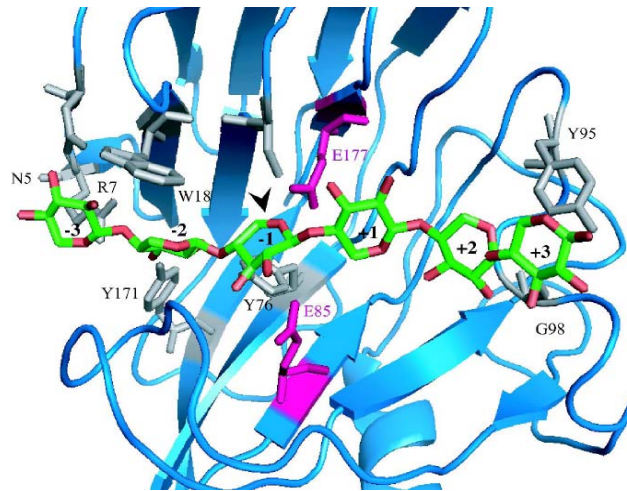


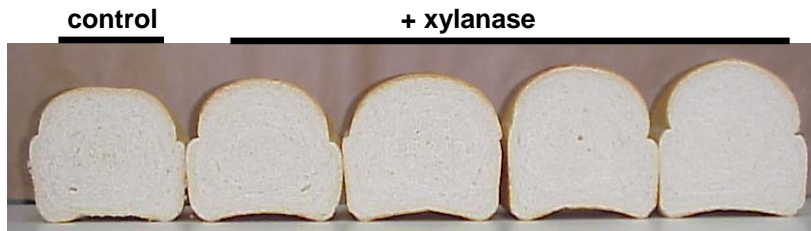
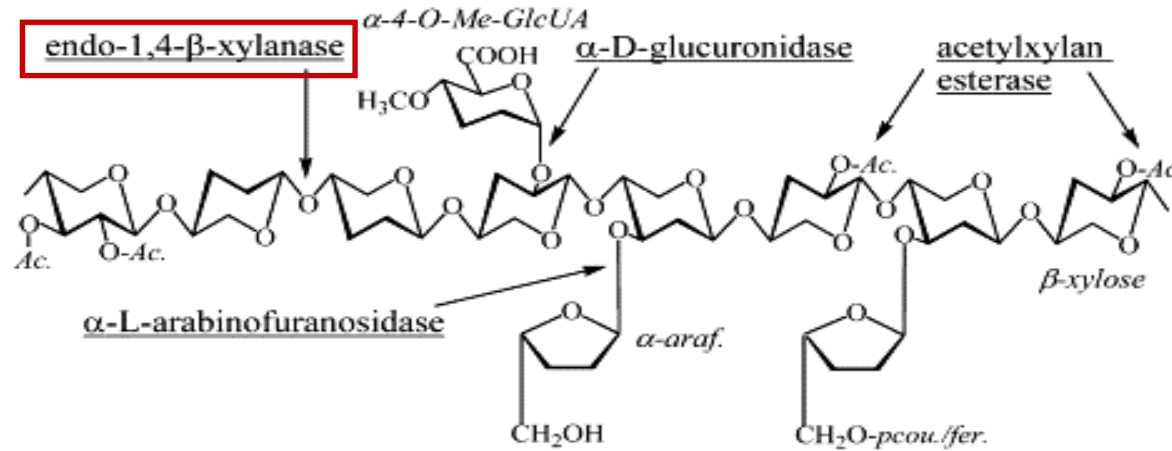
Development and improvement of xylanases for the degradation of cereal arabinoxylan

Jean-Guy Berrin, Marine Tison, Mickaël Lafond,
Jacques Georis and Nathalie Juge

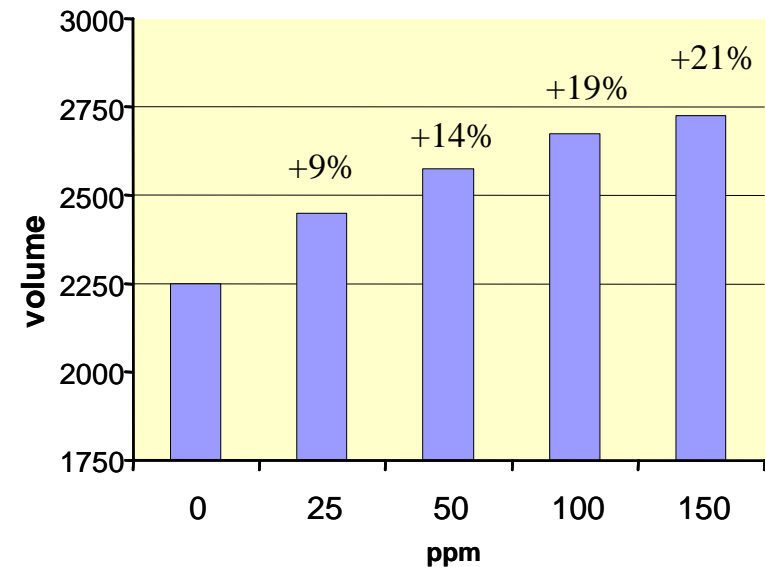


Laboratoire Biosciences
University Paul Cézanne
Marseille, France

Xylanases: importance in cereal processing



- ✗ Increase of bread volume
- ✗ Dough handling and machinability
- ✗ Monitoring of dough stickness
- ✗ Structure of crumb and crust

