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GHATA*base*

(GlycosylHydrolase And Transglycosylase Activity database)

DATABASE OF MULTIPLE CELL WALL ENZYME ACTIVITIES DETECTED IN CRUDE EXTRACTS OF VARIOUS PLANT SPECIES

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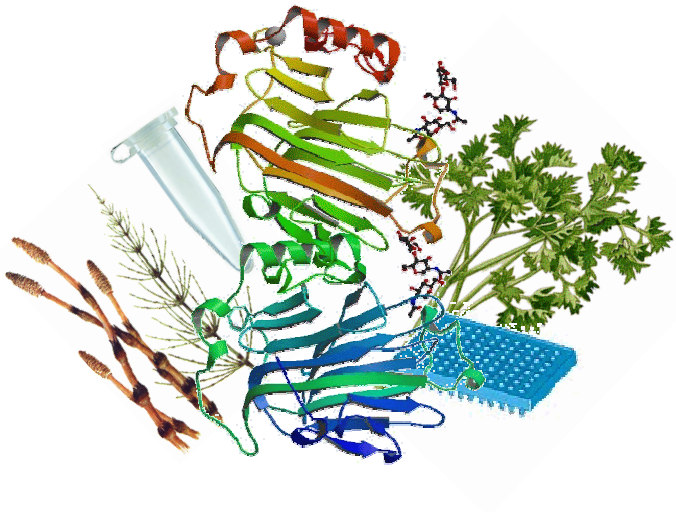
INSTRUCTION MANUAL



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GHATA*base* contains:

- A list of individual enzyme activities for which evidence was obtained in plant protein extracts.
- A hyperlink to a Word file containing scans of 160 TLC plates documenting the presence of various enzymes according to the reaction products generated by their action.
- A code according to which the corresponding TLC scan(s) can be readily found.
- A short description of the experimental conditions under which the analyses were carried out.
- A list of various plant species/organs allowing users to choose the most suitable plant material for preparing extracts with enzyme activity of interest specific to the user.
- Notes and comments about the reaction products.
- More detailed column headers (viewed by hovering over the relevant concise column header)
- Hyperlinks to other enzyme databases (ExPASy, BRENDA and UniProtKB).

The database was created as a simple list in Excel, allowing users to compare the different categories, to choose the row(s) of interest and to extract the necessary information from the spreadsheet.

Abbreviations

(a) Key to code

A code is printed above each TLC scan (e.g. SCX622A, NDXO40C, MDL22E) to describe the specific experimental conditions. This code comprises the following parts, listed in this order:

Extractant

M = 1 M NaCl in 0.2 M MES, pH 5.5

N = 1 M NaCl in 0.2 M succinate, pH 5.5

S = 0.2 M succinate, pH 5.5

Extract

C = crude extract (non-dialysed)

D = crude extract, dialysed against 0.04 M succinate

Substrate

A6 = α-(1→5)-arabinohexaose

A8 = α-(1→5)-arabino-octaose

AX = arabinoxylan

FG = [*Fuc*-^{3}H]XXFG (xyloglucan-derived nonasaccharide, ^{3}H-labelled in the fucose residue)

L = control (no substrate, enzyme extract only)

M6 = β-(1→4)-mannohexaose

X6 = β-(1→4)-xylohexaose

XO = xyloglucan oligosaccharide mixture (mainly XLLG > XXLG > XXXG)

XX = XXXG (xyloglucan-derived heptasaccharide)

Assay conditions

22 = incubation for 24 h at 22°C

40 = incubation for 3.5 h at 40°C

A, B, C, D, E = arbitrary code for particular sub-sections of our enzyme collection.

(b) Abbreviations used in the text of the Excel file and the Word document with TLC scans

+ = intensity of band detected on TLC (+ = least intense, ++++++++++ = most intense)

– = enzyme activity undetectable

A2-A14 = arabino-oligosaccharides of DP 2–14 (e.g., A7 = arabinoheptaose)

Ara6 = arabinohexaose

Ara8 = arabino-octaose

BAW = butan-1-ol/acetic acid/water (2:1:1 by vol.) solvent system

DP = degree of polymerisation

DP? = product with an uncertain or estimated degree of polymerisation

EPEW = ethyl acetate/pyridine/ethanol/water (6:3:1:1 by vol.) solvent system

F = α-Fuc-(1→2)-β-Gal-(1→2)-α-Xyl-(1→6)-β-Glc (as a component of a xyloglucan oligosaccharide)

