BIOINFORMATIC ANALYSIS OF PROPHAGE ENDOLYSINS AND ENDOLYSIN-LIKE GENES FROM THE ORDER LACTOBACILLALES

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Abstract: Endolysins belonging to the group of peptigoglycan hydrolases, which are able to cleave peptidoglycan in bacterial cell walls, become an extensively studied group of enzymes. Thanks to their narrow target specificity and low probability of resistance they are considered to be an appropriate alternative to conventional antibiotics. The present paper concerns the occurrence of endolysin and endolysin-like genes in genomes of bacteria belonging to the order Lactobacillales. Using bioinformatic programmes we compared and analysed protein sequences of catalytic and cell wall binding (CWB) domains of these enzymes, their preferred combinations, their phylogenetic relationship and potential occurence of natural „domain shuffling". The existence of this phenomenon in selected group of enzymes was confirmed only in limited range, so we assume that the natural trend is the distribution of „well-tried" combinations of catalytic and CWB domains of endolysin genes as a whole.

Key words: endolysin, Lactobacillales, bioinformatic analysis, domain shuffling

1. Introduction

Bacterial peptidoglycan hydrolases (PGHs) form a vast and highly diverse group of enzymes of different origin capable of cleaving bonds in polymeric peptidoglycan, which is a major component of the bacterial cell envelope in both Gram-positive and Gram-negative bacteria (VOLLMER *et al.*, 2008; HUMANN and LENZ, 2009). Activity of these enzymes results in a cell wall disruption and consequent bacterial cell lysis.

One subgroup of PGHs are endolysins, which are encoded in genomes of bacteriophages and expressed in the host cells to digest the bacterial cell wall for bacteriophage progeny release at the terminal stage of the phage reproduction cycle. Endolysins as well as the most of other PGHs are composed of at least two clearly separated functional domains: With few exceptions, the N-terminal domain contains the catalytic activity of the enzyme, whereas the C-terminal cell wall binding domain (CWB) directs the enzyme to a specific substrate (FISCHETTI, 2005; LOESSNER, 2005).

According to the specific bond that is split down in the peptidoglycan, catalytic domains can be divided into several classes: (1) glycosidases (glucosaminidases and muramidases) hydrolyze β-1→4 bonds between disaccharide units of glycan chains, (2) peptidases (endopeptidases and carboxypeptidases) cleave short peptide bridges cross-linking glycan chains, and (3) amidases cleave the amide bond between glycan chains and peptide bridges (LAYEC *et al.*, 2008).

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There is also variability among CWB domains given the fact that any compounds present on bacterial cell wall (peptidoglycan subunits, saccharides, proteins, lipoteichoic acid, etc.) could serve as their receptor. Correct binding of whole enzyme molecule to the cell wall of sensitive bacteria requires these specific receptors. CWB domains thus offer to the enzymes a high degree of specificity, since these receptors are only found in enzyme-sensitive bacteria (LOESSNER, 2005). One of the favourable features of endolysins is their narrow target specificity – these enzymes are uniquely specific to their host (or a small number of closely related bacteria), therefore they might represent an effective way to control specific pathogens without disturbing the normal microflora (LÓPEZ *et al.*, 2004). Although endolysin and endolysin-like genes are usually present in genomes of bacteriophages, they can also be found in bacterial genomes, whether as a result of the presence of prophages or as their residua.

This work is focused on study of endolysins from the order Lactobacillales, group of Gram-positive lactic acid bacteria that are ubiquitous in nature. These bacteria have a role as commensals on mucosal surfaces and skin and inhabit the digestive tract of many animal species and humans (STROMPFOVÁ and LAUKOVÁ, 2004). They have also been the focus of substantial research because of their economic importance in food fermentation. Some strains are used as the starter cultures in the cheese industry, other ones are used in the production of yogurt. An important problem in industrial milk fermentation is bacteriophage attack. Bacteriophage infection leads to the lysis of the starter cells and thereby interrupts the fermentation of milk sugar (lactose) into lactic acid by the starter bacteria (BRUSSOW, 2001).