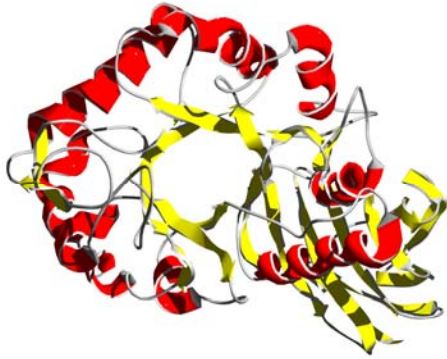




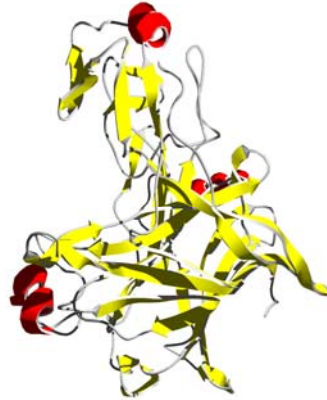
Xylanase classification

www.CAZY.org

GH5



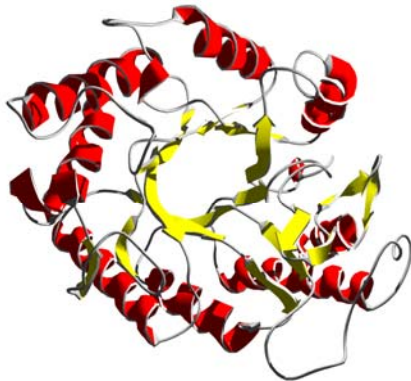
GH7



GH8



GH10



GH11

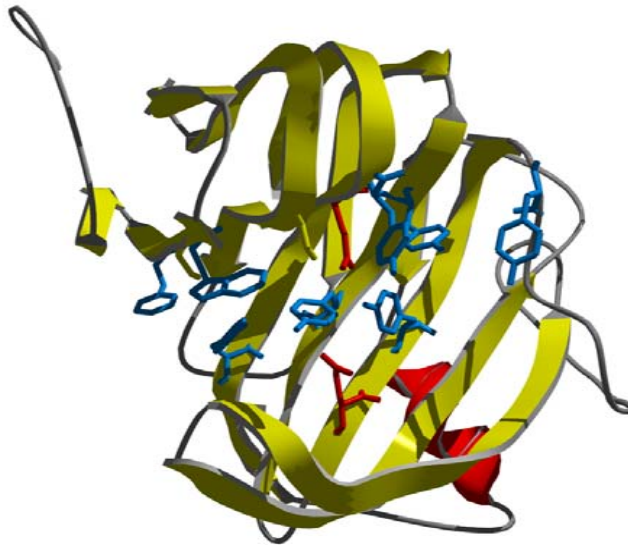


GH43



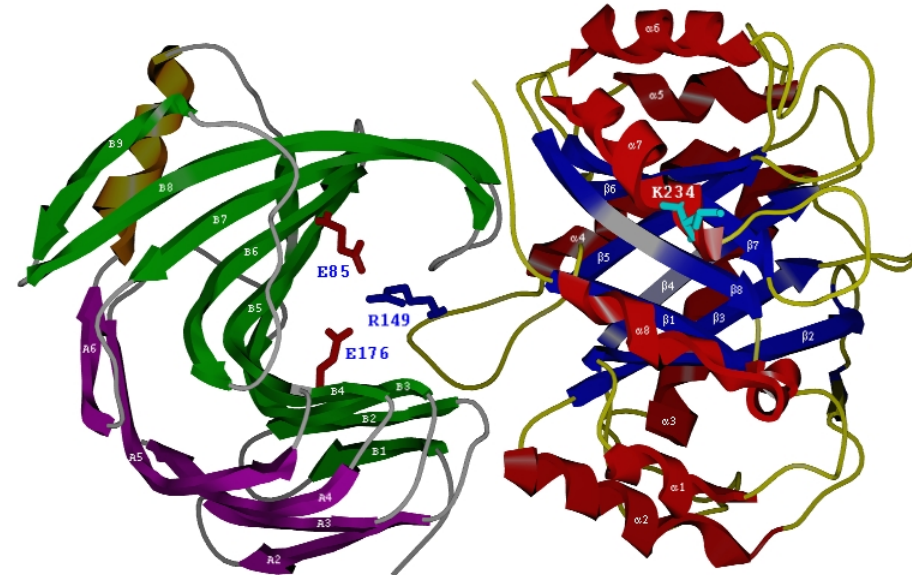
GH11 Xylanases

- ✗ Low MW (20-30 kDa), β -jelly roll
- ✗ True xylanases: exclusively active on xylan
- ✗ Large substrate binding cleft (> 5 subsites)
- ✗ Prefer long oligosaccharides and non substituted region
- ✗ Inhibited by wheat protein inhibitors



