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## Cholate-Stimulated Biofilm Formation by *Lactococcus lactis* Cells

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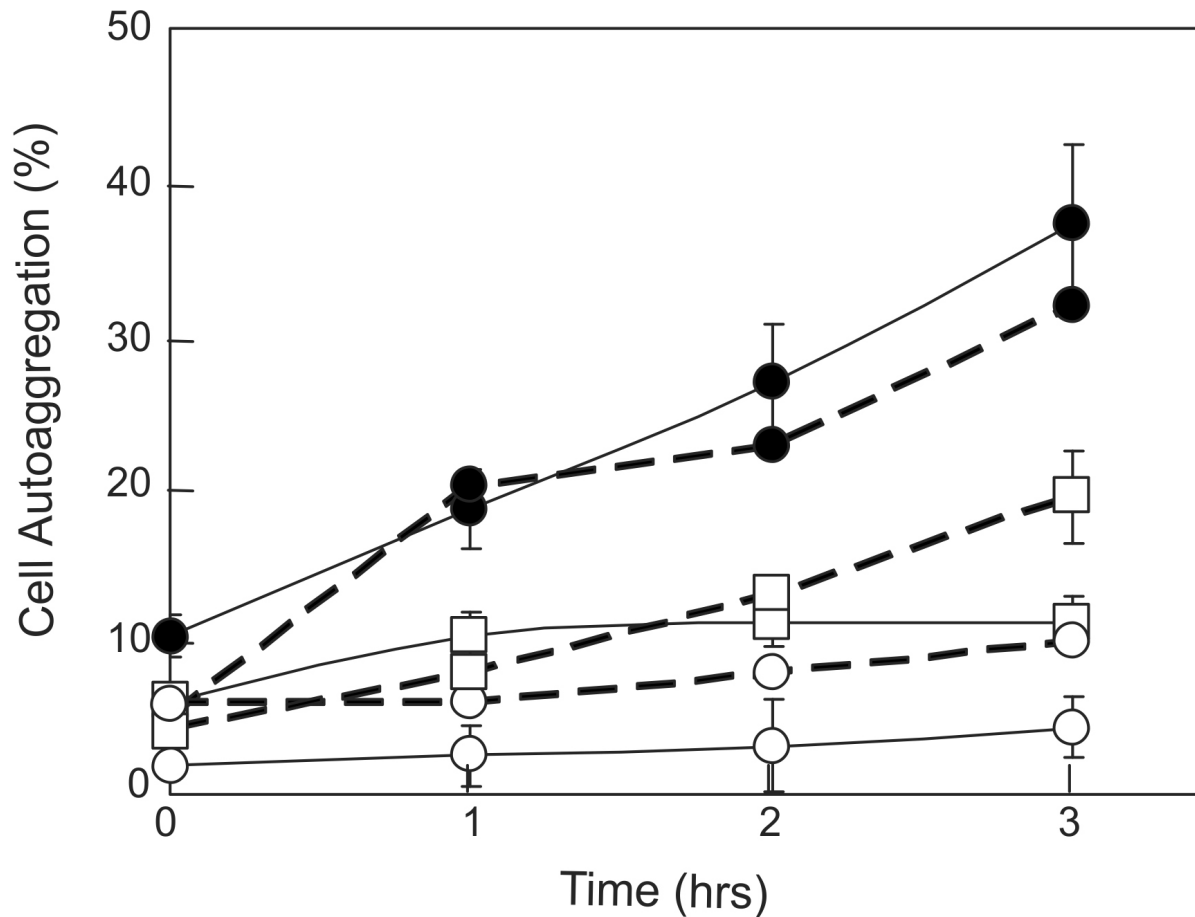
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SUPPLEMENTARY TABLE 1

