Characterization of the two *Neurospora crassa* cellobiose dehydrogenases and their connection to oxidative cellulose degradation

Supplementary Material

Christoph Sygmund,[†] Daniel Kracher, [†] Stefan Scheiblbrandner, Kawah Zahma, Alfons K. G. Felice, Wolfgang Harreither, Roman Kittl and Roland Ludwig[*]

Food Biotechnology Laboratory, Department of Food Sciences and –Technology, BOKU – University of Natural Resources and Life Sciences, Muthgasse 18, A-1090 Vienna, Austria

[†]C.S and D.K. contributed equally

*Corresponding author. Mailing address: Department für Lebensmittelwissenschaften und -technologie, Universität für Bodenkultur, Muthgasse 18/2, A-1190 Wien, Austria. Phone: +431 47654 6149. Fax: +431 47654 6199 E-mail: roland.ludwig@boku.ac.at

Table S1. Purification of recombinant CDH IIA and CDH IIB produced by *P. pastoris*.

	Purification step	Total activity	Spec. Act. [U mg^{-1}]		Ratio cyt *c*/DCIP	Purification [c] [fold]	Yield [%]
		Cyt *c* [a] [U]	Cyt *c* [a]	DCIP [b]			
CDH IIA	Crude extract	1700	1.3	6.1	0.21	1	100
	Phenyl-Sepharose	1410	7.8	20	0.39	6.0	83
	DEAE-Sepharose	1250	8.3	21.2	0.39	6.4	73
CDH IIB	Crude extract	590	0.20	0.63	0.32	1	100
	Phenyl-Sepharose	255	0.41	0.79	0.52	2.1	43
	Q Source	140	1.1	2.1	0.52	5.5	24
	Gel filtration	65	3.0	5.1	0.59	15	11

[a] Enzymatic activity was measured at pH 6.0

[b] Enzymatic activity was measured at pH 5.0

[c] The purification factor is calculated from the specific activity obtained with the cyt *c* assay, which is specific for CDH.

TABLE S2. Substrate specificities of CDH IIA and CDH IIB for various carbohydrates (final concentration 100 mM) measured with the indicated electron acceptors at pH 5.0 (DCIP) or pH 6.0 (cyt *c*, 1,4-benzoquinone. Relative activities (%) are compared to the activity obtained with cellobiose.

Substrate	**CDH IIA**			**CDH IIB**		
	DCIP	**Cyt *c***	**1,4-Benzo-quinone**	**DCIP**	**Cyt *c***	**1,4-Benzo-quinone**
Cellobiose	100	100	100	100	100	100
Lactose	121	91	94	79	122	108
Maltotriose	13.3	28.4	6.6	23.1	27.3	8.8
Maltose	2	4.5	2.1	12.8	19.2	7.0
Glucose	6	12.6	3.8	42.7	52.5	25.6
Galactose	3	7	1.6	5.5	11.8	3.4
Xylose	1	1.3	1.3	2.5	5.7	2.1
Mannose	1.3	1.5	1.1	6.8	10	3.7

TABLE S3. Substrate inhibition values of *N. crassa* CDH IIA and CDH IIB for cellobiose and lactose in the presence of 300 µM DCIP as electron acceptor for pH values between 4.0 – 7.0.