G = β-Glc (as a component of a xyloglucan oligosaccharide)

Gal = galactose

GH = glycosyl hydrolase activity

Glc = glucose

L = β-Gal-(1→2)-α-Xyl-(1→6)-β-Glc (as a component of a xyloglucan oligosaccharide)

M2-M6 = manno-oligosaccharides of DP 2–6

Man = mannose

Man6 = mannohexaose

NaCl = enzyme extract prepared in the high-salt extractant 'B or C'

Succ = enzyme extract prepared in the succinate-buffered extractant 'A'

TG = transglycosylation activity

X = α-Xyl-(1→6)-β-Glc (isoprimeverose) (as a component of a xyloglucan oligosaccharide)

X2–X11 = xylo-oligosaccharides of DP 2–11.

XG = xyloglucan-derived trisaccharide [α-Xyl-(1→6)-β-Glc-(1→4)-Glc]

XGOs = xyloglucan oligosaccharide mixture (mainly XLLG > XXLG > XXXG)

XLLG = xyloglucan-derived nonasaccharide (see individual abbreviations for G, L and X)

XXFG = xyloglucan-derived nonasaccharide (see individual abbreviations for G, F and X)

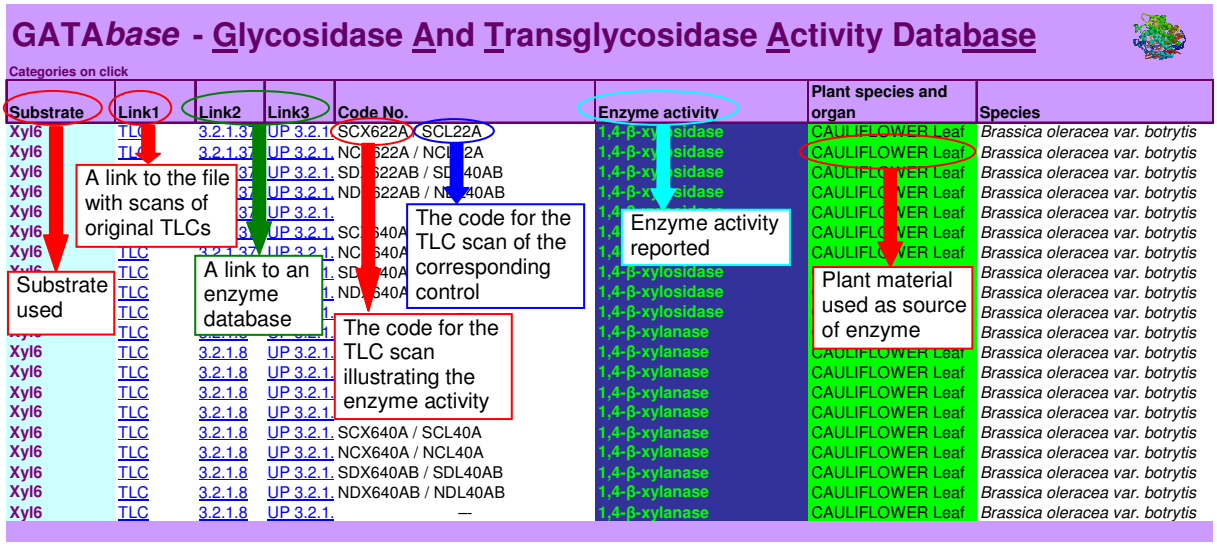
XXLG = xyloglucan-derived octasaccharide (see individual abbreviations for G, L and X)