Fortunately, we can also find several favourable features of bacteriophages and enzymes encoded by their genomes (as mentioned above), which made them an interesting tool for medicine. Decades of antibiotic misuse have resulted in increasing bacterial resistance to many modern antibiotics and generating potentially dangerous multiresistant strains of lactic acid bacteria. This antibiotic resistance can cause significant danger for many people with common bacterial infections (MATHUR and SINGH, 2005). Infections caused by antibiotic resistant bacterial strains could be treatable by PGHs, including bacteriophage endolysins.

Using bioinformatic analysis of protein sequences of PGHs obtained from GenBank database we studied natural trends in combinations of their individual domains and potential occurence of natural „domain shuffling“.

2. Material and methods

In the first step we searched GenBank database (Benson *et al*., 2011) for protein sequences of endolysins using pair of key words “endolysin” and “Lactobacillales” (or “lysin” and “Lactobacillales”). Every obtained sequence we used as the query in the PROTEIN BLAST search (Altschul *et al.*, 1990) and this way we obtained several more sequences of endolysin-like proteins from the order Lactobacillales.

From all sequences obtained in the first GenBank database search and subsequent PROTEIN BLAST search (sequences with similarity score 500 or more) we chose those with known type of both catalytic and CWB domain and this way we created a set of sequences for consequent analyses.

CDD outputs from PROTEIN BLAST, graphic representation of conserved domains (Marchler-Bauer *et al.*, 2011) we used for determination of protein domain boundaries.

Using MEGA 4 software and its component Tree Explorer (TAMURA *et al.*, 2007) we performed the phylogenetic analysis, we constructed two phylogenetic trees (maximum parsimony method) of studied proteins, individually according to the sequences of catalytic and CWB domains. Finally we compared these trees to verify the degree of difference that is proportional to the intensity of natural "domain shuffling". All sequences were analysed as one set regardless of their origin (whether they were obtained from phage or bacterial gemomes).

3. Results and discussion

Domain structure of PGHs represents the potential for constructing new recombinant enzymes with artificially created combinations of catalytic and CWB domains ("domain shuffling"). Such approach allows one to prepare novel enzymes with desired target specificity (provided by CWB domain) and concrete catalytic activity targeting selected type of chemical bonds in the peptidoglycan (CROUX *et al*., 1993; SANZ *et al*. 1996).

In our work we analysed the selected protein sequences of endolysins and endolysin-like genes from order Lactobacillales and using bioinformatic software we compared their domain structure (Table 1). We identified 116 sequences from 33 bacterial species; the most of sequences was obtained from genomes of *Streptococcus pyogenes* (23 sequences), *Lactobacillus reuteri* (16 sequences) and *Enterococcus faecalis* (10 sequences). On the other hand, genomes of several species (e.g. *Enterococcus faecium*, *Lactobacillus helveticus* or *Streptococcus gordonii*) were especially poor of endolysin-like sequences. The results of sequence analysis revealed 9 types of catalytic domains (glycosidases: Glucosaminidase, GH25_muramidase and Lysozyme_like; amidases: Amidase_2, Amidase_3, Amidase_5 and CHAP; peptidases: endopeptidase and SCP_bacterial) and 5 types of CWB domains (the most frequent were LysM and SH3_5 domain). Several analysed protein sequences contain one catalytic and two different CWB domains and vice versa. The most preferred domain combinations were glycosidase catalytic domain with LysM (~35%), glycosidase catalytic domain with SH3_5 (25%) and amidase catalytic domain with SH3_5 (~20%). While glycosidase and amidase domains were combined with both LysM and SH3_5 CWB domains, peptidase domains were present only in combination with LysM domain (~17% of all sequences). We observed some differences between preferred domain combinations among individual bacterial species. Enterococci for example prefer combinations of glycosidase domain with LysM or amidase domain with SH3_5; in lactococcal sequences we found only LysM domain in combination with glycosidase or peptidase domains; in contrast streptococci prefer SH3_5 domain with glycosidase or amidase domains. The greatest variability in domain combinations was observed among lactobacilli. Given the small number of sequences analysed it is not clear, how significant these differences are.

Table 1. Domain structure of endolysin and endolysin-like proteins from the order Lactobacillales.