		CDH IIA	**CDH IIB**
pH	**Carbohydrate**	**K_i [mM]**	**K_i [mM]**
4.0	Cellobiose	340±27	150±12
	Lactose	900±65	210±13
5.0	Cellobiose	250±16	240±17
	Lactose	600±38	420±24
6.0	Cellobiose	320±19	150±11
	Lactose	850±44	210±12
7.0	Cellobiose	700±26	120±10
	Lactose	580±21	150±9

```
1     ATG AAG ACT GGT TCC ATC TTG GCT GCT TTG GTT GCT TCT GCT TCC   45
1     Met Lys Thr Gly Ser Ile Leu Ala Ala Leu Val Ala Ser Ala Ser   15

46    GCT CAC ACT ATC TTC CAA AAG GTT TCT GTT AAC GGT GCT GAC CAG   90
16    Ala His Thr Ile Phe Gln Lys Val Ser Val Asn Gly Ala Asp Gln   30

91    GGT CAG TTG AAG GGT ATT AGG GCT CCA GCT AAC AAC AAC CCA GTT   135
31    Gly Gln Leu Lys Gly Ile Arg Ala Pro Ala Asn Asn Asn Pro Val   45

136   ACT GAC GTT ATG TCC TCC GAC ATC ATC TGT AAC GCT GTT ACT ATG   180
46    Thr Asp Val Met Ser Ser Asp Ile Ile Cys Asn Ala Val Thr Met   60

181   AAG GAC TCC AAC GTT TTG ACT GTT CCA GCT GGT GCT AAG GTT GGT   225
61    Lys Asp Ser Asn Val Leu Thr Val Pro Ala Gly Ala Lys Val Gly   75

226   CAT TTT TGG GGT CAC GAA ATT GGT GGT GCT GCT GGT CCA AAC GAT   270
76    His Phe Trp Gly His Glu Ile Gly Gly Ala Ala Gly Pro Asn Asp   90

271   GCT GAT AAT CCA ATT GCT GCT TCC CAC AAG GGT CCA ATC ATG GTT   315
91    Ala Asp Asn Pro Ile Ala Ala Ser His Lys Gly Pro Ile Met Val   105

316   TAC TTG GCT AAA GTT GAC AAC GCT GCT ACT ACT GGT ACT TCC GGT   360
106   Tyr Leu Ala Lys Val Asp Asn Ala Ala Thr Thr Gly Thr Ser Gly   120

361   TTG AAG TGG TTC AAG GTT GCT GAA GCT GGT TTG TCC AAC GGA AAG   405
121   Leu Lys Trp Phe Lys Val Ala Glu Ala Gly Leu Ser Asn Gly Lys   135

406   TGG GCT GTT GAT GAC TTG ATC GCT AAC AAC GGT TGG TCC TAC TTC   450
136   Trp Ala Val Asp Asp Leu Ile Ala Asn Asn Gly Trp Ser Tyr Phe   150

451   GAC ATG CCA ACT TGT ATT GCT CCA GGT CAG TAC TTG ATG AGA GCT   495
151   Asp Met Pro Thr Cys Ile Ala Pro Gly Gln Tyr Leu Met Arg Ala   165

496   GAG TTG ATC GCT TTG CAC AAC GCT GGT TCT CAG GCT GGT GCT CAA   540
166   Glu Leu Ile Ala Leu His Asn Ala Gly Ser Gln Ala Gly Ala Gln   180

541   TTC TAC ATT GGT TGT GCT CAG ATC AAC GTT ACT GGT GGT GGT TCT   585
181   Phe Tyr Ile Gly Cys Ala Gln Ile Asn Val Thr Gly Gly Gly Ser   195

586   GCT TCT CCA TCC AAC ACT GTT TCT TTC CCT GGT GCT TAC TCT GCT   630
196   Ala Ser Pro Ser Asn Thr Val Ser Phe Pro Gly Ala Tyr Ser Ala   210

631   TCT GAC CCA GGT ATC TTG ATC AAC ATC TAC GGT GGT TCC GGT AAG   675
211   Ser Asp Pro Gly Ile Leu Ile Asn Ile Tyr Gly Gly Ser Gly Lys   225

676   ACT GAC AAC GGT GGT AAG CCA TAC CAA ATT CCA GGT CCA GCT TTG   720
226   Thr Asp Asn Gly Gly Lys Pro Tyr Gln Ile Pro Gly Pro Ala Leu   240

721   TTC ACT TGT CCT GCT GGT GGT TCA GGT GGA TCT TCT CCA GCT CCT   765
241   Phe Thr Cys Pro Ala Gly Gly Ser Gly Gly Ser Ser Pro Ala Pro   255

766   GCT ACA ACT GCT TCT ACT CCA AAG CCA ACT TCC GCT TCT GCT CCT   810
256   Ala Thr Thr Ala Ser Thr Pro Lys Pro Thr Ser Ala Ser Ala Pro   270

811   AAG CCT GTT TCT ACT ACT GCT TCC ACA CCT AAG CCT ACA AAC GGT   855
271   Lys Pro Val Ser Thr Thr Ala Ser Thr Pro Lys Pro Thr Asn Gly   285

856   TCT GGT TCT GGT ACA GGT GCT GCT CAC TCT ACT AAG TGT GGT GGA   900
286   Ser Gly Ser Gly Thr Gly Ala Ala His Ser Thr Lys Cys Gly Gly   300

901   TCT AAG CCA GCT GCT ACA ACA AAG GCT TCT AAC CCA CAG CCT ACT   945
301   Ser Lys Pro Ala Ala Thr Thr Lys Ala Ser Asn Pro Gln Pro Thr   315

946   AAT GGT GGT AAC TCC GCT GTT AGA GCT GCT GCT TTG TAC GGT CAA   990
316   Asn Gly Gly Asn Ser Ala Val Arg Ala Ala Ala Leu Tyr Gly Gln   330

991   TGT GGT GGT AAA GGT TGG ACT GGT CCA ACT TCT TGT GCT TCC GGT   1035
331   Cys Gly Gly Lys Gly Trp Thr Gly Pro Thr Ser Cys Ala Ser Gly   345

1036  ACT TGT AAG TTC TCC AAC GAC TGG TAC TCC CAG TGT TTG CCA TAA   1080
346   Thr Cys Lys Phe Ser Asn Asp Trp Tyr Ser Gln Cys Leu Pro End
```