GH 11

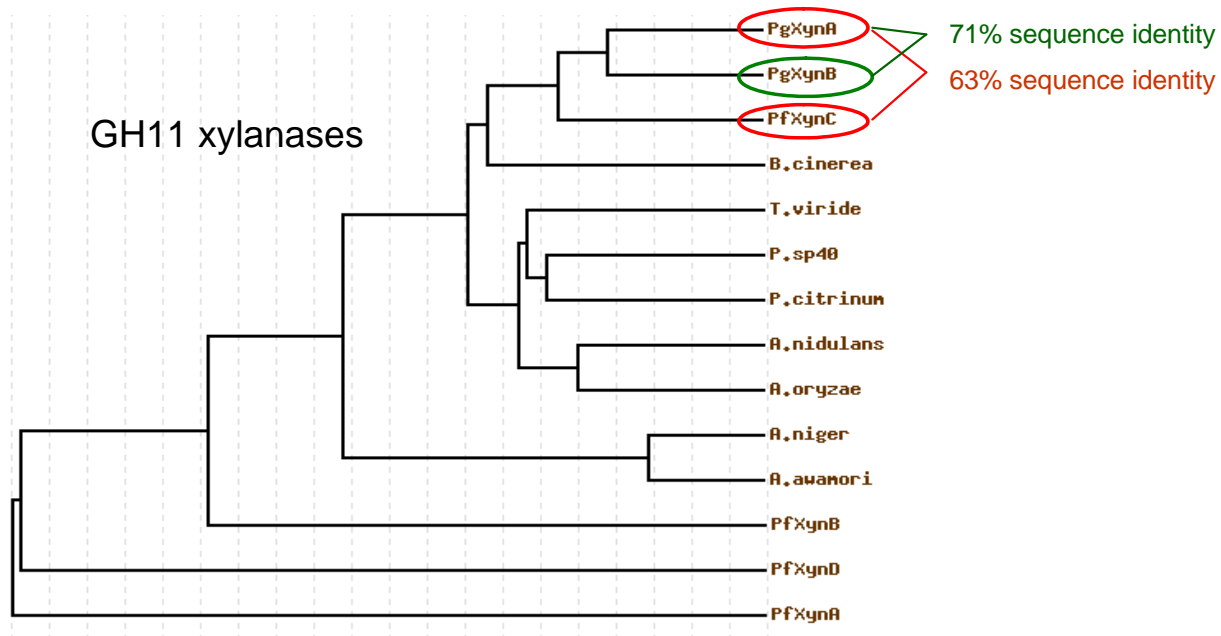
Penicillium funiculosum (PfXynC)



PfXynC / XIP-I

Focus on GH11 xylanases from *Penicillium*

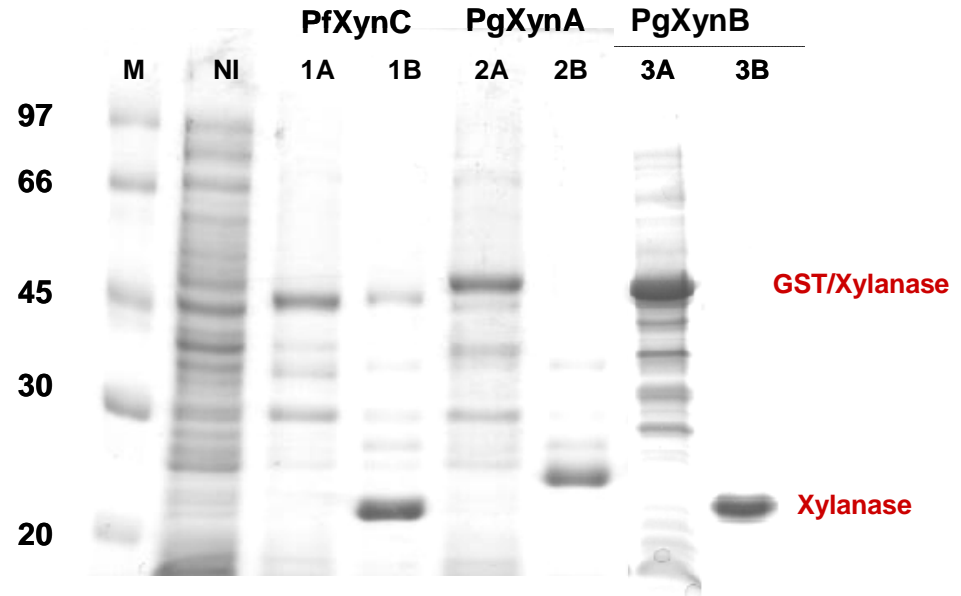
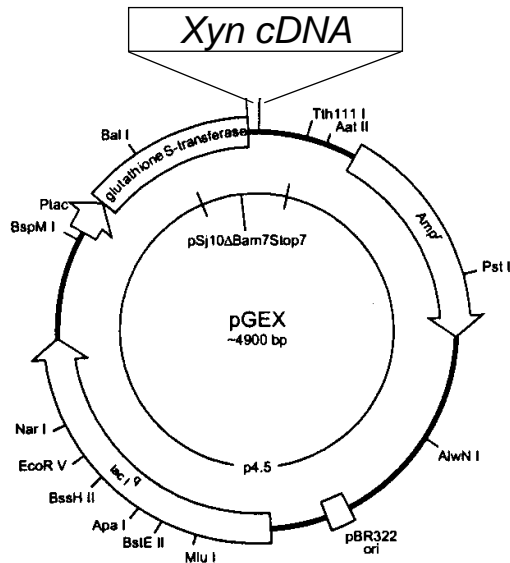
- × *P. funiculosum* xylanase C: **PfXynC** (Furniss et al., 2004)
- × *P. griseofulvum* xylanase A: **PgXynA** (Berrin et al., 2007) (André-Leroux et al., 2008)
- × *P. griseofulvum* xylanase B: **PgXynB**



