Lactococcus lactis, a new bacterial factory for functional expression of membrane proteins

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In spite of the functional and biotechnological importance of membrane proteins (MP), their structural study remains difficult because of their hydrophobicity and their low abundance in the cells. Moreover, in the well-known heterologous systems, these proteins are often produced at very weak rates, toxic and/or not correctly folded. *Lactococcus lactis*, a Gram-positive lactic bacterium, traditionally used in food fermentation, is now largely used in biotechnology for the production on a large scale of prokaryotic and eukaryotic proteins. In the last years, *L. lactis* proved to be an alternative system for expression of MPs [1-5]. First, all chloroplast MPs of *Arabidopsis thaliana* tested could be produced at levels compatible with further biochemical analyses [1,4] and several proteins were active [4,6]. It was also the case for other plant and human proteins [1,7, unpublished data]. These recent data suggest that *L. lactis* is an attractive system for effective and functional production of 'difficult-to-express' membrane proteins.

L. lactis = Emerging and alternative expression system for MPs

Fast and cheap growth; easy to handle and scale-up

Comparison with *E. coli*:

- ✓ No extracellular proteinases
- ✓ **ONLY 1 glycolipid-rich membrane**
- ✓ Different in phospholipid composition
- ✓ Possible screens for functional expression
- ✓ **NO formation of inclusion bodies**
- ✓ Facultative anaerobic culture: no vigorous agitation and no aeration
- ✓ Expression of genes whose products are potentially toxic
- ✓ Expression of MPs without signal peptides and codon optimization

Gram⁺ bacteria (production of fermented food) [5,7]

A tightly controlled expression = **NICE**

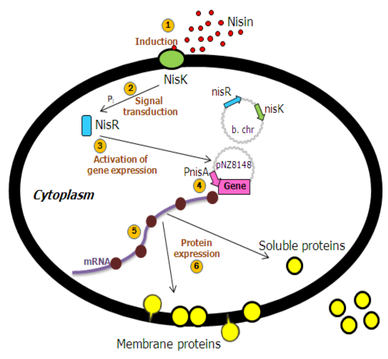


Figure 1: Schematic representation of the NIsin Controlled Expression system. Nisin induces tightly controlled expression of genes placed under the control of the PnisA promoter via signal transduction. Depending on presence or absence of targeting signals, protein is expressed into cytoplasm, secreted or in cell envelope [7].

Gene cloning : Gateway-compatible strategy [4]

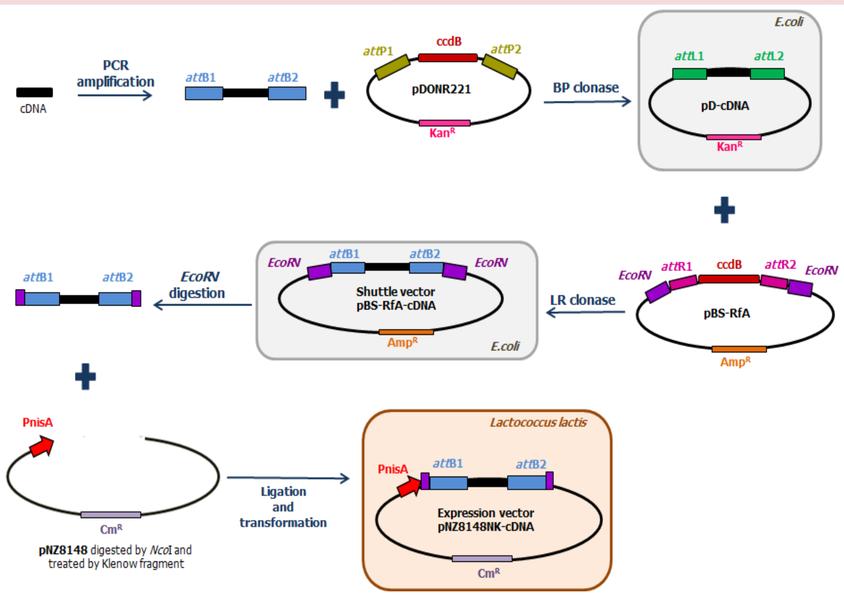


Figure 3. Gateway-compatible strategie to facilitate cDNA transfer into *L. lactis* vectors. The cDNA fragment generated after PCR amplification is inserted into the entry vector pDONR221 by a BP reaction. Afterwards, it's transferred by a LR reaction into pBS-RfA, generating the ''shuttle'' vector pBS-RfA-cDNA. Then, after excision from the pBS-RfA-cDNA by digestion with *Eco*RV, it is ligated into pNZ8148NKle. Amp^R, Kan^R, Cm^R, resistance to ampicilline, kanamycine and chloramphenicol respectively; *att* X: recombinaison sites of the Gateway technology; MCS: multicloning site of pNZ8148 [7].