FIG. S1. Codon optimized nucleotide sequence and translated polypeptide of *N. crassa* GH61-3 NCU02916 for heterologous expression in *P. pastoris*.

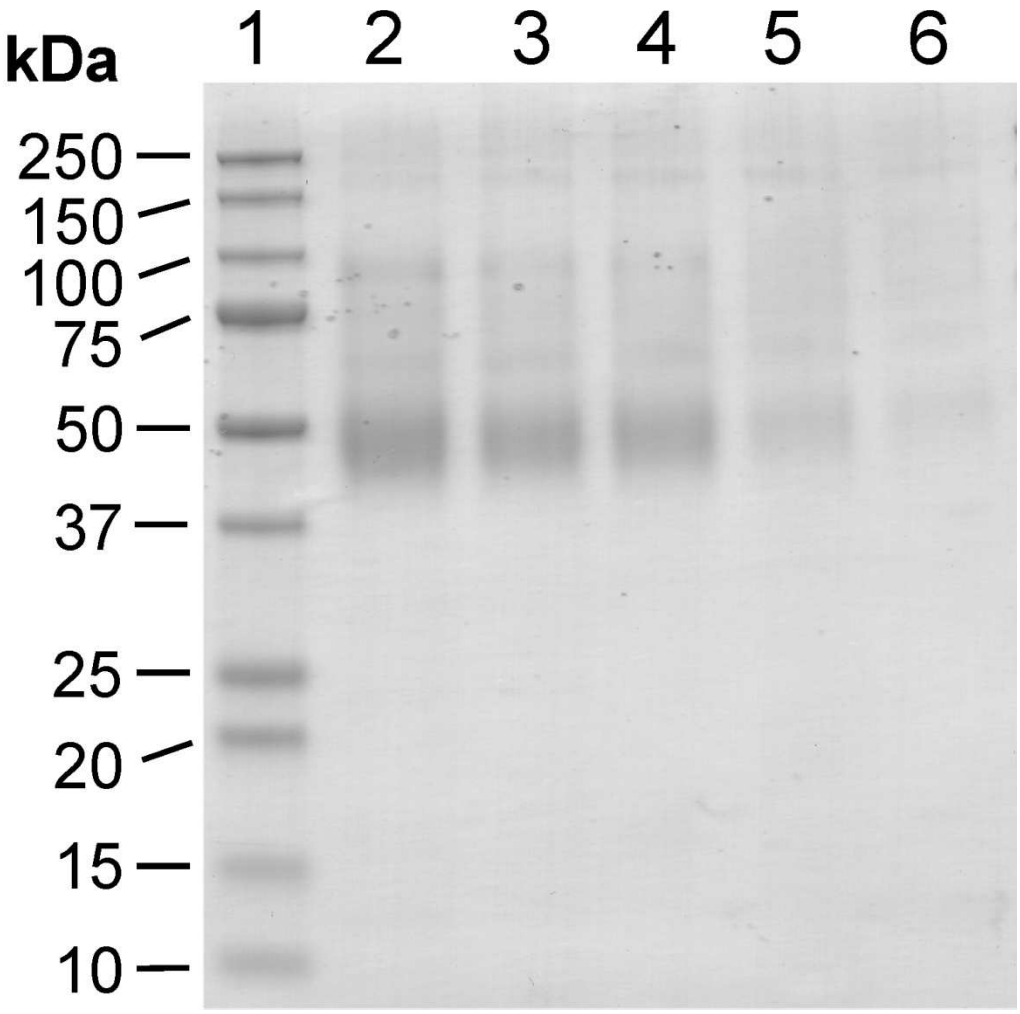

FIG. S2. Supernatant of fermentation samples of *N. crassa* GH61-3 NCU02916 applied on an SDS-PAGE and developed with Coomassie blue (Precision Plus protein standard, lane 1). Samples were taken 24 h (lane 6), 30 h (lane 5), 48 h (lane 4), 55 h (lane 3) and 73 h (lane 