Curative treatment of candidiasis by the live biotherapeutic microorganism Lactobacillus rhamnosus Lcr35® in the invertebrate model Caenorhabditis elegans: first mechanistic insights

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Scientific background

Proven probiotic effects of **Lcr35®**: anti-***Candida albicans*** (opportunistic pathogenic yeast in humans) properties demonstrated by *in vitro* approaches [1], clinical trials and by an *in vivo* preventive approach [2]
→ **molecular mechanisms not elucidated**

in vivo mechanistic study

Caenorhabditis elegans, innovative animal model for studying microorganisms-microorganisms and microorganisms-host interactions
Pertinent model between *in vitro* and *in vivo* models using mammals.

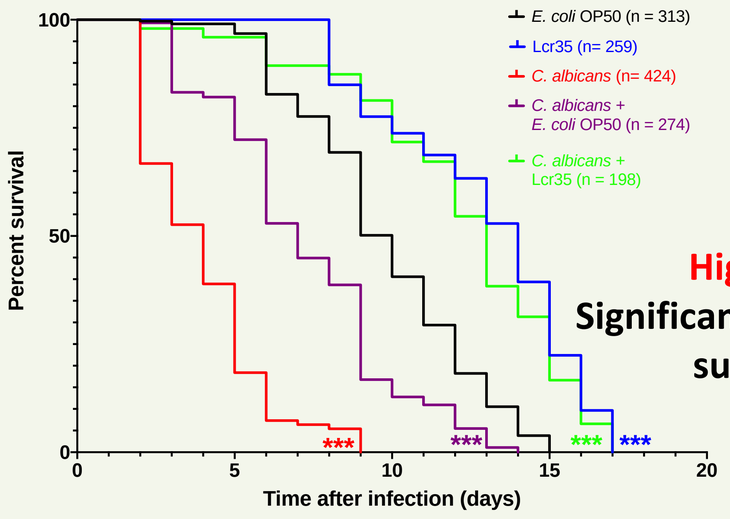
Methods

L4 / young adult worms →
- *E. coli* OP50 or Lcr35® or *C. albicans* (4h)
- *C. albicans* (2h) + *E. coli* OP50 (4h)
- *C. albicans* (2h) + Lcr35® (4h)

→
- *C. elegans* survival assay
- Determination of *C. elegans* DAF-16 cellular localization
- *C. elegans* transcriptomic assay
- Evaluation of the presence of *C. albicans* in the worm's gut

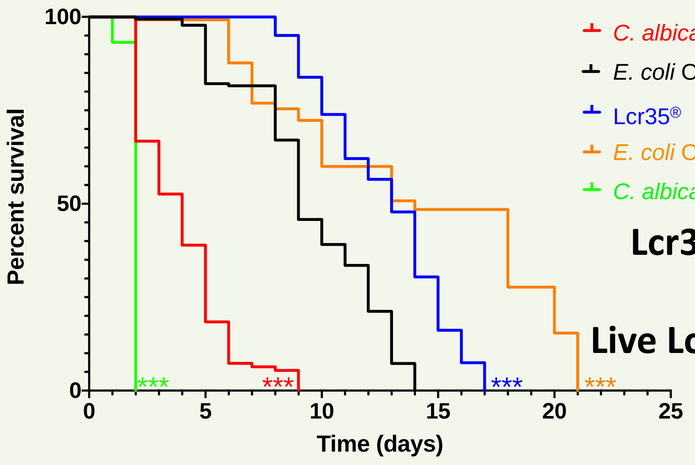
Results

C. elegans survival assay with live Lcr35®



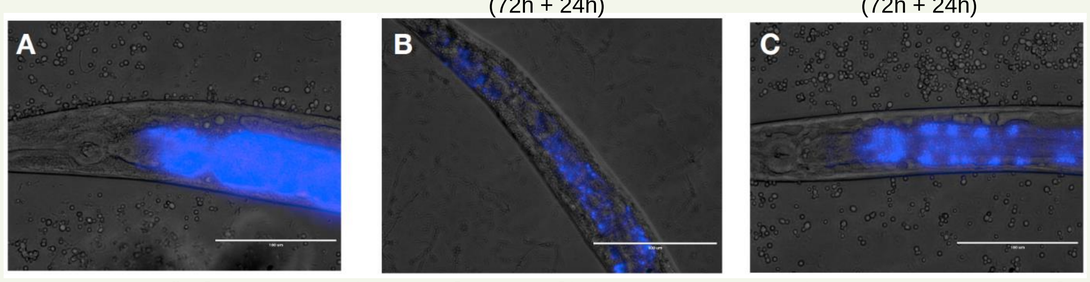
High mortality rate of *C. albicans*.
Significant increase of *C. elegans* longevity and survival with Lcr35® ± *C. albicans*
Effective curative treatment