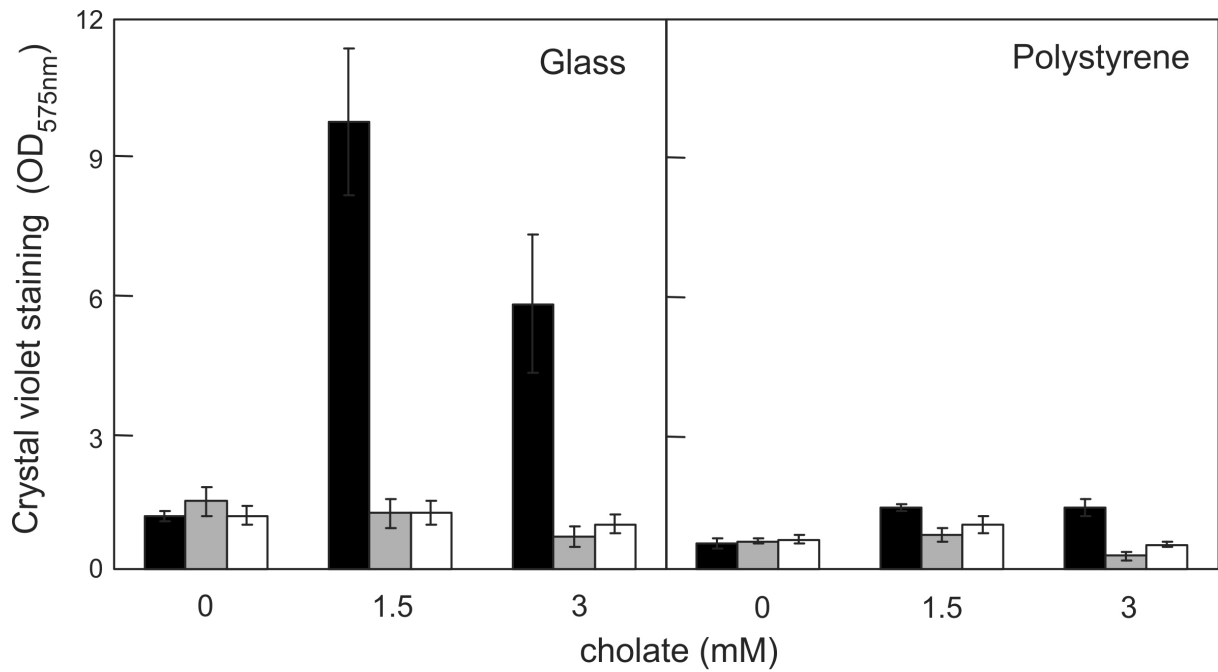
Comparison of the cholate susceptibility of biofilm derived, associated and planktonic *L. lactis* cells.

	IC <sub>50</sub> (mM)		
	Planktonic	Biofilm derived	Biofilm associated
Without cholate exposure:			
$\Delta lmrCD^f$	2.7	3.4	>14
$\Delta lmrCD$	2.1	1.8	2.3
Wild type	2.4	2.8	7.4
With cholate exposure:			
$\Delta lmrCD^f$	2.1	2.9	>14
$\Delta lmrCD$	1.5	1.8	>14
Wild type	2.5	2.4	>14