2) after induction with methanol. The production of GH61-3 NCU02916 is shown by the formation of a strong band at approx. 50 kDa.

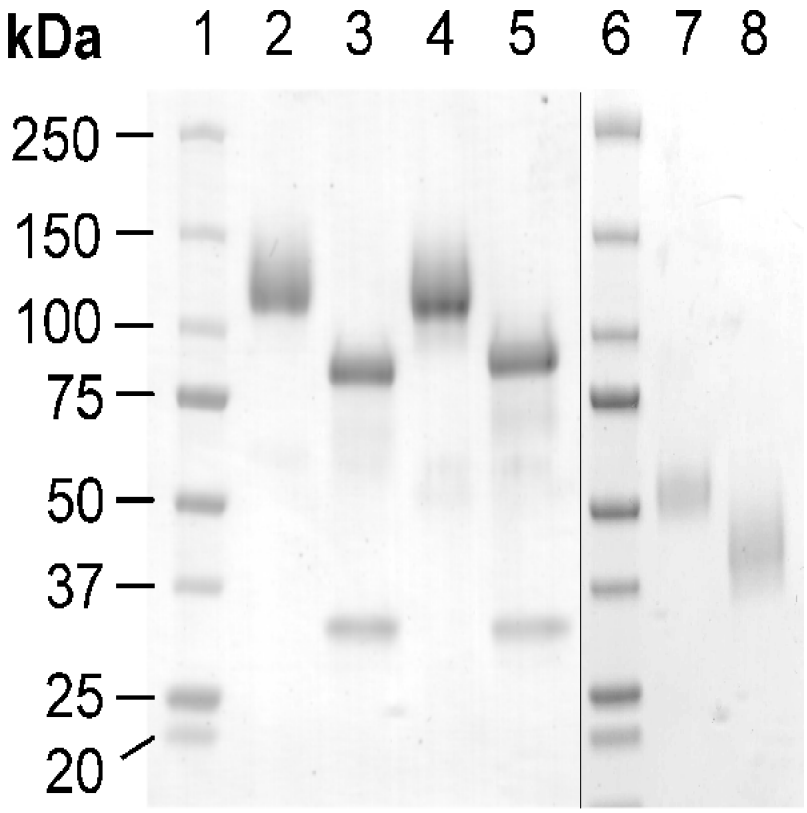

FIG. S3. SDS-PAGE: lane 1, molecular mass marker (Precision Plus Protein, Biorad); lane 2, purified CDH IIA; lane 3, deglycosylated CDH IIA; lane 4, CDH IIB; lane 5, deglycosylated CDH IIB; lane 6, molecular mass marker; lane 7, purified GH61-3; lane 8, partially deglycosylated GH61-3.