- Characteristics ?
- Use in applications ?

Expression of *Penicillium* xylanases in *E. coli*

- ✗ *P. funiculosum* xylanase C: **PfXynC**
- ✗ *P. griseofulvum* xylanase A: **PgXynA**
- ✗ *P. griseofulvum* xylanase B: **PgXynB**



- (NI) cell extracts of *E. coli* BL21 cells non induced
- (1A, 2A and 3A) cell extracts of *E. coli* BL21 cells after induction
- (1B, 2B and 3B) thrombin cleaved fusion proteins after purification

- Efficient system of expression
- Purification in one step



Biochemical characterization of *Penicillium* GH11 xylanases

		PfXynC	PgXynA	PgXynB
Mr (Da) (Maldi-Tof) ^a	Experimental	21143	20829	21301
	Theoretical	21145	20831	21300
pI (IEF)	Experimental	4.2	8.6	6.4
	Theoretical	4.4	8.4	6.6
N-terminus sequence^b		GSPEFQS	GSQITQN	N.A.
Optimum T(°C)		50°C	50°C	30°C
Optimum pH		5.5	5.5	4.5

^a Faculté de pharmacie, La Timone, Marseille

^bInstitut Pasteur, Paris

N.A. Not Available

Substrate specificity

Low and Medium Viscosity Wheat Arabinoxylan

		K_m (mg.ml ⁻¹)	V_{max} (U.mg ⁻¹)	k_{cat} (s ⁻¹)	k_{cat}/K_m (ml. s ⁻¹ .mg ⁻¹)
PgXynA	LVAX	3.8 ± 0.7	1589 ± 84	545	143
	MVAX	16.9 ± 3.7	2014 ± 259	691	41
PgXynB	LVAX	69.5 ± 7.4	4402 ± 84	1563	23
	MVAX	N.D.	N.D.	N.D.	N.D.
PfXynC	LVAX	14.4 ± 0.7	8559 ± 250	2939	204
	MVAX	27.2 ± 4.5	10662 ± 550	3646	134

DNS assay method

Reactions carried out in McIlvaine's buffer pH 5.5 at 30°C

^a concentration of arabinoxylan: 10 mg/ml, 1 Unit represents the release of 1 μmol of xylose per min

N.D. Not Determined

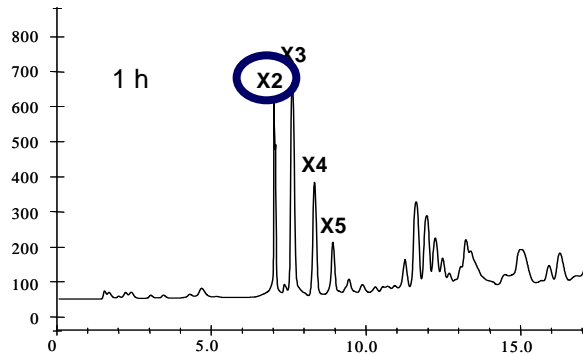
➤ **Activity PfXynC > PgXynA > PgXynB**



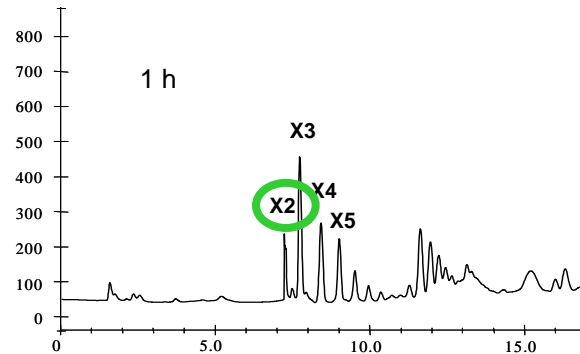
Wheat arabinoxylan hydrolysis

Low Viscosity Wheat Arabinoxylan (Separation by HPAEC-PAD, Dionex)