ID	source	domains	
		catalytic	CWB
ZP_02184645	*Carnobacterium sp.* AT7	Amidase_3	LysM PG_binding_1
ZP_02185877	*Carnobacterium sp.* AT7	Amidase_2	LysM PG_binding_1
AAA67325	*Enterococcus faecalis*	Glucosaminidase	LysM
BAG12399	*Enterococcus faecalis*	Lysozyme_like Amidase_5	SH3_5
NP_814147	*Enterococcus faecalis* V583	GH25_muramidase	LysM
NP_814543	*Enterococcus faecalis* V583	Glucosaminidase	LysM
NP_815016	*Enterococcus faecalis* V583	Amidase_2	SH3_5
NP_815207	*Enterococcus faecalis* V583	Amidase_2	SH3_5
NP_815299	*Enterococcus faecalis* V583	Glucosaminidase	LysM
NP_815667	*Enterococcus faecalis* V583	GH25_muramidase	LysM
NP_816267	*Enterococcus faecalis* V583	Glucosaminidase	LysM
NP_816427	*Enterococcus faecalis* V583	GH25_muramidase	LysM
ZP_00602624	*Enterococcus faecium* DO	Glucosaminidase	LysM
P39046	*Enterococcus hirae*	Glucosaminidase	LysM
AY581208	*Enterococcus sp.* phage 1	Amidase_5	SH3_5
YP_194209	*Lactobacillus acidophilus* NCFM	GH25_muramidase	SLAP
YP_795546	*Lactobacillus brevis* ATCC 367	endopeptidase	LysM
YP_805691	*Lactobacillus casei* ATCC 334	GH25_muramidase	LysM
YP_805885	*Lactobacillus casei* ATCC 334	GH25_muramidase	SH3_5 LysM
YP_807109	*Lactobacillus casei* ATCC 334	GH25_muramidase	LysM
YP_001986445	*Lactobacillus casei* BL23	GH25_muramidase	LysM
YP_001986643	*Lactobacillus casei* BL23	GH25_muramidase	SH3_5 LysM
YP_001987288	*Lactobacillus casei* BL23	GH25_muramidase	SH3_5 LysM
YP_812161	*Lactobacillus delbrueckii* ATCC BAA-	GH25_muramidase	SLAP
YP_812257	*Lactobacillus delbrueckii* ATCC BAA-	endopeptidase	LysM
YP_812312	*Lactobacillus delbrueckii* ATCC BAA-	Glucosaminidase	SLAP
YP_618265	*Lactobacillus delbrueckii* ATCC 11842	GH25_muramidase	SLAP
YP_618351	*Lactobacillus delbrueckii* ATCC 11842	endopeptidase	LysM
YP_001843302	*Lactobacillus fermentum* IFO 3956	GH25_muramidase	LysM
YP_001843358	*Lactobacillus fermentum* IFO 3956	endopeptidase	LysM
YP_001844636	*Lactobacillus fermentum* IFO 3956	Glucosaminidase	LysM
YP_814010	*Lactobacillus gasseri* ATCC 33323	GH25_muramidase	SH3_5
YP_814525	*Lactobacillus gasseri* ATCC 33323	GH25_muramidase	SH3_5
YP_815282	*Lactobacillus gasseri* ATCC 33323	GH25_muramidase	Cpl-7
YP_001577714	*Lactobacillus helveticus* DPC 4571	GH25_muramidase	SLAP
NP_964172	*Lactobacillus johnsonii* NCC 533	GH25_muramidase	SH3_5
BAE02832	*Lactobacillus plantarum*	GH25_muramidase	SH3_5 LysM
NP_784445	*Lactobacillus plantarum* WCFS1	GH25_muramidase	SH3_5 LysM
NP_785858	*Lactobacillus plantarum* WCFS1	GH25_muramidase	SH3_5 LysM
NP_786050	*Lactobacillus plantarum* WCFS1	Glucosaminidase	SH3_5
NP_786398	*Lactobacillus plantarum* WCFS1	GH25_muramidase	SH3_5
NP_786644	*Lactobacillus plantarum* WCFS1	endopeptidase	LysM
NP_964349	*Lactobacillus prophage* Lj928	GH25_muramidase	SH3_5
NP_965218	*Lactobacillus prophage* Lj928	GH25_muramidase	SH3_5
AAY86796	*Lactobacillus reuteri*	endopeptidase	LysM
AAY86900	*Lactobacillus reuteri*	endopeptidase	LysM

Continuation of Table 1.

