

Phenotypic Characterization of Exopolysaccharide Production in *Lactococcus*

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Lactic acid bacteria (LAB) play a significant role in the production of several food products for human consumption. Humans exploit and maximize the various benefits from LAB characteristics, especially in foods such as cheese, yogurt and other fermented products. More recently, research discovered that certain LAB, such as *Lactococcus* species, produce unique exopolysaccharides (EPS). Prior research by E. Knoshaug, J. Ahlgren and J. Trempy with the bacterial strain *Lactococcus lactis* subsp. *cremoris* Ropy352 shows the production of a unique EPS in 10 w/w% nonfat milk, where it increased the milk's viscosity to 24 Pa•s after 24 hours. Lyophilized samples of other *Lactococcus* species were retrieved from the Oregon State University Department of Microbiology Bacterial Strain Collection to investigate if other *Lactococcus* strains exhibit an EPS phenotypically similar to the EPS produced by *L. Lactis* subsp. *cremoris* Ropy352.

Background:

What is a EXOPLYSACCHARIDE (EPS)?

Microbial exopolysaccharides (EPS) are a diverse group of secreted polymers that are attached to the cell surface and released as extracellular slime in the cells environment. EPS in Lactic acid bacteria are associated with cell protection against antibiotics, toxic compounds and other adverse environmental conditions³.

Importance?

With food consumers demanding a more “natural” product, LAB secreted EPS has been suggested to potentially replace synthetically produced stabilizers and thickener.

Methods:

Culture lyophilized samples in M17 (5%Lactose) broth ≈24 hours, in 30°C.

Streak 10uL of samples in whey agar for isolation.

Make overnight cultures, 5mL of M17 broth at 30°C.

Inoculate 5mL samples of milk and lactose free milk (Lactaid) with 50uL of overnight culture. Placed in 30°C incubator.

≈24 hours, check for change in viscosity

Results



Figure 1: Images above show the drastic change in viscosity of milk after ≈24 hours incubation. 5mL sample of 2% fat milk was inoculated with 50uL overnight cultures of Ropy 352 in M17 broth at 30°C. Inoculated milk was incubated in the same condition.

Vial #	Culture	Lactaid	2% Milk
163	<i>Lactococcus cremoris</i> Da-1	+++	++
178	<i>Lactococcus cremoris</i> ATCC 9596	+++	++
187	<i>Lactococcus cremoris</i> R-1	+++	+
189	<i>Lactococcus cremoris</i> C-13	+++	++
217	<i>Lactococcus cremoris</i> 4R-7	+++	++
220	<i>Lactococcus cremoris</i> 26-1	++	+++
223	<i>Lactococcus cremoris</i> 18-1	+++	+
67	<i>Lactococcus diacetylactis</i> DRC-1	+++	+
70	<i>Lactococcus diacetylactis</i> DRC-3	+++	+
1	<i>Lactococcus lactis</i> E	+++	+++
182	<i>Lactococcus diacetylactis</i> ATCC 11007	+++	++

Table 1. Tabular data of eleven LAB strains producing ropy EPS similar to the EPS produced by *L. lactis* subsp. *cremoris* Ropy352. Results after ≈ 24 hours of inoculation of overnight culture at 30°C into 5mL of Lactaid® (lactose free milk) and 2% milk. + and – signs are based on the viscosity of the products compared to ROPY 352 (positive control). +++ being as viscous as ROPY 352 and – being little to no change in viscosity