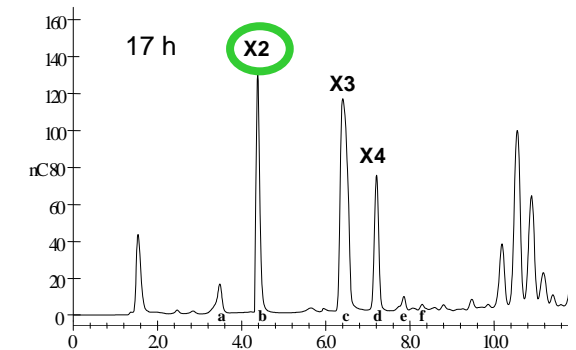
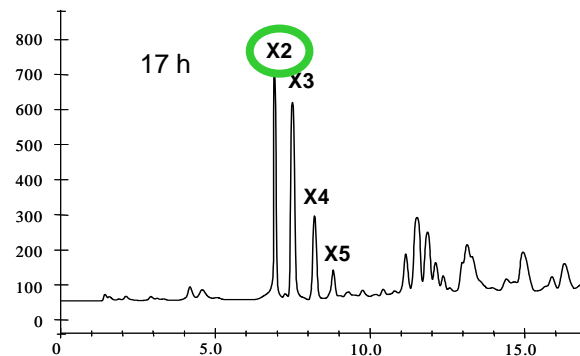
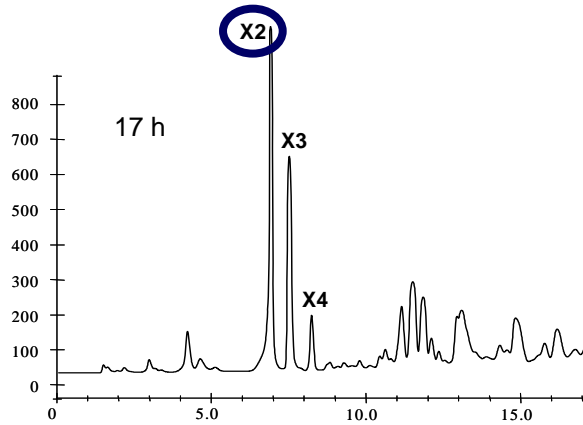
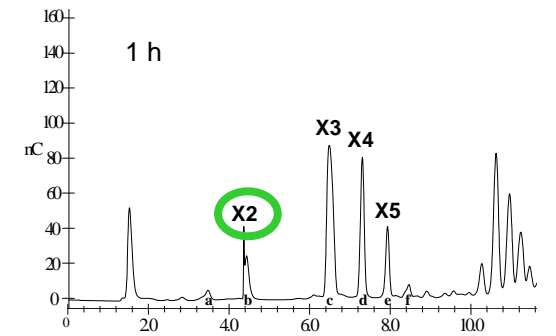
PfXynC



