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Publication date:
2011

Document Version
Submitted manuscript

LhrC – a quintuplets sRNA of *Listeria monocytogenes* and its role during infection and antimicrobial conditions

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Introduction

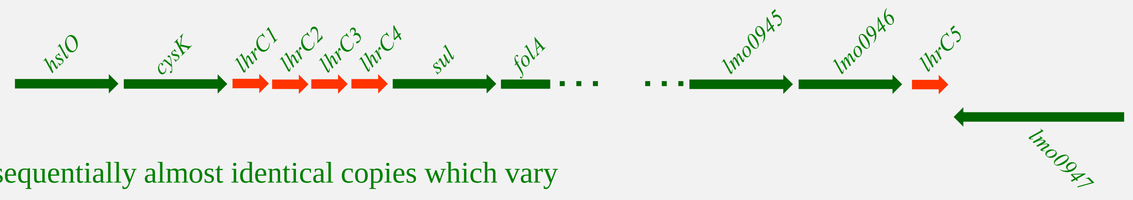

Fig. 1

The small non-coding RNA LhrC is conserved among all Listeria species and present in five sequentially almost identical copies which vary from 111 to 114 bp in size (Christiansen *et al*. 2006, RNA) . In 2009 all five copies of LhrC were found to be highly expressed in blood (Toledo-Arana *et al*. 2009, Nature), and very recently it was shown that the sRNA is also expressed when *L. monocytogenes* resides within a macrophage cell (Mraheil *et al*. 2011, Nucleic Acid Research). It can therefore be assumed that LhrC is very important for the pathogen when dealing with the harsh conditions within a host and thus relevant for a successful infection from the pathogen point of view.

Aims of this study are an identification of the exact conditions leading to an increased expression of LhrC, determination of what role each of the five LhrC copies plays, elucidation of the regulatory network behind LhrC expression, and eventually the detection of targets of this sRNA.

LhrC is induced after cell surface stress

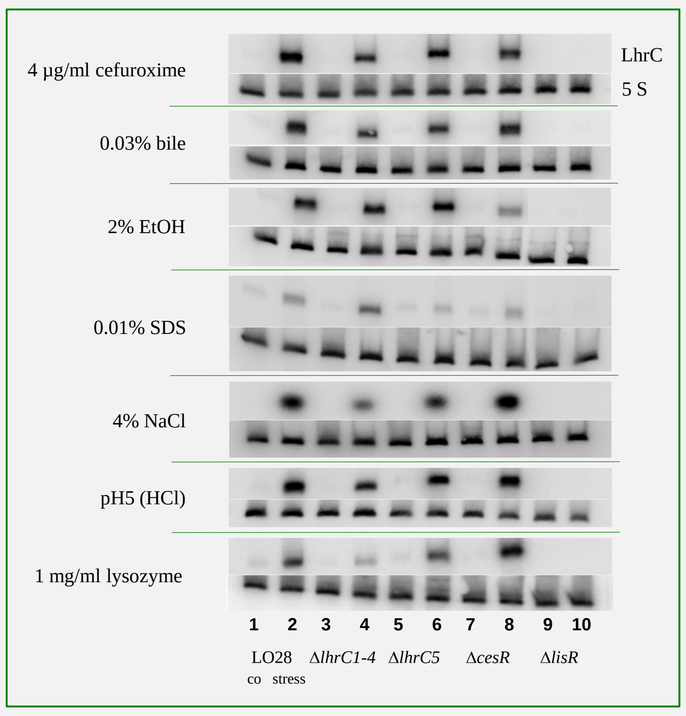

Fig. 2

A whole range of cell surface acting agents (cefuroxime, bile salts, ethanol, etc.) induce an increased expression of LhrC in LO28 (lane 2: stress, 1: control) as seen from Northern Blots (NBs), (Fig.2). Besides the wild type (WT), mutants lacking *lhrC1-4* (lanes 3, 4) and *lhrC5* (5 and 6) have been analyzed. Apparently both LhrC5 and LhrC1-4 are expressed under all tested conditions.

