

Distribution of aerobic and oxidative stress related genes in *Lactobacillus casei* group

ZOTTA Teresa^{1*}, IANNIELLO Rocco G.¹, RICCIARDI Annamaria¹, GUIDONE Angela¹, PARENTE Eugenio^{1,2}

¹ Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Potenza, Italy
² Istituto di Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche, Avellino, Italy

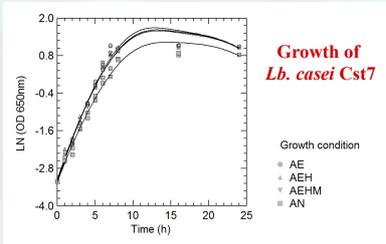
INTRODUCTION

The *Lactobacillus casei* group includes three closely related species, *Lactobacillus casei*, *Lb. paracasei* and *Lb. rhamnosus*, isolated from different ecological niches (foods and humans) and used as probiotics in a number of pharmaceutical and food preparations. Since several studies demonstrated that aerobic/respiratory pathways in lactic acid bacteria (LAB; Pedersen *et al.*, 2005; Zotta *et al.*, 2012) offer helpful traits for industrial applications, we performed for the *casei* group a comparative *in silico* analysis of the main aerobic (*pox*, *nox*, *npr*, *cydABCD*, *menFDXBEC*) and oxidative stress (*kat*, *sod*) related genes against the well characterized respiring LAB species (*Lactococcus lactis* and *Lb. plantarum*) using the Integrated Microbial Genomes (IMG) System (<http://img.jgi.doe.gov/cgi-bin/w/main.cgi>). Additionally, we evaluated the effect of aerobiosis on the growth and stress resistance in one strain of each *casei* group species.

RESULTS

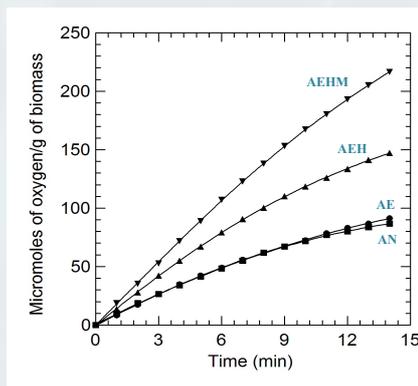
Kinetic of growth and metabolite production

Aerobiosis slightly increased the specific growth rate of *Lb. casei* and *Lb. paracasei* (4% to 12%) when hemin and menaquinone were added, whereas no appreciable differences were found in *Lb. rhamnosus*. Even if the *in silico* analysis revealed the presence of genes encoding for the key enzymes of oxygen metabolism (*pox*, *nox*, *npr*) some typical traits of aerobic pathway such as increase in biomass yield, production of H₂O₂ and acetic acid were not detected.



Oxygen uptake

A significant O₂ uptake was measured in *Lb. rhamnosus* (AEH=9.2 μmol/min g biomass; AEHM=14.6 μmol/min g biomass) and a weaker one in *Lb. casei* (AEHM=3.2 μmol/min g biomass), possibly due to the expression of genes involved in the respiratory activity (cytochrome quinol oxidase/transporter and ubiquinone/menaquinone biosynthesis), while no respiration was found in the *Lb. paracasei* strain.



Oxidative stress

All strains were sensitive to H₂O₂ because of the inability to synthesize an heme-dependent catalase, but *Lb. casei* and *Lb. paracasei* were tolerant to superoxide (up to 2 mM menadione) probably due to the expression of *sod* gene. These data were recently supported by the results on 200 "casei group" strains screened for their tolerance to H₂O₂ and superoxide generators (menadione and pyrogallol; data not shown).

References

Pedersen M.B., Iversen S.L., Sørensen K., Johansen E. 2005. *FEMS Microbiol Rev*, 29:611-624.
 Zotta T., Ricciardi A., Guidone A., Sacco M., Muscarelli L., Mazzeo M.F., Cacace G., Parente E. 2012. *Int J Food Microbiol*, 155: 51-59.

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MATERIALS AND METHODS

Strains and culture conditions: The kinetics of growth of *Lb. casei* Cst7, *Lb. paracasei* DSM4905 and *Lb. rhamnosus* DSM20021 were evaluated in buffered (pH 6.5) MRS broth under static (anaerobiosis, AN) and shaking (aerobiosis without supplements, AE; with 2.5 μg/mL hemin, AEH; with 2.5 μg/mL hemin and 1 μg/mL menaquinone, AEHM) conditions.

Chemical and biochemical analyses: Biomass yield, glucose consumption, production of lactic and acetic acids, O₂ uptake (from 5.5 mmol/L glucose), H₂O₂ concentration and catalase activity were measured on the stationary phase (S, 16 h) cells and supernatants.

Oxidative stress treatments: Tolerance to different (from 0.8 to 0.0015 mol/L) H₂O₂ concentrations was tested in microplate experiments, while survival to menadione (a superoxide generating compound, from 0.3 mM to 0.018 mM) was evaluated by spotting cell suspensions on MRS agar plates.

In silico analysis: Comparative analysis of selected genes was performed by IMG platform tools.