ID	source	domains	
		catalytic	CWB
AAY86914	*Lactobacillus reuteri*	Glucosaminidase	LysM
YP_001842732	*Lactobacillus reuteri* F275	Glucosaminidase	LysM
YP_001841599	*Lactobacillus reuteri* JCM 1112	endopeptidase	LysM
YP_001842145	*Lactobacillus reuteri* JCM 1112	endopeptidase	LysM
YP_001842697	*Lactobacillus reuteri* JCM 1112	endopeptidase	LysM
ZP_03072312	*Lactobacillus reuteri* 100-23	GH25_muramidase	SH3_5
ZP_03072387	*Lactobacillus reuteri* 100-23	GH25_muramidase	LysM
ZP_03072513	*Lactobacillus reuteri* 100-23	GH25_muramidase	SH3_5
ZP_03072609	*Lactobacillus reuteri* 100-23	endopeptidase	LysM
ZP_03072731	*Lactobacillus reuteri* 100-23	GH25_muramidase	SH3_5
ZP_03073251	*Lactobacillus reuteri* 100-23	endopeptidase	LysM
ZP_03073284	*Lactobacillus reuteri* 100-23	Glucosaminidase	LysM
ZP_03073901	*Lactobacillus reuteri* 100-23	GH25_muramidase	LysM
ZP_03074398	*Lactobacillus reuteri* 100-23	endopeptidase	LysM
ZP_03212068	*Lactobacillus rhamnosus* HN001	GH25_muramidase	LysM
ZP_03212389	*Lactobacillus rhamnosus* HN001	GH25_muramidase	LysM
YP_396046	*Lactobacillus sakei* subsp. sakei 23K	Glucosaminidase	LysM
YP_534994	*Lactobacillus salivarius* UCC118	endopeptidase	LysM
YP_535201	*Lactobacillus salivarius* UCC118	GH25_muramidase	LysM
YP_535698	*Lactobacillus salivarius* UCC118	GH25_muramidase	LysM
YP_001031636	*Lactococcus lactis* MG1363	Glucosaminidase	LysM
YP_001031859	*Lactococcus lactis* MG1363	Glucosaminidase	LysM
YP_001032179	*Lactococcus lactis* MG1363	GH25_muramidase	LysM
YP_808554	*Lactococcus lactis* subsp. cremoris SK11	Glucosaminidase	LysM
YP_809109	*Lactococcus lactis* subsp. cremoris SK11	GH25_muramidase	LysM
YP_811362	*Lactococcus lactis* subsp. cremoris SK11	GH25_muramidase	LysM
NP_266428	*Lactococcus lactis* subsp. lactis Il1403	Glucosaminidase	LysM
NP_266697	*Lactococcus lactis* subsp. lactis Il1403	Glucosaminidase	LysM
YP_001727510	*Leuconostoc citreum* KM20	SCP_bacterial	LysM
YP_817821	*Leuconostoc mesenteroides* ATCC 8293	SCP_bacterial	LysM
YP_817823	*Leuconostoc mesenteroides* ATCC 8293	SCP_bacterial	LysM
YP_818129	*Leuconostoc mesenteroides* ATCC 8293	endopeptidase	LysM
YP_810395	*Oenococcus oeni* PSU-1	endopeptidase	LysM
YP_810757	*Oenococcus oeni* PSU-1	Glucosaminidase	LysM
YP_810956	*Oenococcus oeni* PSU-1	CHAP (amidase)	LysM
YP_804483	*Pediococcus pentosaceus* ATCC 25745	GH25_muramidase	SH3_5 LysM
YP_804659	*Pediococcus pentosaceus* ATCC 25745	endopeptidase	LysM
ZP_00787586	*Streptococcus agalactiae* CJB111	Glucosaminidase Amidase_3	LysM
NP_688827	*Streptococcus agalactiae* 2603V/R	Amidase_5 Glucosaminidase	Cpl-7
ABV55414	*Streptococcus dysgalactiae* subsp. equisimilis	Amidase_3 CHAP (amidase)	LysM
YP_001449532	*Streptococcus gordonii* CH1	CHAP (amidase)	LysM
ZP_02920165	*Streptococcus infantarius* ATCC BAA-	CHAP (amidase)	LysM
NP_720819	*Streptococcus mutans* UA159	CHAP (amidase)	LysM
YP_598205	*Streptococcus pyogenes* MGAS10270	Glucosaminidase CHAP (amidase)	SH3_5
YP_059383	*Streptococcus pyogenes* MGAS10394	Glucosaminidase CHAP (amidase)	SH3_5
YP_060515	*Streptococcus pyogenes* MGAS10394	CHAP (amidase)	SH3_5
YP_060862	*Streptococcus pyogenes* MGAS10394	CHAP (amidase)	SH3_5