PgXynA



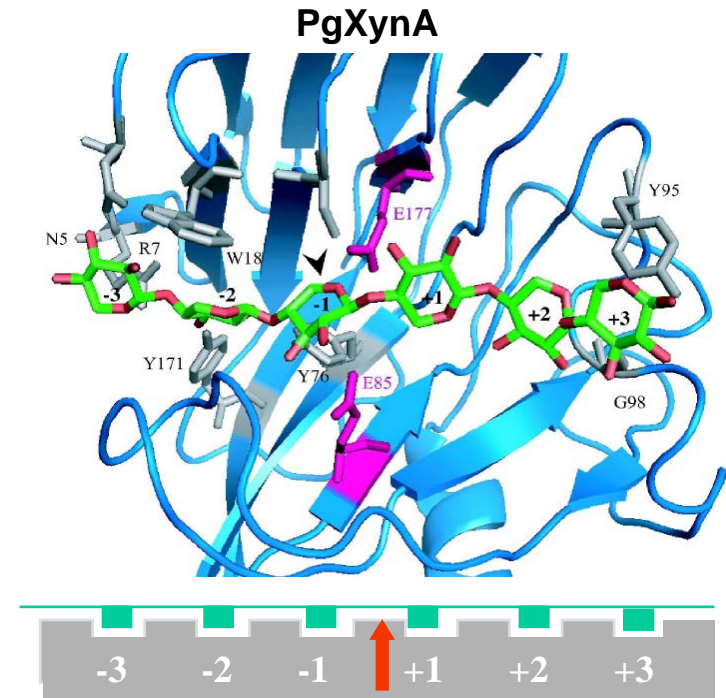
PgXynB



➤ Major end products from LVAX = X2 and X3

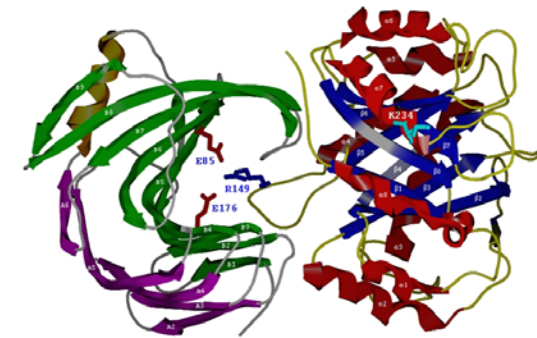
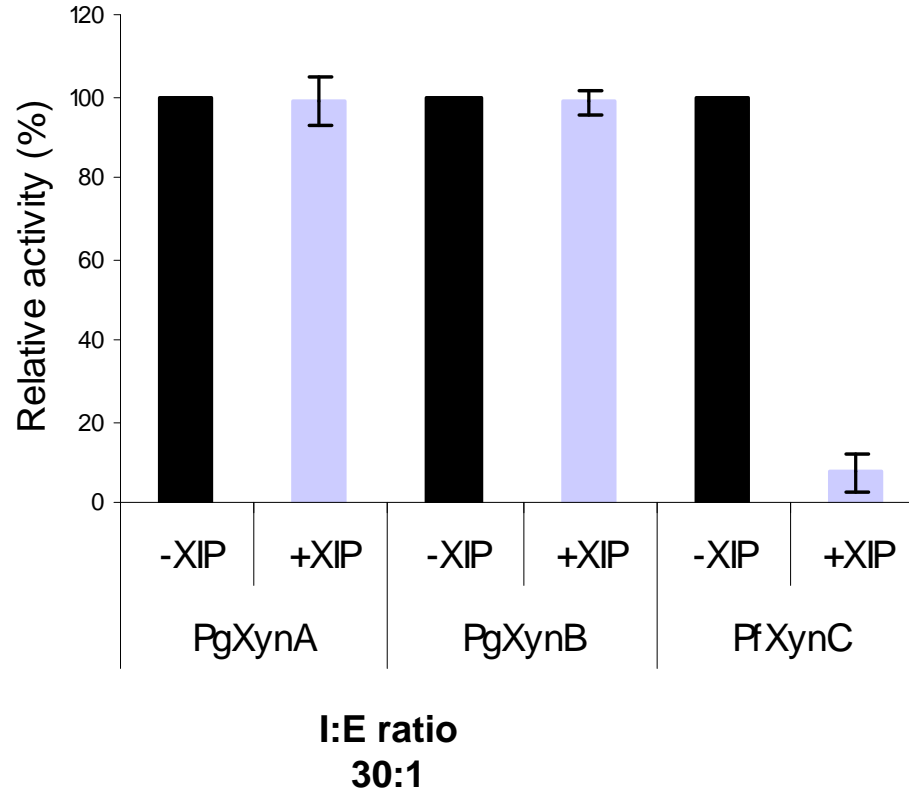
Xylo-oligosaccharides hydrolysis

	k_{cat}/K_m (min ⁻¹ .M ⁻¹)		
	PfXynC	PgXynA	PgXynB
X6	6.49×10^6	2.55×10^6	8.0×10^5
X5	3.06×10^6	4.6×10^5	7.4×10^4
X4	1.83×10^5	3.0×10^4	6.2×10^3
X3	1.52×10^3	1.5×10^2	N.D.