Gene insertion in pNZ8148 : classical cloning

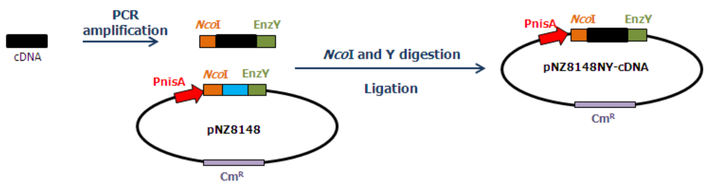


Figure 2: Classical cloning of cDNA in pNZ8148. After amplification by PCR, the cDNA is digested by *Nco*I and Enzyme Y (another enzyme from the multicloning site). Then, the digested fragment is ligated into pNZ8148 previously digested with the same endonucleases, giving rise to pNZ8148NY-cDNA [7].

Expression of eukaryotic MPs in *L. lactis* with diverse functions, origins and topologies

Protein	Function	Size (kDa)	TM helices	Organism	Expression yields	Functional
MPC1/MPC2	mitochondrial pyruvate carrier	12.3+14.3	2x2	*M. musculus*	<1%	
SAM5	mitochondrial S-adenosyl methionine carrier	30.9	4	*S. cerevisiae*	<1%	
Mdl1	mitochondrial ATP-dependent permease	76	5	*S. cerevisiae*	<0.1%	
MIR1	mitochondrial phosphate carrier protein	32.8	6	*S. cerevisiae*	<1%	
CTP1	tricarboxylate transport protein	32.9	6	*S. cerevisiae*	5%	
DIC1	mitochondrial dicarboxylate transporter	33	6	*S. cerevisiae*	10%	
GGC1	mitochondrial GTP/GDP carrier protein	33.2	6	*S. cerevisiae*	4%	
PIC2	mitochondrial phosphate carrier protein 2	33.5	6	*S. cerevisiae*	1-2%	
AAC3	mitochondrial ADP/ATP carrier protein 3	33.7	6	*S. cerevisiae*	5%	
ODC2	mitochondrial 2-oxodicarboxylate carrier 2	34	6	*S. cerevisiae*	10%	
AAC1	mitochondrial ADP/ATP carrier protein 1	34.1	6	*S. cerevisiae*	<1%	
ODC1	mitochondrial 2-oxodicarboxylate carrier 1	34.2	6	*S. cerevisiae*	8%	
AAC2	mitochondrial ADP/ATP carrier protein 2	34.4	6	*S. cerevisiae*	<1%	
★ AAC1	mitochondrial ADP/ATP carrier protein 1	34	6	*H. sapiens*	0.5-1%	
★ ceQORH	quinone oxidoreductase - electron transfer	33.1	p	*A. thaliana*	30%	
LPR1	multi-copper oxidase	60.5	p	*A. thaliana*	<0.1%	
PHF	phosphate transport regulation	42.4	1	*A. thaliana*	1.5%	
★ CYP715A1	cytochrome-mono-oxygenase	59	1	*A. thaliana*	15%	
AAC hyd	hydrogenosomal carrier	33.9	6	*N. patriciarum*	<1%	
AtHMA1	heavy metal transporter	80.1	6	*A. thaliana*	3%	
AtHMA3	heavy metal transporter	81.4	8	*A. thaliana*	1%	
★ AtHMA6	heavy metal transporter	100	8	*A. thaliana*	3%	
AtHMA4	heavy metal transporter	126.7	8	*A. thaliana*	0.75%	
★ NTT1	chloroplast ADP/ATP transporter	57.5	12	*A. thaliana*	0.2%	
SUT1	sucrose transporter	54.8	12	*S.tuberosum*	1-2%	
Bcl-Xl	apoptosis regulation	24.7	1	*H. sapiens*	1%	
★ CYP3A4	cytochrome-mono-oxygenase	57.4	1	*H. sapiens*	5%	
★ MGST1	microsomal glutathione S-transferase 1	17.6	4	*R.norvegicus*	3%	
★ MGST1	microsomal glutathione S-transferase 1	17.6	4	*H. sapiens*	3%	
ABCG2	breast cancer resistance protein	72	6	*H. sapiens*	0.5-1%	
Erd2	KDEL receptor	24.4	7	*H. sapiens*	<0.1%	
CXCR4	chemokine receptor type 4	37.9	7	*H. sapiens*	<0.1%	
CCR5	chemokine receptor type 5	38.7	7	*H. sapiens*	<0.1%	
PS1Δ9	human alpha secretase component	55	9	*H. sapiens*	0.1-0.2%	
CFTR = ABCC7	cystic fibrosis transmembrane conductance regulator	168	12	*H. sapiens*	<0.1%	