A lot of cell surface stress is sensed through the two-component-systems (TCSs) LisRK and CesRK in *L. monocytogenes* [Kallipolitis and Ingmer 2001, FEMS Microbiol Lett]. To find out whether these TCSs are involved in the regulation of the sRNA LhrC, a Δ*cesR* (7 and 8) and Δ*lisR* (9 and 10) have also been investigated in the NBs. In Δ*lisR* no LhrC could be detected at any of the stress conditions meaning that a functional LisRK TCS is mandatory for LhrC expression. The picture of Δ*cesR* resembles the one of the WT except for ethanol stress where the LhrC signal is weaker in Δ*cesR* than in the WT indicating an involvement of the CesRK TCS in LhrC expression for this specific stress (Fig. 3). However, LisRK is still essential.

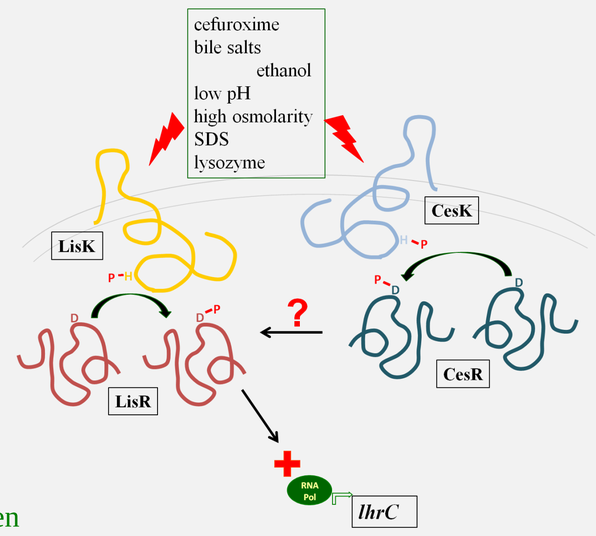

Fig. 3

Growth defects of ΔlhrC1-5

In order to determine the importance of LhrC for growth under various stress conditions LO28 WT, Δ*lhrC1-4*, Δ*lhrC5*, Δ*lisR*, Δ*cesR* and a full *lhrC* mutant lacking all five copies of the sRNA (Δ*lhrC1-5*) were grown in the presence of several surface-acting stressors.

In 4 µg/ml cefuroxime Δ*lhrC1-5* exhibits a defect in growth (Fig. 4a) compared to the WT, Δ*lisR* and Δ*cesR* mutant are not growing at all.

Also at the presence of 0.07% bile salts the full lhrC mutant lags clearly behind in growth compared to the WT, and even Δ*lhrC1-4* is hampered.