➤ PfXynC / PgXynA / PgXynB = 6 catalytic subsites

Sensitivity of *Penicillium* xylanases to XIP-I

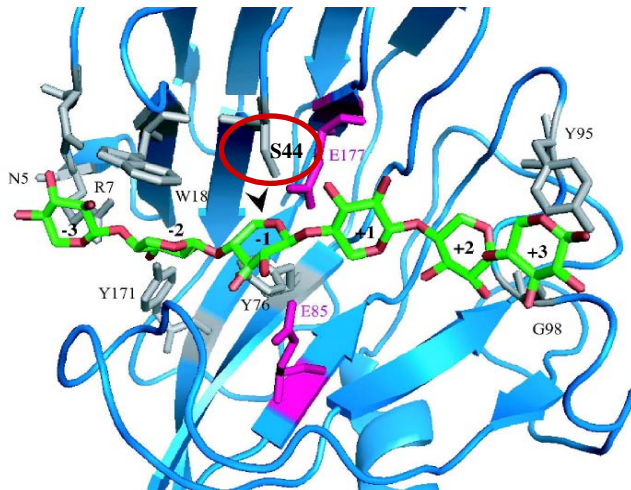


PfXynC / XIP-I
 $K_i = 3.4 \text{ nM}$

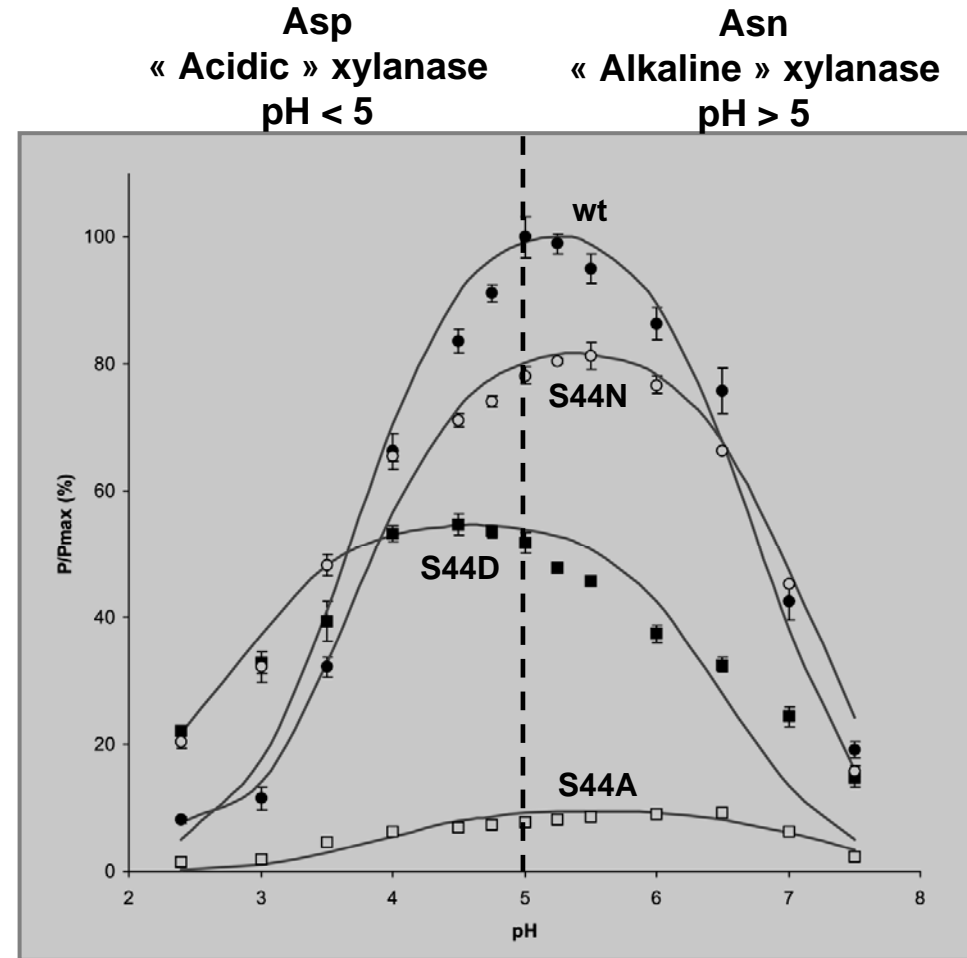
(Payan et al., 2004)
(Furniss et al., 2004)

