Efficient Delivery of Vitamin K2 by Bacteria Involved in Food Fermentation Processes

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Background

Vitamin K2 is a fat-soluble vitamin that is essential to human health [1]. It acts as an enzyme co-factor required for the activation of proteins involved in vital physiological processes including haemostasis, calcium metabolism and cell growth regulation. Intake of vitamin K2 was recently shown to have beneficial effects on cardiovascular and bone health [2].
Vitamin K2 (menaquinones, MK-n, Fig. 1A) is produced by bacteria and accumulates in the cytoplasmic membrane where it acts as electron carrier in the respiratory electron transport chain [3] (Fig. 1B).

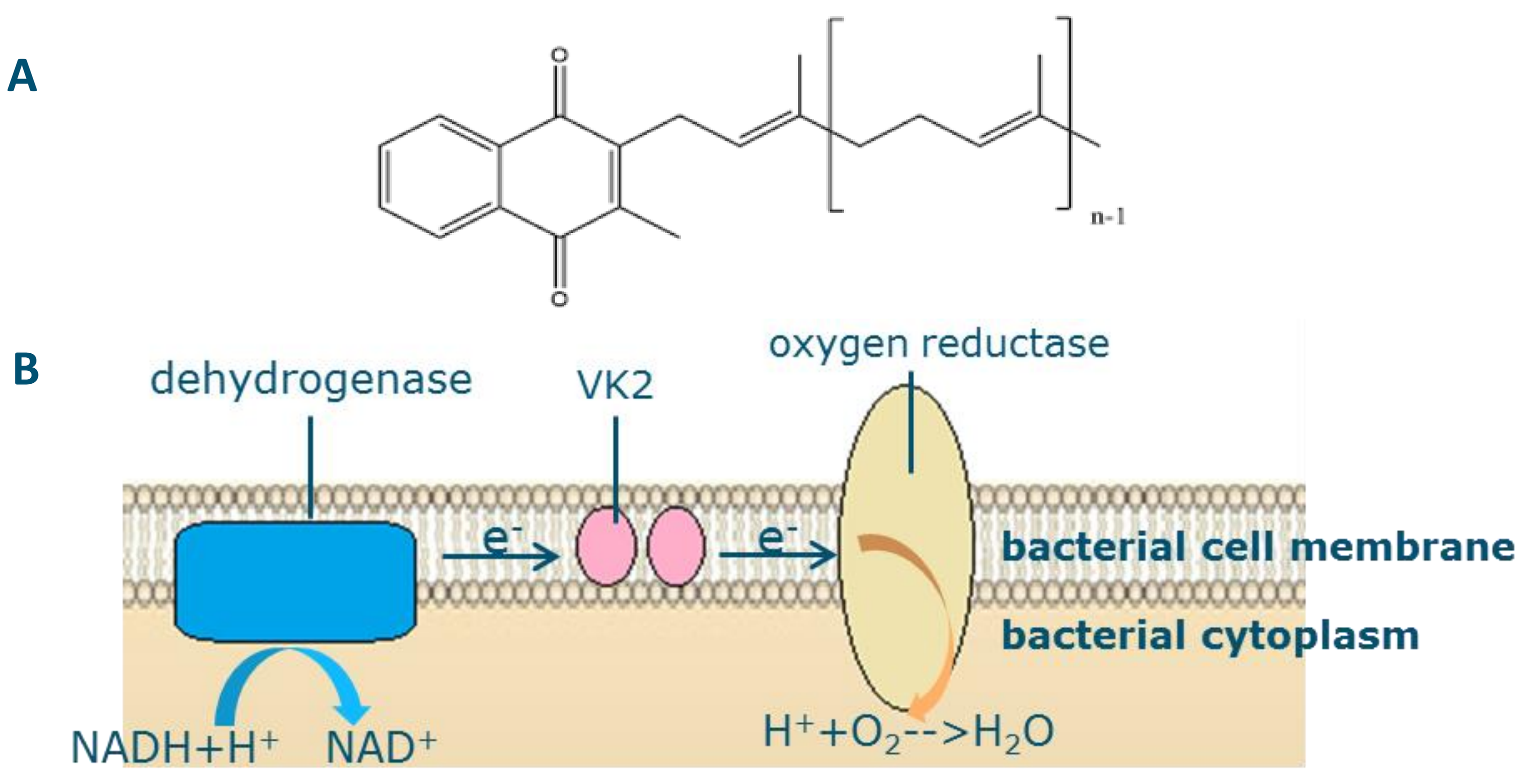

Figure 1. A) The chemical structure of vitamin K2 , MK-n. **B)** Vitamin K2 as part of the electron transport chain between NADH dehydrogenase and a oxygen reductase in bacterial cell membrane.

