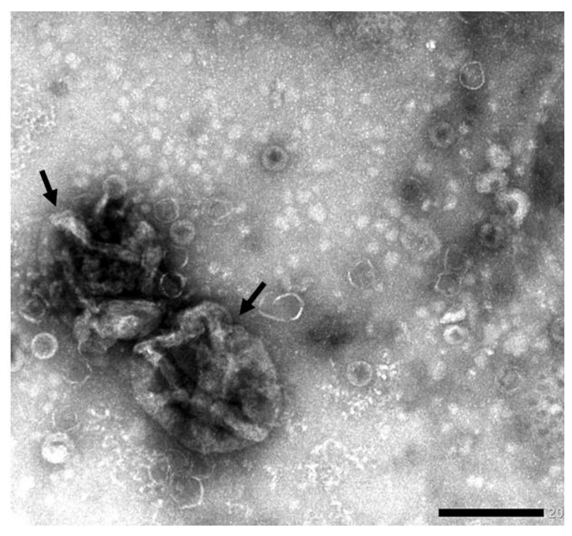
Thus, demonstrating the delivery of bioactive vitamin K2 by lactococcal EVs to human cells by the *in vitro* assay.



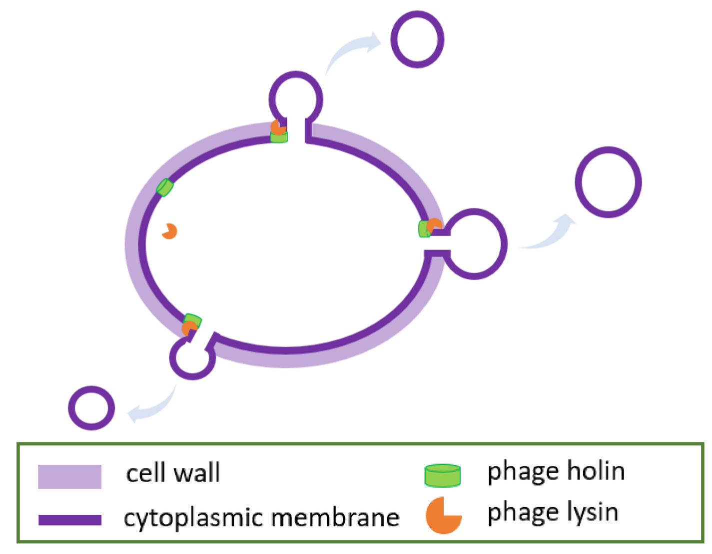
How are Lactococcal EVs produced?

For Gram-positive bacteria including *L. lactis*, the thick cell wall had been historically presumed to be a strong physical barrier preventing EV release. The mechanism of EV release from Gram-positive bacteria has been an intriguing question.

We demonstrated that the prophage encoded holin-lysin system stimulates EV formation in L. lactis strain FM-YL11, by partially degrading the cell envelop allowing EVs to escape.



Transmission electron microscopic picture of lactococcal EVs



Proposed EV production mechanism in *L. lactis*

Perspectives

Investigation on EVs produced by bacteria with GRAS status that are key players in food fermentations, will promote the applications of bacterial EVs in efficient delivery of bioactive, nutritional compounds from the microbial origins to the human host, contributing to improved human nutrition and conceivable health benefits.

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