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**The Ester Hydrolytic and Synthetic
Activities of X-Prolyl Dipeptidyl
Peptidase from *Streptococcus
thermophilus***

A thesis presented in partial fulfillment of the requirements for
the degree of Master of Science in Biochemistry
at Massey University, New Zealand

**Matthew David Bennett
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Abstract

X-prolyl dipeptidyl peptidase (EC 3.4.14.11), or PepX, is a dipeptidase found in most dairy lactic acid bacteria that hydrolyses N-terminal dipeptides from larger peptides where proline is the residue penultimate to the scissile bond. It has recently been found that PepX will also catalyse the hydrolysis of some chromogenic esters and synthesise esters via an acyltransferase mechanism that uses ethanol as the acceptor molecule and tributyrin as the donor molecule.

In this study, the *pepX* gene from *Streptococcus thermophilus* strain B2513 was cloned and sequenced. This sequence was found to differ in several positions from the recently published *pepX* sequence of *S. thermophilus* strain ACA-DC4. None of the observed substitutions occurred in the catalytic domain of the enzyme, all being localised to the C-terminal β -sheet domain.

An activity assay using a chromogenic peptide substrate with tributyrin as an was used to prove that PepX binds peptide substrates and acylglycerides at the same binding site, implying that the same catalytic machinery carries out both peptide hydrolysis and activities involving acylglycerides.

PepX was found to form esters only from the acylglyceride tributyrin, and was not active on any of the larger triglycerides tested. The chemical mechanism for this ester formation is proposed to involve the direct transfer of an acyl group from the donor to an acceptor, rather than acyl hydrolysis followed by the separate transfer of a carboxylic acid product onto an acceptor, as the enzyme does not form esters when provided with butyric acid and ethanol.

PepX was found to be incapable of hydrolysing milkfat and tributyrin in aqueous solution. This contrasts with the ability of PepX to hydrolyse the synthetic ester *p*-nitrophenyl butyrate, which probably is a reflection of the lability of the ester bond in this substrate.

The results of this study show that PepX is a peptidase that has a secondary acyltransferase activity, with no hydrolase activity on natural acylglyceride substrates.

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Abbreviations

Amp	Ampicillin
BLAST	Basic Local Alignment Search Tool
BSA	Bovine serum albumin
BTP	1, 3-Bis[Tris (hydroxymethyl) methylamino] propane
°C	Degrees Centigrade
CAPS	(3-[cyclohexylamino]-1-propanesulfonic acid)
CMC	Critical micelle concentration
ddH ₂ O	Double distilled water
DEE	Diethyl ether
DIG	Digoxigenin
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNTP	Deoxyribose nucleotide triphosphate
ECL	Enhanced Chemiluminescence System (Amersham)
EDTA	Ethylene diamine tetraacetic acid
EtBr	Ethidium bromide
EtOH	Ethanol
FAA	Free amino acids
FFA	Free Fatty Acids
FID	Flame Ionisation Detector
FPLC	Fast Protein Liquid Chromatography
GLC	Gas Liquid Chromatography, Gas Liquid Chromatograph
HEPES	n-(2-hydroxyethyl)piperazine-n-(2-ethanesulfonic acid)
IEX	Ion Exchange Chromatography
IMAC	Immobilised Metal Affinity Chromatography
kDa	kilo Daltons
LB	Luria Broth
MCS	Multiple cloning site / polylinker (vector)
MWt	Molecular mass
NaCl	Sodium chloride


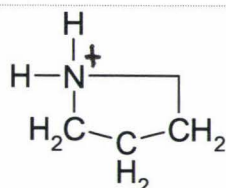
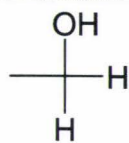
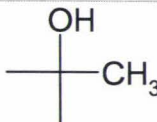
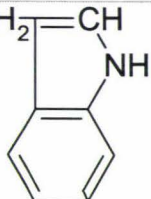
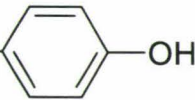
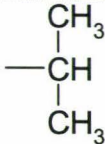
Abbreviations