Due to the hydrophobic nature and association with cell membrane of vitamin K2, the delivery route to the human host is not known. Evidence of extracellular membrane vesicle production in bacteria accumulated in recent years [4] and the vesicles may serve as vehicles for efficient vitamin K2 delivery (Figure 2).

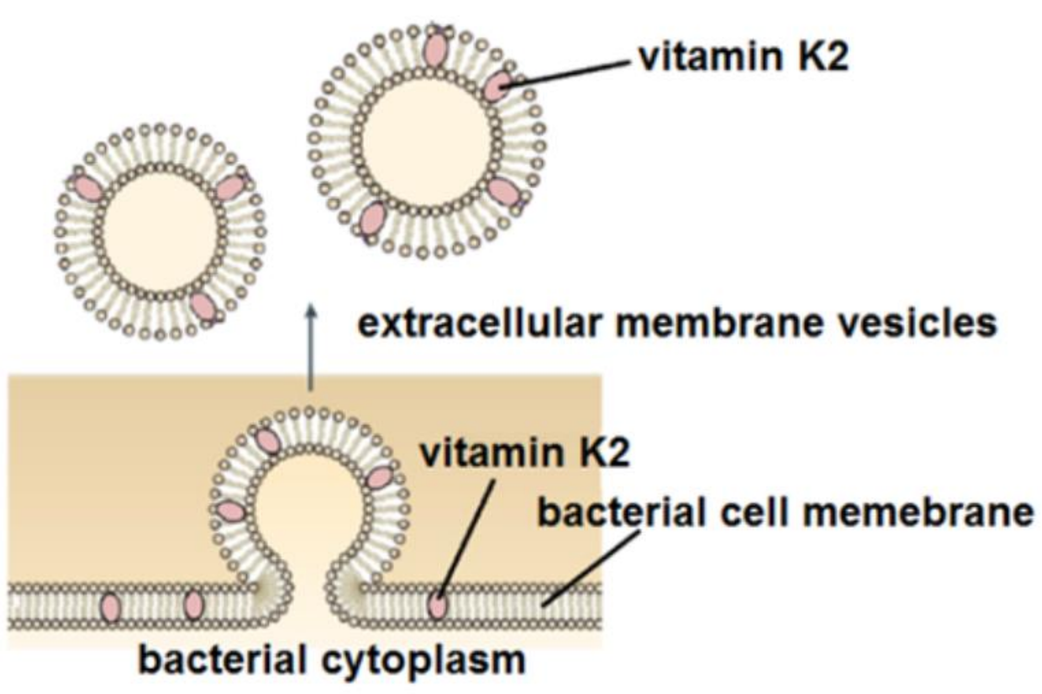

Figure 2. Hypothetical model: secretion of vitamin K2-containing extracellular membrane vesicles from producing bacteria.

Aim

This study aims to examine vitamin K2 production in various bacterial species and strains (*Lactococcus lactis, Bacillus subtilis* and *Propionibacterium freudenreichii*) involved in food fermentation proesses, and to explore the potential of efficient delivery of vitamin K2 to the human host.

Vitamin K2 Production

Selected strains were cultivated for 48h in the following conditions:

L. lactis – M17 with 0.5% glucose, 30°C static
B. subtilis – nutrient broth with 0.5% glucose, 37°C aerobic
P. freudenreichii – DSMZ medium 91 (1% lactate), 30°C anaerobic

Vitamin K2 was extracted from the biomass using hexane and measured by UPLC-MS (Fig. 3).