Continuation of Table 1.

ID	source	domains	
		catalytic	CWB
YP_602773	*Streptococcus pyogenes* MGAS10750	Amidase_5 Glucosaminidase	Cpl-7
NP_664535	*Streptococcus pyogenes* MGAS315	Glucosaminidase CHAP (amidase)	SH3_5
NP_664726	*Streptococcus pyogenes* MGAS315	Glucosaminidase CHAP (amidase)	SH3_5
NP_664900	*Streptococcus pyogenes* MGAS315	Amidase_5 Glucosaminidase	Cpl-7
NP_665012	*Streptococcus pyogenes* MGAS315	Glucosaminidase CHAP (amidase)	SH3_5
NP_665215	*Streptococcus pyogenes* MGAS315	CHAP (amidase)	SH3_5
YP_282364	*Streptococcus pyogenes* MGAS5005	CHAP (amidase)	SH3_5
NP_606641	*Streptococcus pyogenes* MGAS8232	Amidase_5 Glucosaminidase	Cpl-7
NP_607527	*Streptococcus pyogenes* MGAS8232	Glucosaminidase CHAP (amidase)	SH3_5
YP_596324	*Streptococcus pyogenes* MGAS9429	Glucosaminidase CHAP (amidase)	SH3_5
NP_268942	*Streptococcus pyogenes* M1 GAS	Glucosaminidase CHAP (amidase)	SH3_5
NP_269522	*Streptococcus pyogenes* M1 GAS	Glucosaminidase CHAP (amidase)	SH3_5
YP_002285797	*Streptococcus pyogenes* NZ131	CHAP (amidase)	SH3_5
YP_002286426	*Streptococcus pyogenes* NZ131	Glucosaminidase CHAP (amidase)	SH3_5
YP_598455	*Streptococcus pyogenes* phage 315.5	CHAP (amidase)	SH3_5
NP_801715	*Streptococcus pyogenes* SSI-1	CHAP (amidase)	SH3_5
NP_802383	*Streptococcus pyogenes* SSI-1	CHAP (amidase)	SH3_5
YP_001128106	*Streptococcus pyogenes* str. Manfredo	Glucosaminidase CHAP (amidase)	SH3_5
YP_001128256	*Streptococcus pyogenes* str. Manfredo	Amidase_5 Glucosaminidase	Cpl-7
YP_001034311	*Streptococcus sanguinis* SK36	CHAP (amidase)	LysM
ZP_00874987	*Streptococcus suis* 89/1591	CHAP (amidase)	LysM
YP_819956	*Streptococcus thermophilus* LMD-9	CHAP (amidase)	LysM
YP_138970	*Streptococcus thermophilus* LMG 18311	CHAP (amidase)	LysM

Consecutively we performed a detailed phylogenetic analysis of catalytic and CWB domain sequences and the resulting phylogenetic trees we compared with each other by connecting the catalytic and CWB domains together representing one protein (Fig. 1). The results indicate that the degree of relatedness of the catalytic domains of proteins in most cases corresponds to the degree of relatedness of their CWB domains (approximately parallel flowlines between clusters of phylogenetic trees).