C. elegans survival assay with Lcr35® Cell Free Supernatant



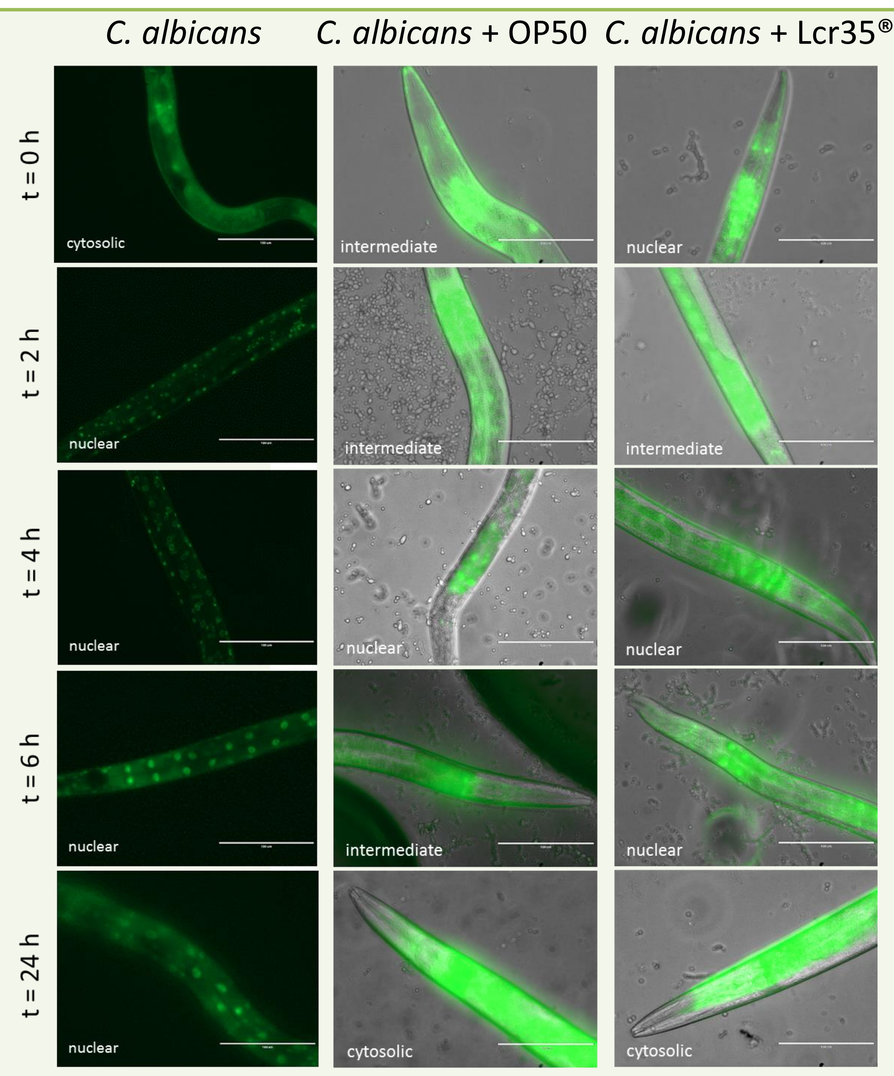
Lcr35® CFS not toxic (increased longevity)
No anti *C. albicans* activity
Live Lcr35® bacterial cells are required for anti pathogen activity

Gut colonizing *C. albicans* staining (DAPI)



Presence of the yeast in each conditions. A curative treatment does not allow the elimination of the pathogen

C. albicans / *C. albicans* + OP50 / *C. albicans* + Lcr35®



DAF-16 activity: activation of the transcription factor during a curative treatment

	Genes of interest relative expression						
	Insulin-like pathway		p38 MAPK pathway		Antimicrobials		
Conditions	*daf-2*	*daf-16*	*sek-1*	*pmk-1*	*abf-2*	*cnc-4*	*fipr-22 / fipr-23*
Lcr35®		5.84 ***	0.50	3.05		8.48	
C. albicans		0.31	4.57	4.66	3.58	14.31	3.45
C. albicans + *E. coli* OP50		0.41		2.57		7.04	0.26
C. albicans + Lcr35®			11.24 **	2.42	8.00 **		4.29 *

Lcr35® as a curative treatment induced an upregulation of p38 MAPK and antimicrobials encoding genes

Conclusion

- **This is the unique study using the *C. elegans* model for deciphering the molecular mechanisms of a candidiasis curative treatment.**
- Lcr35® protects the animal from the fungal infection (+225% of survival, $p < 2 \times 10^{-16}$) even if the yeast is detectable in its intestine. In contrast, the Lcr35® cell-free supernatant does not appear to have any antipathogenic effect. At the mechanistic level, the DAF-16/Forkhead Box O transcription factor is activated by Lcr35® and genes of the p38 MAP Kinase signaling pathway and genes involved in the antifungal response are upregulated in presence of Lcr35® after *C. albicans* infection.
- These results suggest that the probiotic strain acts by stimulating its host *via* DAF-16 and the p38 MAPK pathway.

Scientific outlook

- Global transcriptomic study on *C. elegans* by RNA sequencing
- Study of the modulation of virulence genes in *C. albicans*
- Characterization of metabolites involved in host-microorganism interactions by metabolomics
- Study of the strain-dependent aspect of the results by testing other probiotic / pathogenic couples

References

[1] Nivoliez A *et al.* (2012) Influence of manufacturing processes on *in vitro* properties of the probiotic strain *Lactobacillus rhamnosus* Lcr35®. J Biotechnol
[2] Poupet C *et al.* (2019) *Lactobacillus rhamnosus* Lcr35 as an effective treatment for preventing *Candida albicans* infection in the invertebrate model *Caenorhabditis elegans*: First mechanistic insights. PLoS One
[3] Poupet C *et al.* (2019) "Curative Treatment of Candidiasis by the Live Biotherapeutic Microorganism *Lactobacillus rhamnosus* Lcr35® in the Invertebrate Model *Caenorhabditis elegans*: First Mechanistic Insights," *Microorg.*

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