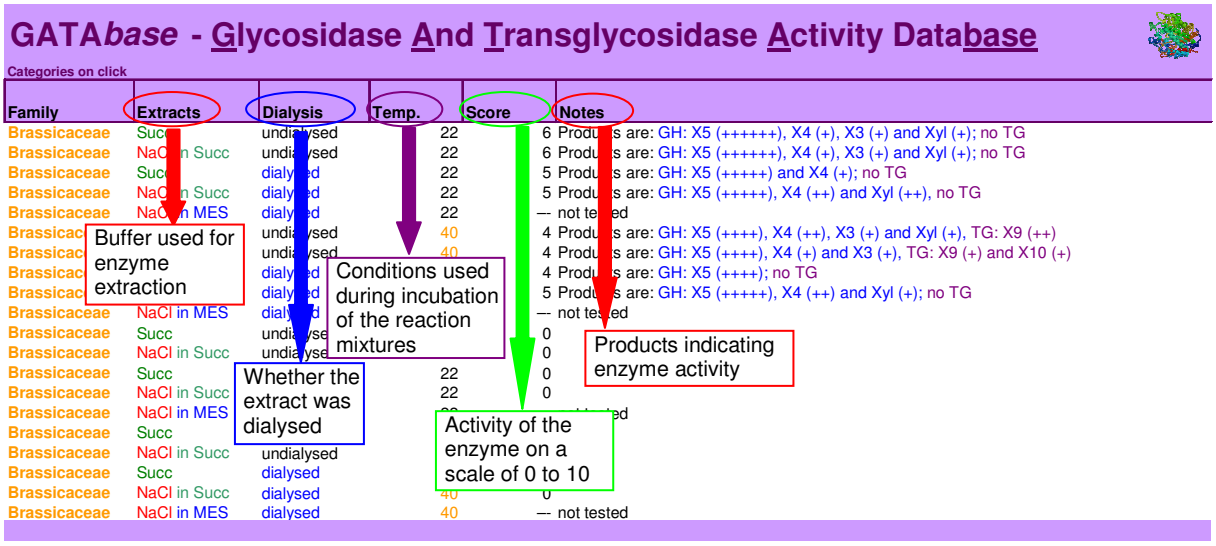
XXXG = xyloglucan-derived heptasaccharide (see individual abbreviations for G and X)

Xyl = xylose

Xyl6 = xylohexaose

Categories listed in the Excel spreadsheet





Explanation of the semi-quantitative procedure for reporting enzyme activities

General approach

(1) The intensity of the spots was semi-quantified as + (faint) to ++++++++++ (intense) or – (absent), always compared with the substrate-free control.

(2) Reported enzyme activity is based on scale of 0 to 10.

(3) GH (glycosylhydrolase activity) is reported as exo- and endo-activities separately when possible.

(4) TG (transglycosylase activity) is reported when products were found with DP greater than that of the substrate.

(5) The final activity (scale range 0–10) was estimated mainly according to the intensity of the product bands, usually judged by the product with the highest number of pluses. For example, when Man6 was the substrate for GH activity, and the products were scored as M5 (++++), M4 (++) and M3 (+), an activity score of '4' was returned.

(6) In addition, however, the decrease in intensity of the substrate band was considered. For example, when Man6 was the substrate for GH activity, and the products were scored as M5 (+), M4 (+++), M3 (+++++), M2 (+++) and Man (++++), and the remaining substrate (M6) was scored as (–), then an activity score of '10' was returned.

Specific rules for scoring individual activities

The following account provides general comments on how the four classes of activity (glycosidase, glycanase, transglycosidase and transglycanase; see Fig. 1a–d) that potentially act on a given substrate were identified, followed by a selected representative pattern that would receive a particular score. It is not possible to describe here all possible permutations of band intensities.

Substrate: Man6

1,4-β-Mannosidase activity on Man6 would first produce M5, later M4, later M3, etc. Thus a high yield of M3 and M4 could represent either mannanase activity or high activity of a mannosidase that requires a substrate of DP ≥ 3. 1,4-β-Mannanase evidence requires (M3 or M4)≥M5, and M6>M5.

Representative patterns:

1,4-β-Mannosidase activity = 4 if	M6≥80% of M6 detected at time 0 M5=(++++) M4≥(++) M3≥(+) M2≤(+) *or* M6≥80% of M6 detected at time 0 M5=(+++++) M4=(+++)
1,4-β-Mannanase activity = 4 if	M6≥85% of M6 detected at time 0 M5≤(++) M4=(+++) M3≥(++) but ≤(++++) M2=(+++) Man=(–)

Substrate: Xyl6

1,4-β-Xylanase evidence requires Xyl3>Xyl5, and Xyl6>Xyl5; or (Xyl3 and Xyl4)≥Xyl5, and Xyl6≥Xyl5. Transglycosylation reactions were indicated by products larger than X6. The intensity of the X7 band in the sample was corrected for that present as a contaminant in the substrate (contaminating X7 in most X6 samples received would have been scored as (++)).The intensity of other TG products (such as X8, X9 and X10) is also mentioned in the database notes, but was not considered in scoring the trans-1,4-β-xylosidase activity.