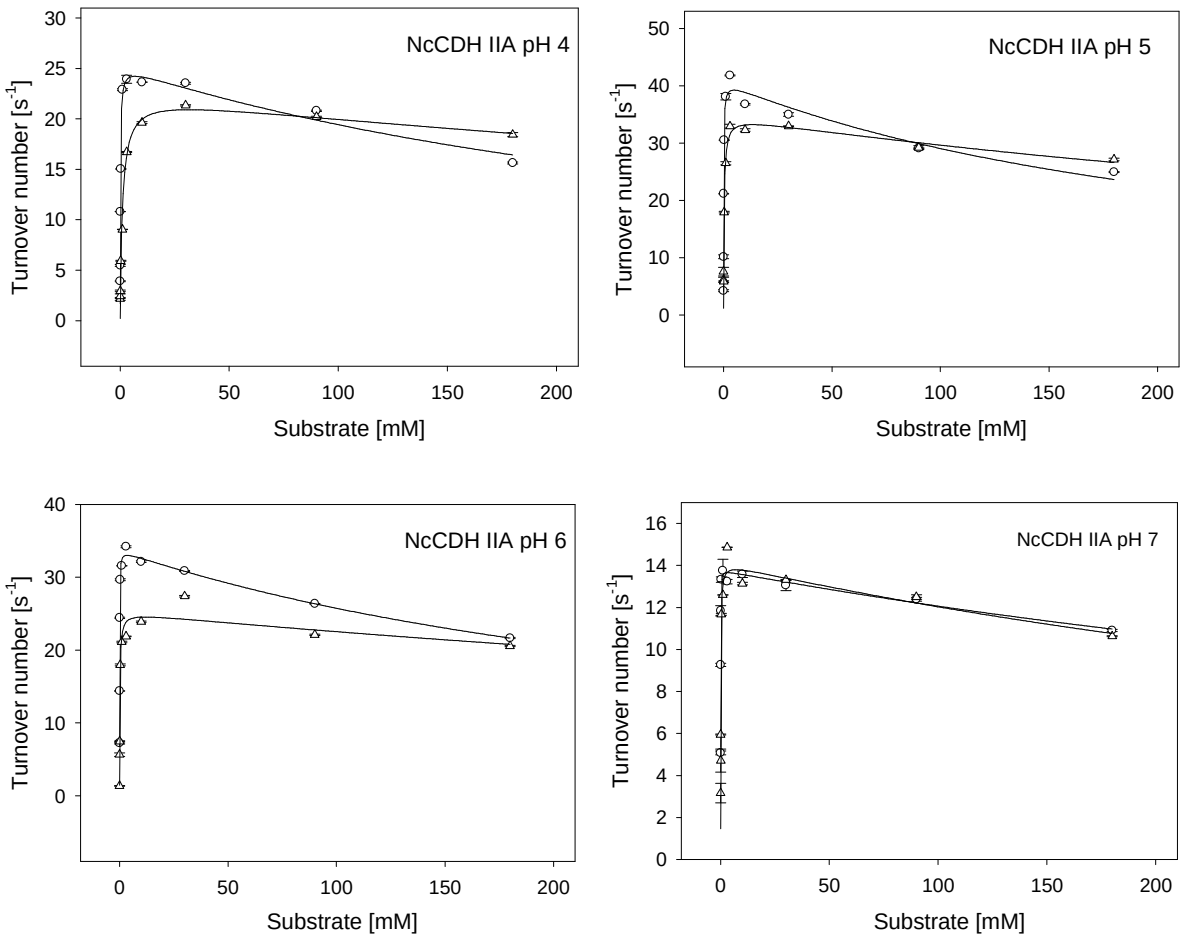

FIG. S4. Inhibition of *N. crassa* CDH IIA by cellobiose (triangles) and lactose (circles) in the presence of DCIP as electron acceptor at pH values between 4.0 – 7.0.