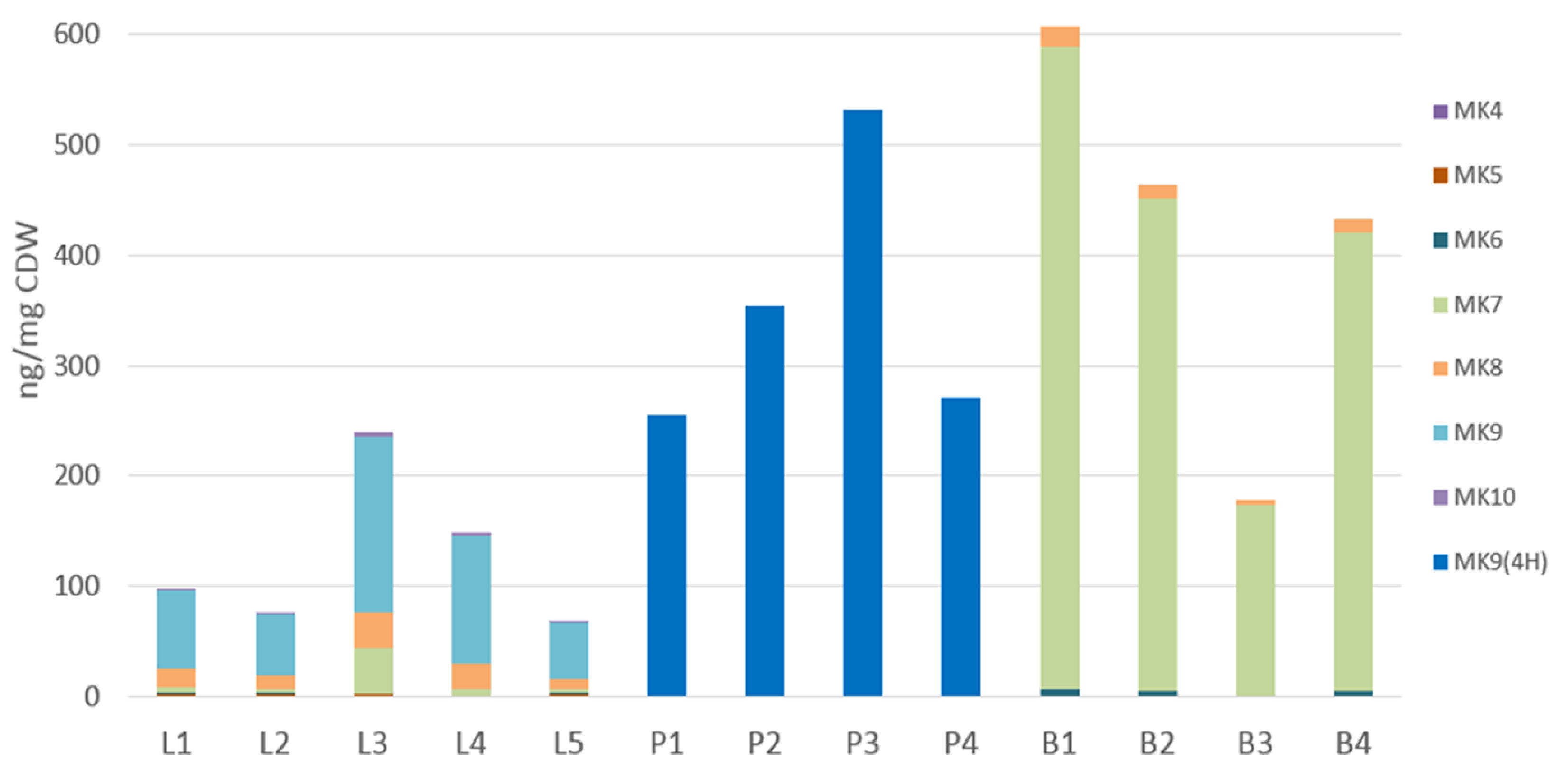

Figure 3. Vitamin K2 production in strains of *L. lactis* (L1-L5), *P. freudenreichii* (P1-P4) and *B. subtilis* (B1-B4).

Membrane Vesicle Production

Natural membrane vesicle production was observed in *L. lactis*, and artificial membrane vesicle generation was demonstrated possible by applying mechanical forces on bacterial biomass (Fig. 4).