Figure 2 illustrates more detailed view to phylogenetic trees from Fig. 1 and demonstrates one example of domain relatedness, as mentioned above. Seven proteins from four different bacterial strains, and thus regardless of their origin (see Fig. 2 caption) show very similar relatedness of their catalytic domains (GH25_muramidase) and CWB domains (LysM), which belong to the same phylogenetic clusters.

On the other hand, there are few exceptions. As you can see in Fig. 1 several flowlines between clusters of phylogenetic trees show remarkably different direction from other groups of flowlines. These examples represent few proteins with proven natural “domain shuffling”.

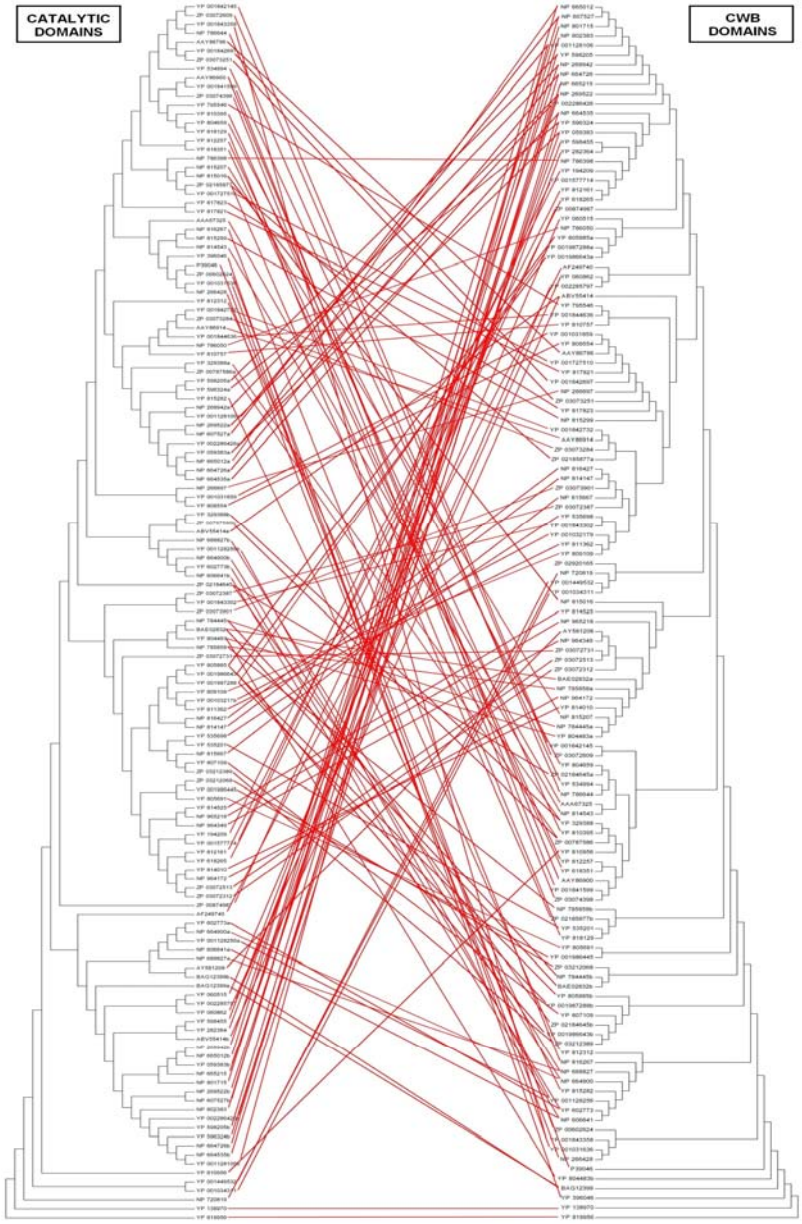

Fig. 1. Comparation of phylogenetic trees of catalytic and CWB domains of selected endolysins and endolysin-like proteins from order Lactobacillales. Groups of approximately parallel flowlines between clusters of phylogenetic trees demonstrate that degree of relatedness of the catalytic domains of proteins in most cases corresponds to the degree of relatedness of their CWB domains. Individually running flowlines with different direction between clusters demonstrate few proteins representing exceptions with proven natural domain shuffling.