Results: Continued

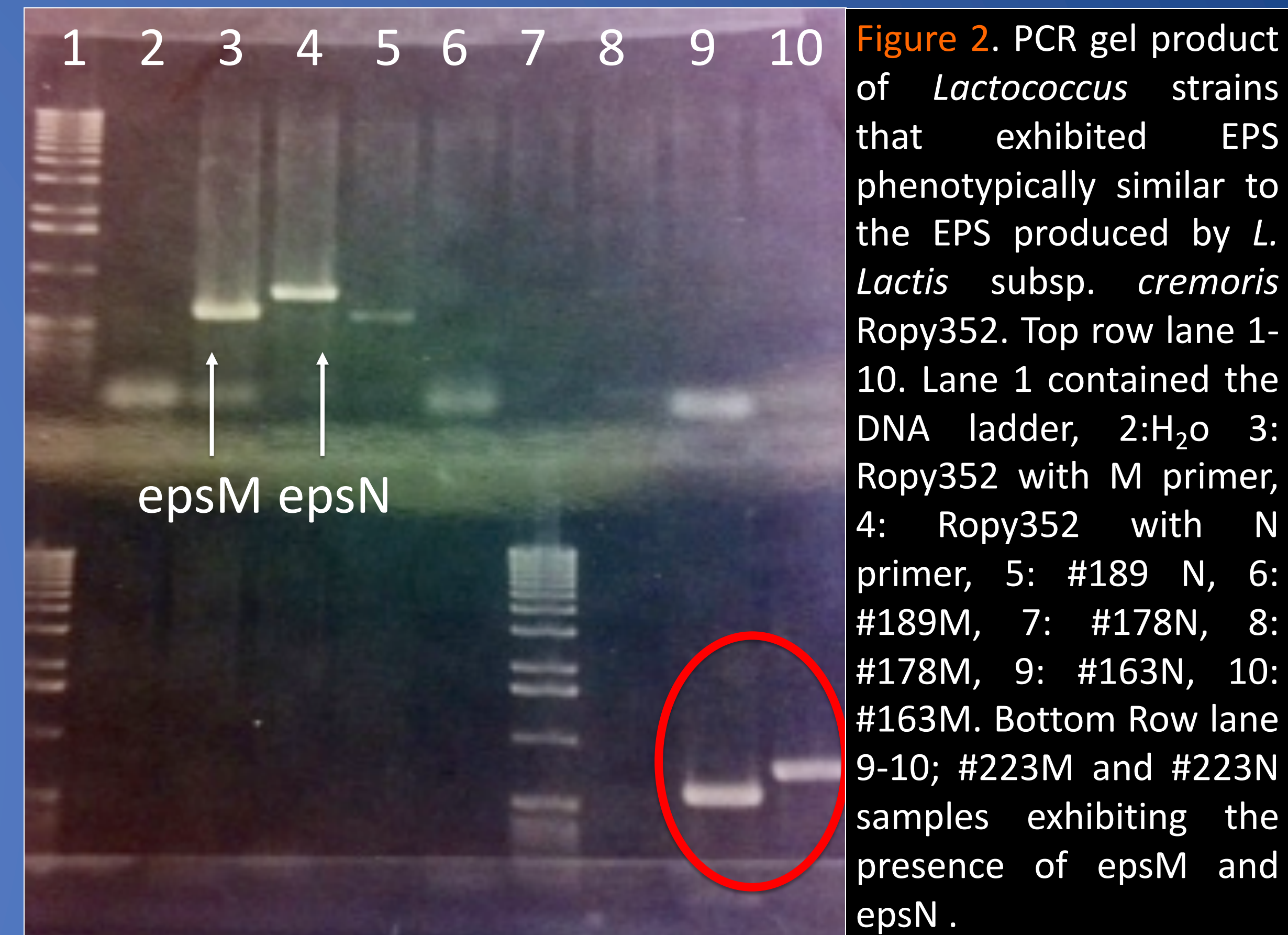


Figure 2. PCR gel product of *Lactococcus* strains that exhibited EPS phenotypically similar to the EPS produced by *L. Lactis* subsp. *cremoris* Ropy352. Top row lane 1-10. Lane 1 contained the DNA ladder, 2:H₂O 3: Ropy352 with M primer, 4: Ropy352 with N primer, 5: #189 N, 6: #189M, 7: #178N, 8: #178M, 9: #163N, 10: #163M. Bottom Row lane 9-10; #223M and #223N samples exhibiting the presence of epsM and epsN.

Conclusions:

- Eleven *Lactococcus* strains produced EPS phenotypically similar to the unique ropy EPS produced by *L. Lactis* subsp. *cremoris* Ropy352 when inoculated in 2% milk.
- L. Lactis* subsp. *cremoris* Ropy352 and the eleven *Lactococcus* strains produced the unique ropy EPS in Lactaid® milk, a lactose free milk, indicating lactose was not required for the production of this unique EPS.
- Lactococcus* strain #223 *Lactococcus cremoris* 18-1 has been characterized and found to contain the genes *epsM* and *epsN*. These genes are needed for the production of the unique ropy EPS in *L. Lactis* subsp. *cremoris*.

References:

1. Dierksen, K. P., Trempy, J. E., & Sandine, W. E. (1997). Expression of ropy and mucoid phenotypes in lactococcus lactis. *Journal of Dairy Science*, 80(8), 1528-1536.
2. Trempy, J. E., Ahlgren, J. A., & Knoshaug, E. P. (2000). Growth associated exopolysaccharide expression in lactococcus lactis subspecies cremoris Ropy352. *Journal of Dairy Science*, 83(4), 633.
3. Vidhyalakshmi R. and Valli Nachiyar C. (2011). Microbial production of exopolysaccharides. *Journal of Pharmacy Research*, 4(7), 2390-2391.