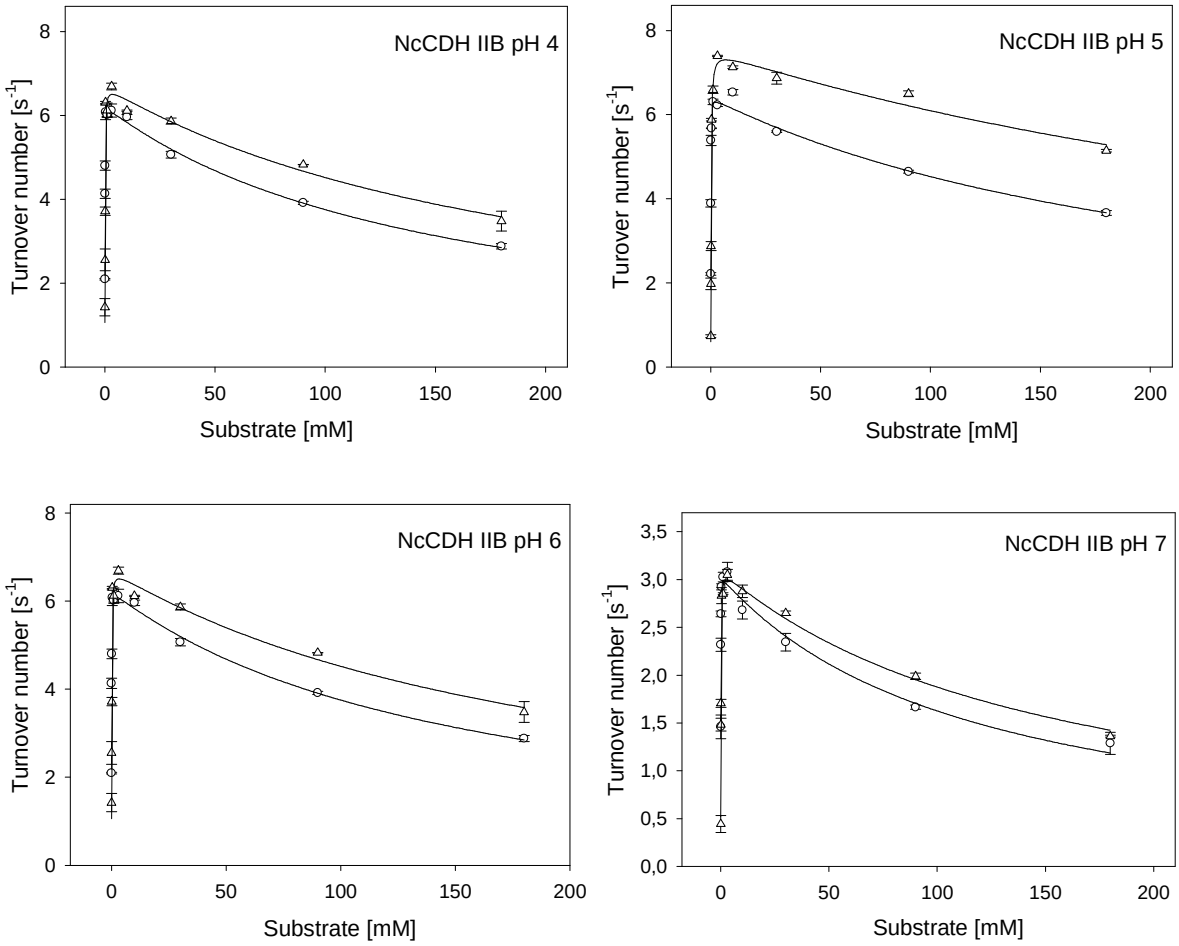

FIG. S5. Inhibition of *N. crassa* CDH IIB by cellobiose (triangles) and lactose (circles) in the presence of DCIP as electron acceptor at pH values between 4.0 – 7.0.