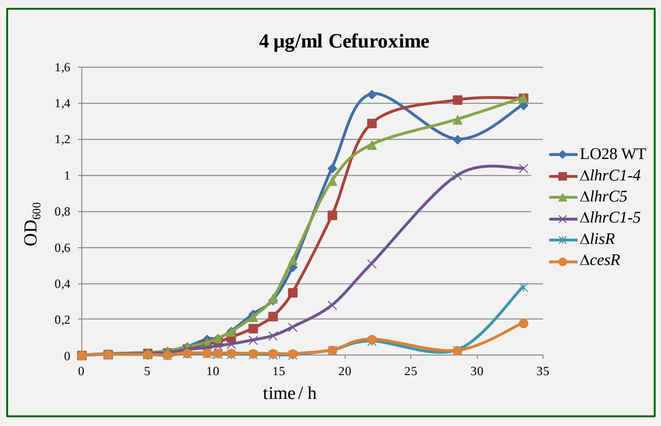

Fig. 4a

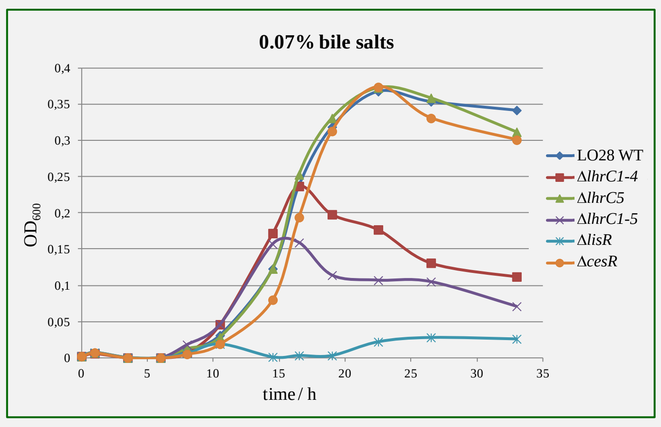

Fig. 4b

Role of the single LhrC copies

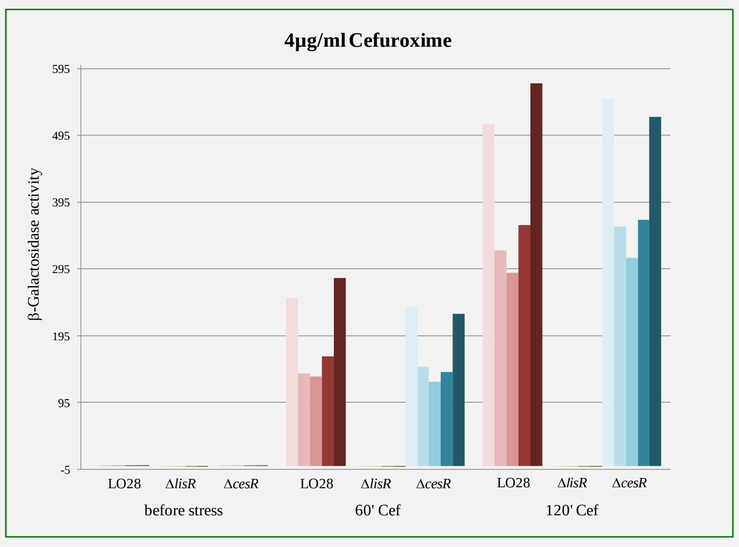

Fig. 5a

LO28 Δ*lisR* Δ*cesR*

p_*lhrC1_lacZ*
p_*lhrC2_lacZ*
p_*lhrC3_lacZ*
p_*lhrC4_lacZ*
p_*lhrC5_lacZ*

Due to the high sequence similarity of the five LhrC homologs the probe used for NBs detects the sum of all LhrC molecules. In order to determine the promotor activity of the single *lhrC* copies each of the five promotors was cloned into the vector pTCV_lac in front of the reporter gene *lacZ* and transformed into LO28 WT, Δ*lisR* and Δ*cesR*. Beta-galactosidase activity was assayed during cefuroxime and ethanol stress (Fig 5a and b). The activity of all five promotors increases dramatically after cefuroxime treatment in the WT as well as in Δ*cesR*, with promotors *lhrC1* and *5* being strongest induced. LhrC induction is entirely lost in Δ*lisR*. Ethanol stress differs from cefuroxime treatment with abolishment of most of the LhrC induction in Δ*cesR* compared to WT.

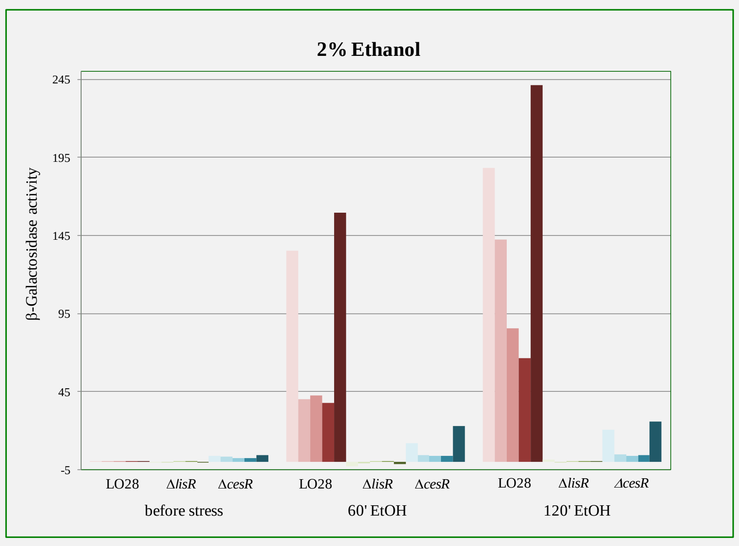

Fig. 5b

Summary

- LhrC is induced during cell surface stress
- LisRK is mandatory for LhrC expression; CesRK involved during ethanol stress
- All five *lhrC* promotors are active with *lhrC1* and *lhrC5* being most active
- LhrC is important for growth in the presence of cefuroxime and bile salts

Perspectively, most effort will be made to identify targets of LhrC implementing all - bioinformatics, global proteomics and transcriptomics techniques.

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