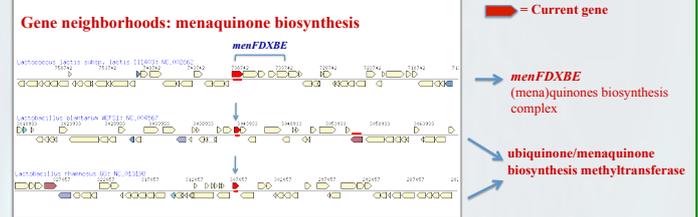
In silico analysis

Lc. lactis II1403 and *Lb. plantarum* WCFS1 were selected for comparative analysis because they are the well characterized respiring LAB species. Only one sequenced strain of each *Lb. casei* and *Lb. rhamnosus* was used, while both genomes of *Lb. paracasei* were compared because are yet in draft status.

Table 1 displays the number of homologs of genes associated with a specific gene tabulated against other genomes. Different values of percent identity and a default E-value of 1e-50 was set in the similarity cut-offs to determine the homologs.

Table 1: Gene occurrence profiles

Gene function	Query gene	<i>Lactococcus lactis</i> II1403 (Finished)		<i>Lactobacillus plantarum</i> WCFS1 (Finished)		<i>Lactobacillus casei</i> Cst7 (Finished)		<i>Lactobacillus paracasei</i> ATCC 25302 (Draft)		<i>Lactobacillus paracasei</i> A'VCC 25302 (Draft)		<i>Lactobacillus rhamnosus</i> GG (Finished)	
		Gene symbol or Locus Tag	Min. % Identity	Gene symbol or Locus Tag	Min. % Identity	Gene symbol or Locus Tag	Min. % Identity	Gene symbol or Locus Tag	Min. % Identity	Gene symbol or Locus Tag	Min. % Identity	Gene symbol or Locus Tag	Min. % Identity
Oxidative stress	<i>kat</i> Heme-dependent Superoxide Dismutase (L. lactis) (L. lactis)	none	/	<i>kat</i>	100%	none	/	none	/	none	/	none	/
	<i>sod</i> Superoxide Dismutase (L. lactis) (L. lactis)	100%		none	/	LCABL_20710	50%	Lppa8_01010000045	50%	HMPREF030_1533	50%	none	/
Aerobic metabolism	<i>poxF</i> Pyruvate oxidase (L. lactis) (L. lactis)	<i>poxL</i>	40%	<i>pox1</i> <i>pox2</i> <i>pox3</i> <i>pox4</i> <i>pox5</i>	40% 40% 40% 40% 100%	<i>poxL</i> <i>poxF</i>	40% 40%	Lppa8_010100010215 Lppa8_010100005291	50% 40%	<i>poxF</i> (HMPREF030_1131) <i>pox5</i> (HMPREF030_2002)	50% 40%	<i>poxL</i> <i>poxF</i>	40% 40%
	<i>nox</i> NADH oxidase (L. lactis) (L. lactis)	<i>noxC</i> <i>noxD</i> <i>noxE</i>	20% 30% 30%	<i>nox1</i> <i>nox2</i> <i>nox4</i> <i>nox5</i>	50% 40% 40% 100%	<i>nox</i> <i>nox5</i>	50% 50%	Lppa8_010100002650 Lppa8_010100008620	50% 50%	<i>nox5</i> (HMPREF030_0383) <i>nox5</i> (HMPREF030_1832)	50% 50%	<i>nox5</i> <i>nox5</i>	50% 50%
	<i>npr</i> NADH dehydrogenase (L. lactis) (L. lactis)	none	/	<i>npr1</i> <i>npr2</i>	40% 100%	<i>npr</i>	40%	none	/	<i>npr</i>	40%	<i>npr</i>	50%
	<i>cyd</i> Cytochrome b558 (L. lactis) (L. lactis)	<i>cydA</i> <i>cydB</i>	50% 50%	<i>cydA</i> <i>cydB</i>	100% 100%	<i>cydA</i> <i>cydB</i>	60% 60%	Lppa8_010100010505 Lppa8_010100010500	60% 60%	<i>cydA</i> (HMPREF030_1072) <i>cydB</i> (HMPREF030_1073)	60% 60%	<i>cydA</i> <i>cydB</i>	60% 60%
	<i>cydC</i> Cytochrome b558 (L. lactis) (L. lactis)	<i>cydC</i> <i>cydD</i>	40% 40%	<i>cydC</i> <i>cydD</i>	100% 100%	<i>cydC</i> <i>cydD</i>	50% 50%	Lppa8_010100010495 Lppa8_010100010490	50% 50%	<i>cydC</i> (HMPREF030_1074) HMPREF030_1075	50% 50%	<i>cydC</i> <i>cydD</i>	50% 40%



In addition to several flavin oxidases, gene analysis revealed the presence of an ubiquinone/menaquinone biosynthesis methyltransferase in the genome of *Lb. rhamnosus* GG but, contrarily to *Lc. lactis* genome in which a complete (mena)quinones biosynthesis gene-set is present, this species (as *Lb. plantarum* WCFS1) probably lacks other genes belonging to (mena)quinones biosynthesis complex.

CONCLUSIONS

This study shows that, with the exception of *Lb. rhamnosus*, strains of the *Lb. casei* group have limited ability to use oxygen although genes for oxidation of pyruvate and NADH and for a partial respiratory chain are present in the genome. Since technologically important properties of microorganisms, even within the same species, are often strain-specific and the data available on the aerobic metabolism of LAB are related to a small number of strains and species, further investigations are needed to clarify the diversity of aerobic growth/oxidative stress response in this important technological LAB group.