➤ PgXynA and PgXynB are both resistant to XIP-I inhibition

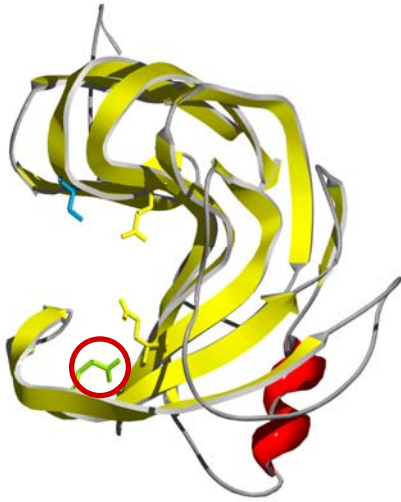
Mutation of Serine 44



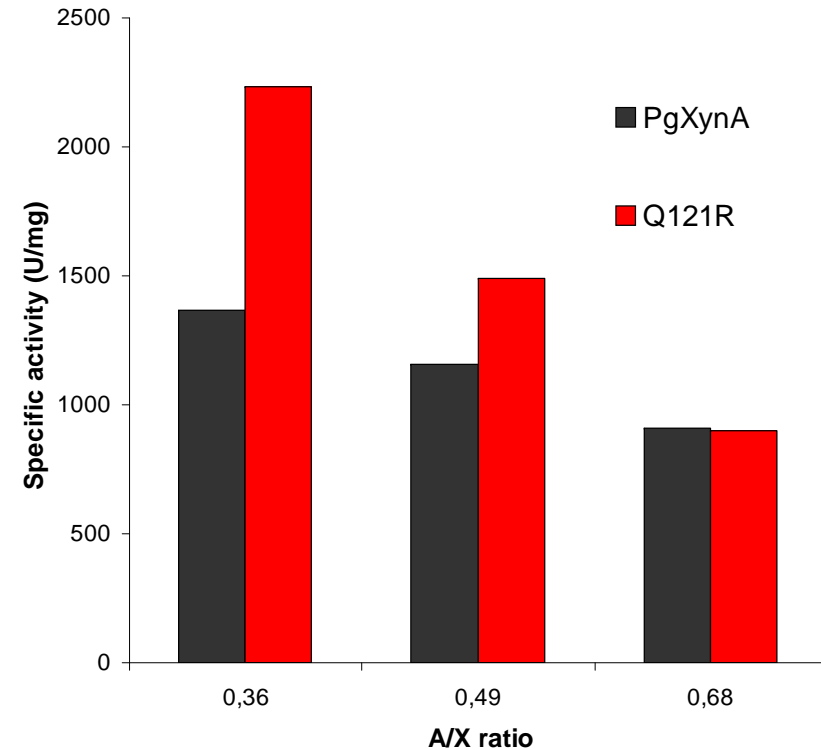
	$K_{m(app)}$ (mg.ml ⁻¹)	k_{cat} (s ⁻¹)	$k_{cat}/K_{m(app)}$ (ml. s ⁻¹ .mg ⁻¹)
wt	3.8 ± 0.7	545 ± 29	143
S44D	4.4 ± 0.7	298 ± 17	66
S44N	3.9 ± 0.4	454 ± 24	116
S44A	4.6 ± 0.8	27 ± 2	6



Mutation of Glutamine 121

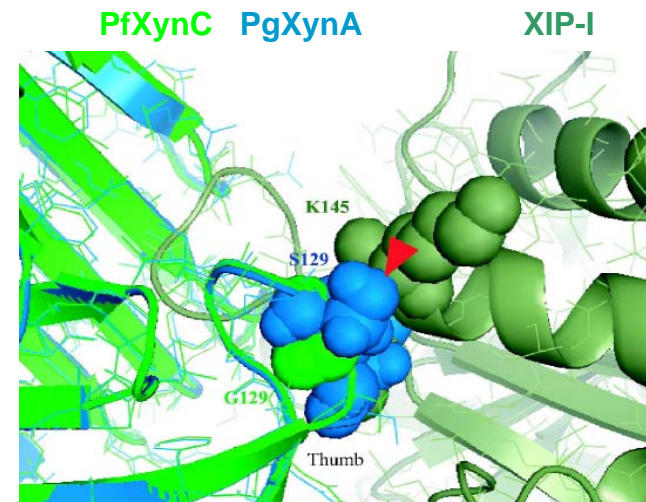
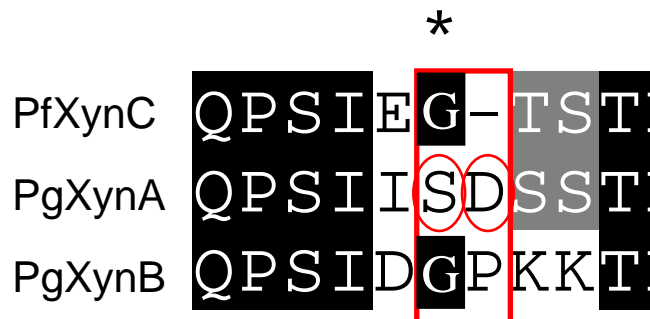


	$K_{m(app)}$ (mg.ml ⁻¹)	k_{cat} (s ⁻¹)	$k_{cat}/K_{m(app)}$ (ml.s ⁻¹ .mg ⁻¹)
wt	3.8 ± 0.7	545 ± 29	143
Q121R	7.7 ± 1.5	1063 ± 86	138



Mutation of « Thumb » residues

	$K_{m(app)}$ (mg.ml ⁻¹)	k_{cat} (s ⁻¹)	$k_{cat}/K_{m(app)}$ (ml. s ⁻¹ .mg ⁻¹)	K_i (nM)
wt	3.8 ± 0.7	545 ± 29	143	N/A
S129G	7.5 ± 0.9	484 ± 28	64	82
ΔD130	5.7 ± 1.0	601 ± 42	105	N/A
S129G/ΔD130	6.4 ± 0.3	457 ± 8	71	3.9





Main conclusions

- **GH11 *P. griseofulvum* xylanases are resistant to XIP-I**
- **PgXynA: unique specificity**
- **Serine 44**
 - ✗ Role in catalysis
 - ✗ Alteration of pH profile
- **Glutamine 121**
 - ✗ Improvement of overall catalytic activity
- **« Thumb » residues**
 - ✗ Modification of inhibitor specificity
 - ✗ Importance of the « thumb » in interaction with XIP-I

Acknowledgments

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