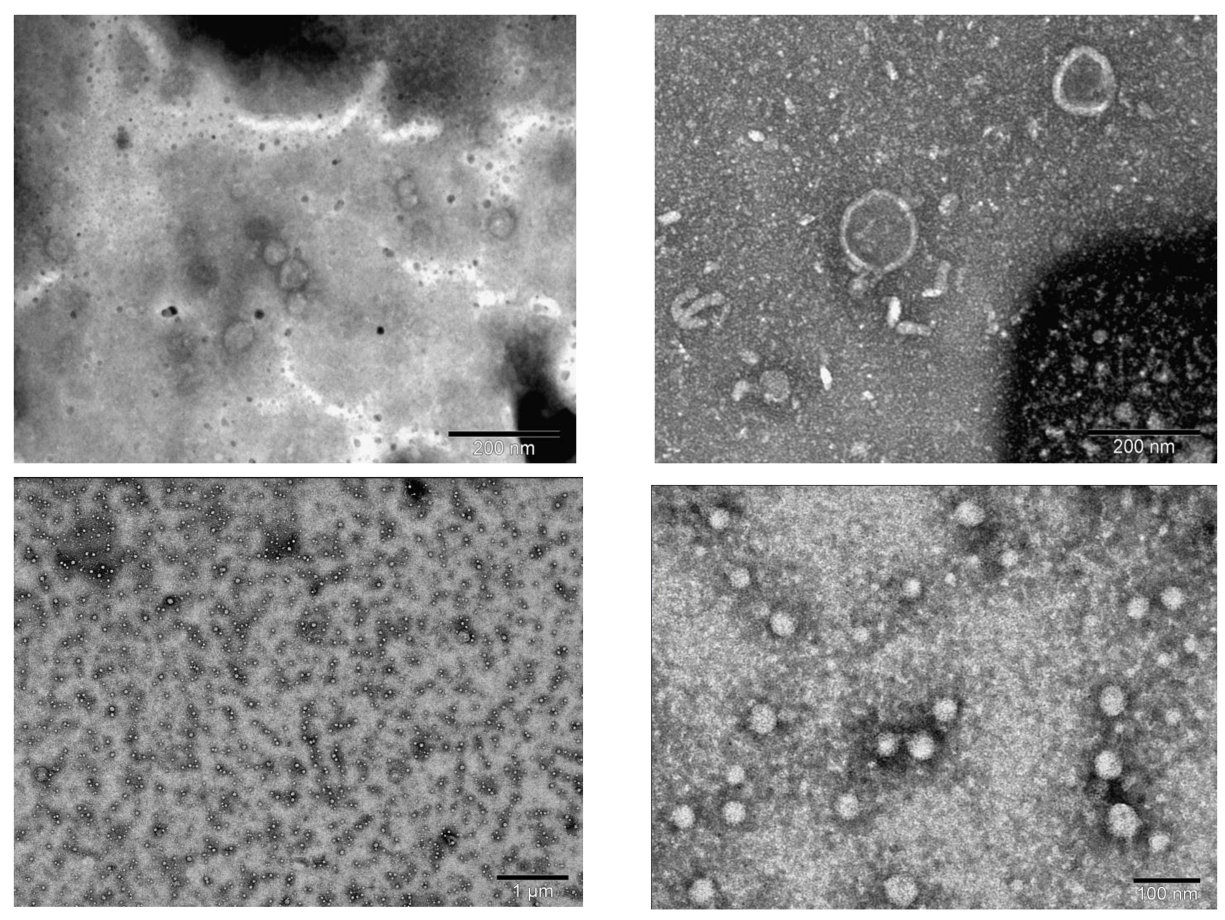

Figure 4. A, B) Natural membrane vesicles from *L. lactis*. **C, D)** Artificial membrane vesicles generated by sonicating the biomass of *L. lactis*.

Conclusions

- The tail length of the produced menaquinons is species specific
- The level of vitamin K2 production varies at strain level
- Evidence of natural membrane vesicle production is provided for *L. lactis*
- Artificial generation of membrane vesicles is feasible and can potentially contribute to the delivery of vitamin K2

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