From these examples we can mention two pairs of proteins: (1) YP_001844636 from *Lactobacillus fermentum* IFO 3956 and YP_795546 from *Lactobacillus brevis* ATCC 367, which both have the same type of CWB domains (LysM) belonging to the same phylogenetic clustre and exhibiting 55% sequence similarity, but their catalytic domains (glucosaminidase and endopeptidase, respectively) show no significant similarity; (2) proteins YP_001844636 from *Lactobacillus fermentum* IFO 3956 and NP_786050 from *Lactobacillus plantarum* WCFS1 have the same type of catalytic domains (glucosaminidase) from the same phylogenetic clustre and exhibiting 68% sequence similarity, but they differ in the type of CWB domains (LysM and SH3_5, respectively) with no significant similarity.

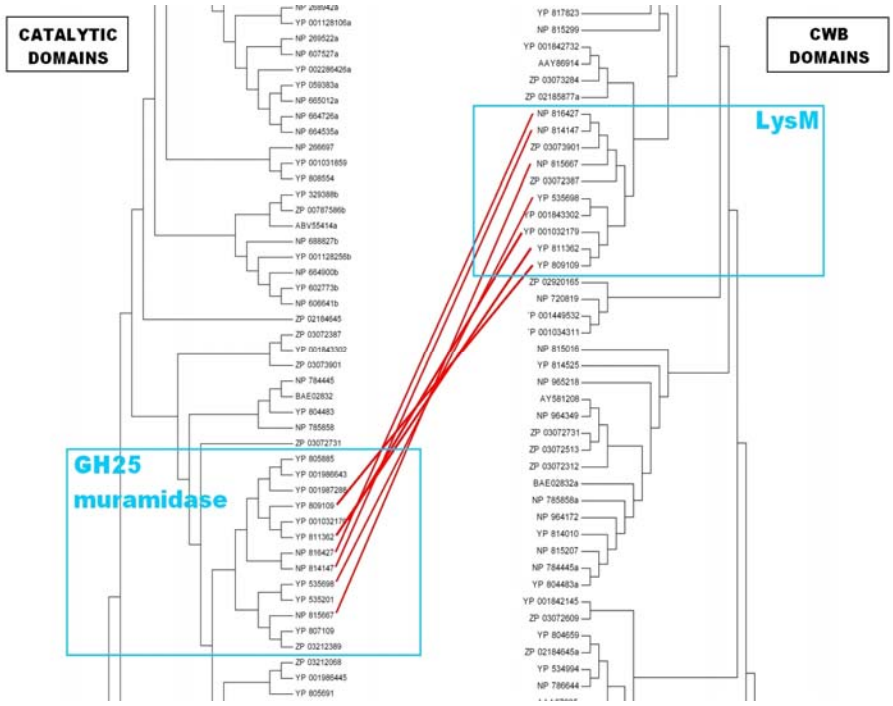

Fig. 2. More detailed view to phylogenetic trees from Fig. 1. Seven proteins from four different bacterial strains (YP_809109 and YP_811362 from *Lactococcus lactis* subsp. *cremoris* SK11, YP_001032179 from *Lactococcus lactis* MG1363, NP_814147, NP_815667 and NP_816427 from *Enterococcus faecalis* V583, and YP_535698 from *Lactobacillus salivarius* UCC118) show very similar relatedness of their catalytic domains (GH25_muramidase) and CWB domains (LysM), which belong to the same phylogenetic clusters.

4. Conclusions

In present work we analysed domain structure of endolysins and endolysin-like proteins from the order Lactobacillales. Understanding the system of the natural domain distribution is the base for consequent experiments with artificial domain

shuffling and production of novel recombinant PGHs. Based on our findings we conclude, that the natural trend is distribution of endolysin genes as a whole and natural "domain shuffling" occurs in this case only in limited degree. Nevertheless, an artificial "domain shuffling" may be regarded as an indispensable method in enzyme engineering.

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