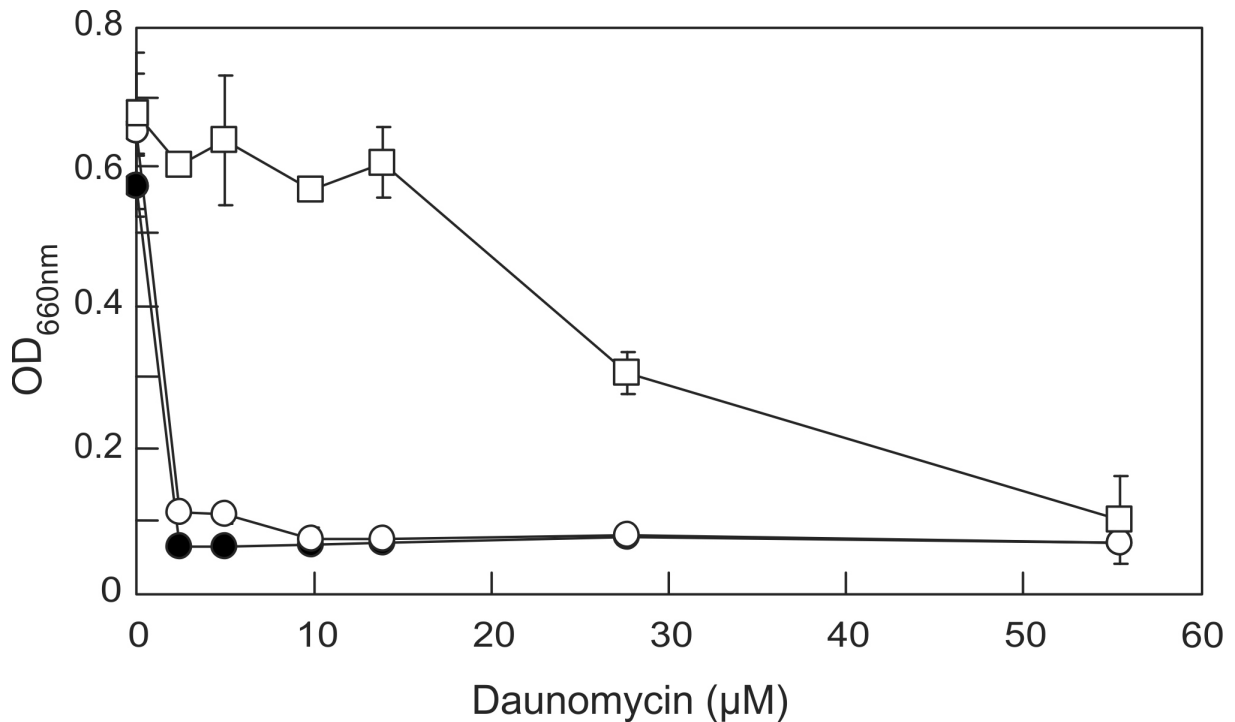
Cells were grown as described in the Materials and Methods section



SUPPLEMENTARY FIG. 1. Autoaggregation (flocculation) of *L. lactis* wild type (open squares),  $\Delta lmrCD$  (open circles) and  $\Delta lmrCD^f$  (filled circles) cells grown in the absence (solid line) or presence (dashed line) of cholate. Cultures were grown overnight in GM17 medium, collected by centrifugation and resuspended in PBS in the absence or presence of 1.5 mM cholate. Suspensions were incubated for 6 h in 15 ml test tubes at 30°C and the  $OD_{650nm}$  measured at 1 hours intervals. The degree (%) of auto aggregation (1) was determined from the  $OD_{660nm}$  values using the equation:  $100 * (1/4 OD_{t=0} - OD_{t=x} / OD_{t=0})$ , with  $OD_{t=0}$  as the initial optical density, and  $OD_{t=x}$  as the optical density of the cell suspension after a short centrifugation step (2,000 rpm for 2 min) at the indicated times. Data presented are averages of three replicates and error bars represent calculated standard deviations.



SUPPLEMENTARY FIG. 2. Biofilm formation by *L. lactis* wild type (open bars),  $\Delta lm r CD$  (gray bars) and  $\Delta lm r CD^f$  (black bars) cells. Cultures were grown for 24 h in GM17 medium containing varying concentrations of cholate in 96-well glass and polystyrene microtiter plates. The OD<sub>650nm</sub> of the resuspended biofilm was measured, and cells were stained with crystal violet whereupon OD<sub>575nm</sub> was measured. Data presented are averages of five replicates and error bars represent the standard deviations.



SUPPLEMENTARY FIG. 3. Susceptibility of biofilm cells of *L. lactis* wild type (open square),  $\Delta lm rCD$  (open circle) and  $\Delta lm rCD^+$  (black circle) to daunomycin. Cultures were grown for 24 h in GM17 medium containing 48 mM taurocholate in 96-well microtiter plates. The peg lids harboring the biofilms were washed and dipped in wells containing varying concentrations of daunomycin for 12 hrs, removed and washed in sterile 10 mM PBS and transferred to fresh GM17 medium. Plates were sealed and incubated for 18 hrs whereupon the OD<sub>650nm</sub> of the wells was measured as a measure of growth.

#### Reference

1. **Basson, A., L. A. Flemming, and H. Y. Chenia.** 2008. Evaluation of adherence, hydrophobicity, aggregation, and biofilm development of *Flavobacterium johnsoniae*-like isolates. *Microb.Ecol.* **55**:1-14.