NCBI	National Center for Biotechnology Information
NEB	New England Biolabs
NH ₂	Amine
(NH ₄) ₂ SO ₄	Ammonium sulfate
OD	Optical Density
PCR	Polymerase Chain Reaction
<i>pepX</i>	X-prolyl dipeptidase gene
PepX	X prolyl dipeptidyl peptidase, X-prolyl dipeptidase protein
ppm	Parts per million
PVDF	Polyvinylidene difluorine
SDS-PAGE	Sodium Dodecyl Sulphate Polyacylamide Gel Electrophoresis
Sp.	Species
SPE	Solid Phase Extraction
SSC	Saline sodium citrate buffer
Subsp.	Subspecies
Tet	Tetracycline
TRIS	Tris (hydroxymethyl) Aminomethane
V _{max}	Maximum reaction velocity
X-Gal	5-bromo-4-chloro-3-indolyl-β-galactoside

Amino Acid Abbreviations

Amino Acid	Sidechain	One letter code	Three letter code
Alanine	—CH_3	A	Ala
Arginine	$\begin{array}{c} \text{NH}_2 \\ \\ \text{—C—C—C—N—} \\ \quad \quad \quad \\ \text{H}_2 \quad \text{H}_2 \quad \text{H}_2 \quad \text{H} \end{array} \quad \text{=NH}_2^+$	R	Arg
Asparagine	$\begin{array}{c} \text{OH} \\ \\ \text{—C—} \\ \\ \text{H}_2 \\ \\ \text{NH}_2 \end{array}$	N	Asn
Aspartate	$\begin{array}{c} \text{OH} \\ \\ \text{—C—} \\ \\ \text{H}_2 \\ \\ \text{=O} \end{array}$	D	Asp
Cysteine	$\text{—CH}_2\text{—SH}$	C	Cys
Glutamine	$\begin{array}{c} \text{OH} \\ \\ \text{—C—C—} \\ \quad \\ \text{H}_2 \quad \text{H}_2 \\ \\ \text{NH}_2 \end{array}$	Q	Gln
Glutamate	$\begin{array}{c} \text{OH} \\ \\ \text{—C—C—} \\ \quad \\ \text{H}_2 \quad \text{H}_2 \\ \\ \text{=O} \end{array}$	E	Glu
Glycine	—H	G	Gly
Histidine	$\begin{array}{c} \text{H} \\ \\ \text{—C—} \\ \\ \text{H}_2 \end{array} \quad \begin{array}{c} \text{N} \\ // \\ \text{NH} \\ \\ \text{+} \end{array}$	H	His
Isoleucine	$\begin{array}{c} \text{CH}_3 \\ \\ \text{—C—CH}_2\text{—CH}_3 \\ \\ \text{H} \end{array}$	I	Ile
Leucine	$\begin{array}{c} \text{CH}_3 \\ \\ \text{—CH}_2\text{—CH} \\ \\ \text{CH}_3 \end{array}$	L	Leu

Amino Acid Abbreviations

Lysine	$\text{—CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$	K	Lys
Methionine	$\text{—CH}_2\text{CH}_2\text{S—CH}_3$	M	Met
Phenylalanine	—CH_2 	F	Phe
Proline		P	Pro
Serine		S	Ser
Threonine		T	Thr
Tryptophan	—CH_2 	W	Trp
Tyrosine	—CH_2  —OH	Y	Tyr
Valine		V	Val

Nucleic Acid Abbreviations

One Letter Code	Base(s) Represented
A	Adenosine
C	Cytosine
G	Guanine
T	Thymidine
U	Uridine
R	G or A
Y	T or C
K	G or T
M	A or C
S	G or C
W	A or T
B	G or T or C
D	G or A or T
H	A or C or T
V	G or C or A
N	Any