List of heterologous eukaryotic MPs expressed in *L. lactis* using the NICE system [1,4,7]. The classification of MPs has been sorted according to numbers of TM (transmembrane) helices (p for peripheral proteins) and origins (plant versus human). Protein sizes are given for full proteins, *i.e.* including the transit peptide for chloroplastic MP (truncated for heterologous expression). M. musculus (Mus musculus); S; cerevisiae (Saccharomyces cerevisiae); *A. thaliana (Arabidopsis thaliana); S. tuberosum (Solanum tuberosum); N. patriciarum (Neocallimastix patriciarum); H. sapiens (Homo sapiens); R. norvegicus (Rattus norvegicus).* The expression yields are given as a percentage of the recombinant protein compared to the total membrane proteins (total MP). The protein is functionally active in *L. lactis* (orange) or the activity has not yet been determined (white). In blue gray, proteins with an expression yield higher than 1% of total MP.

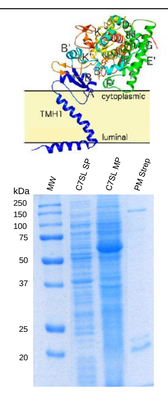
Special cases I : plant MPs [4,6]

Peripheral : ceQORH = 30% [4]

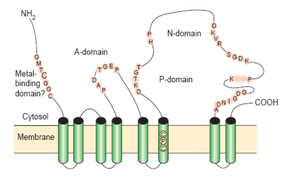
→ ceQORH : active in *L. lactis* and behaves as the native protein [4]

A. Deshydrogenase activity of the His-ceQORH protein produced in *L. lactis*	
System	Specific activity of the ceQORH protein
Complete	19,28 (100%)
Without ceQORH	0 (0%)
Without PC	1,97 (10,2%)
Without NADPH	0 (0%)
Without NBT	1,14 (5,9%)
B. Comparison with other forms of the recombinant ceQORH protein	
ceQORH protein produced in *E. coli*	40%
ceQORHatt protein produced in *L. lactis*	95%
ceQORHnh protein produced in *L. lactis*	90%

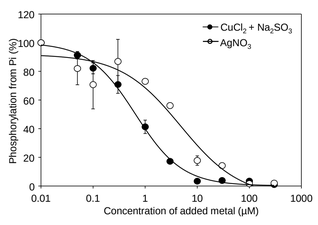
CYP715A1 (1 TM) = 15%



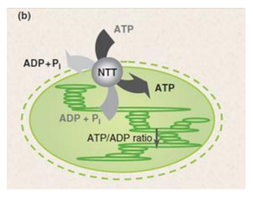
AtHMA6 (8TM) = 3%



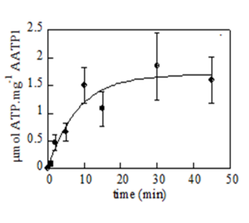
→ AtHMA6 acts as a high affinity Cu(I) transporter [6]