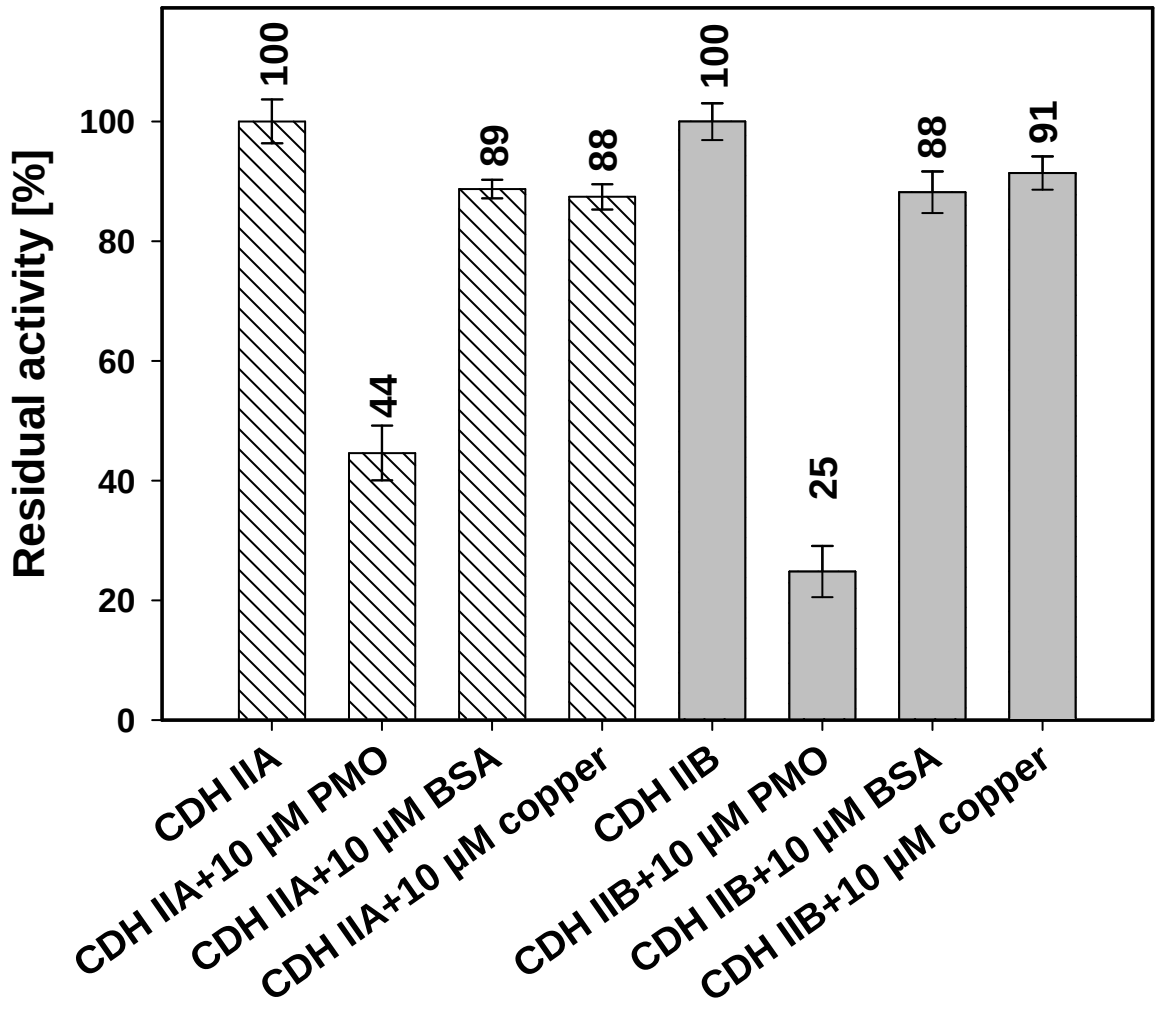

FIG. S6. Inhibition of the cyt *c* reducing activity of CDH IIA and CDH IIB at pH 6.0 by 10 µM GH61-3, 10 µM BSA and 10 µM copper sulfate. The mean value of three measurements is given.