Representative patterns:

β-1,4-Xylanase activity = 4 if	X4≤(++++) X3=(++++)
β-1,4-Xylosidase activity = 4 if	X6≤90% but >85% of X6 detected at time 0 X5=(++++)
Trans-1,4-β-xylosidase activity = 4 if	X7=(++++)
Trans-1,4-β-xylanase activity = 4 if	X9=(++++)

Substrate: Ara6

Representative patterns:

1,5-α-Arabinofuranosidase activity = 4 if	A5=(++++) A4≤(+++) Ara≤(++)
Trans-α-arabinosidase activity = 4 if	A7=(++++) [DP7 is an estimate] A10≤(++++) [DP10 is an estimate]

Substrate: Ara8

1,5-α-Arabinanase activity recorded only if (A7 and A6)≤(A5 and A4), and A8>A7. Trans-α-arabinosidase activity was recorded if A9 (either of the two isomers) was detected. Trans-α-arabinanase activity was recorded A11 (either of the two isomers) was detected.

Representative patterns:

Trans-α-arabinosidase activity = 4 if	A9=(++++) [DP9 is an estimate]
Trans-α-arabinanase activity = 4 if	A11=(++++) [DP11 is an estimate]

Substrate: AX (arabinoxylan)

AX-active α-arabinofuranosidase activity was estimated according to the intensity of free Ara; 1,4-β-Xylosidase activity was estimated according to the intensity of free Xyl.

Representative patterns:

AX-active α-arabinofuranosidase = 4 if	Ara=(++++)
1,4-β-Xylosidase activity = 4 if	Xyl=(++++)
1,4-β-Xylanase activity = 4 if	AX of DP>15?=(+++) X4-X8=(+)

Substrate: XXXG

XGO-active α-xylosidase activity was estimated according to the intensity of free Xyl. XGO-active β-glucosidase activity was estimated according to the intensity of free Glc (note that a β-Glc-(1→4)-β-Glc bond in XXXG can be cleaved only after removal of Xyl by the action of α-xylosidase). The intensity of other probable TG products (such as DP6?a, DP6?b, DP5? and DP4) is also mentioned in the database notes, but was not considered in scoring the activity. The intensity of the XXG band in the sample was corrected for that present as a contaminant in the commercial substrate (the contaminating XXG would have been scored as (++)).

Representative patterns:

XGO-active α-xylosidase activity = 4 if	Xyl=(++++)
XGO-active β-glucosidase activity = 4 if	Glc=(++++)
XGO-active trans-α-xylosidase activity = 4 if	DP8=(+++) DP9=(+++) DP10≤(+) *or* DP8=(++++) or (+++++) DP9=(++)

Substrate: XGOs

XGO-active β-galactosidase activity was estimated principally according to the decrease in intensity of the major substrate, XLLG. However, the intensity of free Gal was also considered when possible. Both XXXG and XXLG can be formed from the nonasaccharide XLLG by β-galactosidase action. Thus, XGO-active α-xylosidase could not be estimated according to the disappearance of these substrates but only by the formation of free Xyl.

Representative patterns:

β-Galactosidase activity = 4 if	XLLG = 60–70% of XLLG detected at time 0
α-Xylosidase activity = 4 if	Xyl=(++++)

Substrate: XXFG

XGO-active α-fucosidase activity was expressed as % hydrolysis of [*Fuc*-^{3}H]XXFG to [^{3}H]fucose. XGO-active α-xylosidase activity accompanying the α-fucosidase activity was assayed by TLC and estimated according to the intensity of products formed by α-xylosidase + β-glucosidase (i.e. XFG, and FG). As the radioactive assays employ low concentration of substrate, free Xyl would not be detected by the thymol stain.

Representative patterns:

XGO-active α-fucosidase activity = 4 if	free [^{3}H]fucose = 35–44%
XGO-active α-xylosidase = 4 if	[*Fuc*-^{3}H]XFG=(++++) [*Fuc*-^{3}H]FG≤(+)