NTT1 (12TM) = 0.2%

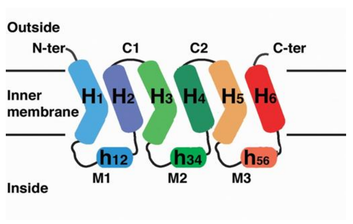


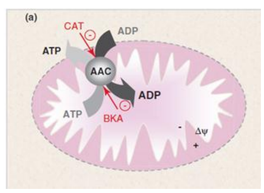
→ NTT1 is active in *L. lactis* [4]

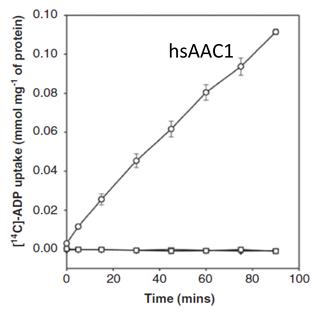


Special cases II : mitochondrial carriers [4,6,8]

Mitochondrial carriers: 6 TM, different oligomeric states exchange of ADP against ATP







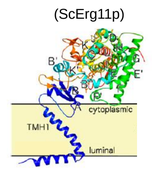
→ Mitochondrial carriers are active in *L. lactis* (transport ADP) and require cardiolipin for function, abundantly present in *L. lactis* membrane [5].

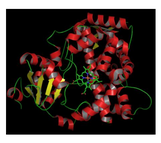
Special cases III : mammalian MPs involved in detoxification processes [9]

P450 cytochromes : CYP3A4

Hemoprotein with 1 TM (57 kDa)
Oxidation (metabolism of 50% of drugs on market)

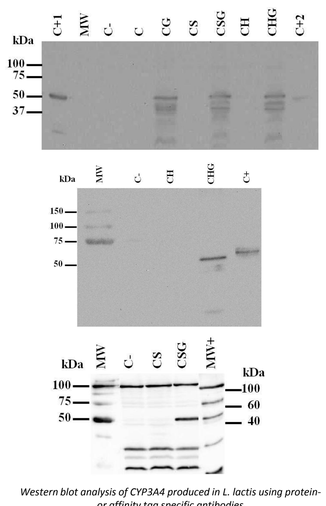
Structure of P450 CYP51 (ScErg11p)



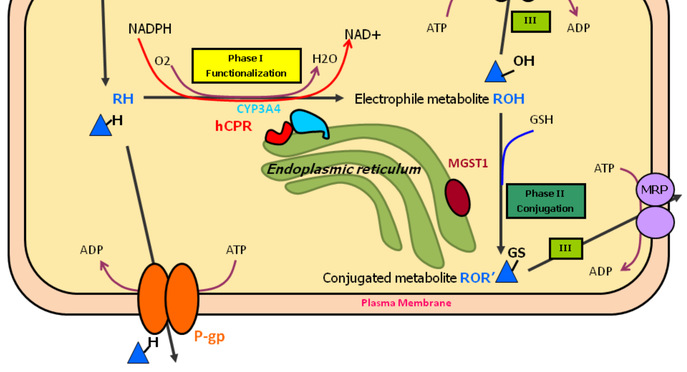


Monk *et al.*, *PNAS*, 2014

CYP3A4 is expressed in *L. lactis* (5%), only after Gateway-compatible strategy



Western blot analysis of CYP3A4 produced in L. lactis using protein- or affinity tag specific antibodies.



Abbreviations of the different constructions generated for the expression in L. lactis of the proteins involved in detoxification (X= C for CYP3A4; rM/hM for rat or human MGST1)

Affinity tag	Cloning	
	classical	Gateway-compatible strategy (G)
no	X	XG
6x His-tag (H)	XH	XHG
Strep-tag II (S)	XS	XSG

Further structure-function analyses

Conclusion

- ✓ **Good alternative to *E. coli* (absence of inclusion bodies, small genome, only one glycolipid rich membrane**
- ✓ **Lower expression yield of MPs vs *E. coli* [1] (likely due to branched-chain amino acid availability)**
- ✓ **Expression of functional proteins from peripheral to intrinsic proteins with up to 12 TM domains**
- ✓ **Since 2001: 2 X-ray structures of prokaryotic MPs expressed in *L. lactis* with the NICE system: ThiT (3RLB, 2Å) and BioY (4DVE, 2,1Å), two *L. lactis* amino acid transporters**

References

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