



FIG S1 Mass loss from enzymatic digestions of *N. gaditana* cell walls. All values are means ($n = 3$) \pm 1 SD. NE = no enzyme; CHI = chitinase, CE1 = cellulase #1; CE2 = cellulase #2; LYT = lyticase; CHS = chitosanase; SUL = sulfatase; LYS = lysozyme; ZYM = zymolyase; PRO = protease. Controls used heat denatured enzyme.

N_gaditana_JU964548	1	YDAGDHVKFNFPMAWSATTTLAWGNHEFYDAWQDAGELNNALDGLKWVTDY
N_gaditana_EKU20567	1	YDAGDHVKFNFPMAWSATTTLAWGNHEWDAWKKAGELSNALDQLKWVADY
N_gaditana_JU964585	1	YDAGDHVKFNFPMAWSATTTLWGVLEFGDAWKKTGEMKNLLDTWKWPLDY
C_thermocellum_AAA20892	1	YDAGDHVKFNFPMSYSAAMLGWAVYEMEDAFKQSGQYNHILNNIKWACDY
N_gaditana_JU973032	1	YDAGDHVKFALTTFGTIVSTLAWSLIEFPDANDSVNERSVMMDITKWGLDN
N_gaditana_JU981247	1	YDAGDFTQFTVPMASMTVLLWGLIRMEDAVAAAGELGHVDRDTWKWGLDN
N_gaditana_AFJ68664	1	HDAGDHTKFTFFGAAATITLLAWGGLAFEEGYKCAKENGNLLDCVKWSTDY
consensus	1	..***...*.....*.*.....

N_gaditana_JU964548	51	LLKCHPDPDTYYVQIGDGNSDHGYWGSAESMT--MHRPSYAVGHGAGROA
N_gaditana_EKU20567	51	LLKCHPDPDTYYVQVGNNSDHGYWGTAESMT--MSRPSYAVGR--GREAA
N_gaditana_JU964585	51	LLKCHPNEDTYVQVGDGNADHSYWGAAESTIN--MNRPSYAITP---NNP
C_thermocellum_AAA20892	51	FLKCHPDKDYVYVQVGDGHADHAWGPAEVMP--MERPSYKVDK---SSP
N_gaditana_JU973032	51	LLKAHYKPDALVYQIAS-FDDHSYWGPEPAFPKKMKRPSLETINP---KHP
N_gaditana_JU981247	51	LLKTHTRNELYVQIAD-EVDHCRWGPEETIN--YLRRSYKVTC---LNP
N_gaditana_AFJ68664	51	LMKLHTGKDEMYVQISDKPTDHFVTVRPEDMT--KTRRSYKVTC---ANP
consensus	51	..*.*... ..*.....**... ..*.*..... ..

N_gaditana_JU964548	99	GADAAAEAAAALAASSLVETQHGDIAYA-----AKLKATA
N_gaditana_EKU20567	97	GSAAICEAAAALAATSVLIFKQQGDSVYA-----AHLIDTA
N_gaditana_JU964585	96	GIEFAAEAGAAALAAGSLIFED--DIAYS-----ALLKAKA
C_thermocellum_AAA20892	96	GSTVVAETSAAALATASLIFK-VDGEYS-----KECLKHA
N_gaditana_JU973032	97	GSDIAGEVSAAEAAASLVFAE-SDSVYS-----HTLLVHS
N_gaditana_JU981247	95	GSQPMNTASAMAACATAFFNHDP-----AYAATILLQHA
N_gaditana_AFJ68664	96	GSQVLESAAALAASHLIFKAHVGEGGGEDAFDRIPDYDETYVASILLQHA
consensus	101	*.*.*.....*.....

N_gaditana_JU964548	134	ISLMAADSTRG-----GFSISEPFYKSSN-WKDEMAWAAALYEATGEE
N_gaditana_EKU20567	132	KSLMALGDGNQG-----GFSVSQPFYSSSG-ERDELAWAAWLYEATGEO
N_gaditana_JU964585	129	PSLFEALADKNRG-----SSDSSPFYKSYSGWDELWAAWVLYKATKDA
C_thermocellum_AAA20892	130	KELFEFADTTKSD---DGYTANGFYNSWSGEYDELSWAANWVLYLATNDS
N_gaditana_JU973032	131	RQLFDFADKHFG-----ICSKDEYGSTGWLDELAWAA-----
N_gaditana_JU981247	129	EBLFAFADMCPCGDWIRNGTIPAGNFYNDGGRWLDEHAFAAAWLHKATGKK
N_gaditana_AFJ68664	146	EBLFAFAVRCPCGHYVEDGKVPADCCYG-GGPGDEEAWAALWLHKATGKD
consensus	151	. *.....**.....*

N_gaditana_JU964548	178	BYLTAAEHWYQEGGCRQA-----WASWDSKAPGVOLLLLKTKDEG
N_gaditana_EKU20567	176	BYLDIAESLYAGGCCGSA-----WSYSWDAKAPGVOLLLYKHTR---
N_gaditana_JU964585	174	KYLKMAENDYDNCCAVTN-----KTEYSWDRKSSAVOLLLYDTK---
C_thermocellum_AAA20892	177	SYLDIAESYSYDKNWGYEPQTNIP-KYKWAQCWDVITYGTYLLLARLKND--
N_gaditana_JU973032		-----
N_gaditana_JU981247	179	BYLDIAIATYQCCYVPGG-----YQVSVLDWADMTMGINLLLYEATQW--
N_gaditana_AFJ68664	195	AYLAFAKATYHTCCSPYKQRDPFNAYTQGWDNKEAGVNLLLYQITQE--
consensus	201

N_gaditana_JU964548	220	KRTMYRDAENFVGGAMKKG-----TPPKGLVYNDOWGSNRYAANAFTI
N_gaditana_EKU20567	215	-KSQYKTDSSNFVGGATCKG-----QTPKGLAYNDKWGSNRYAANGAFI
N_gaditana_JU964585	215	-KEKYADGFAGFVDSWMYETP-----RTPKGLVTFEDKWAIPNRYSAATAFL
C_thermocellum_AAA20892	224	-NGKYKFAIERHLDWWTGTGNGERITYTPPKGLAVLDOWGSIRYATTTAFL
N_gaditana_JU973032		-----
N_gaditana_JU981247	223	-----NFKDAVINRQKAFMN--KIPRTPKGLSYNEWGNRYAANAFTI
N_gaditana_AFJ68664	243	-----EPYAYALKSHLNVWDGDPIARTPAGLSYVSQGSIRVAANQAFI
consensus	251	. .

[illegible]

FIG S2 Multiple sequence alignments of the six putative cellulases from CCMP 526 and a cellulase from *C. thermocellum*.

TABLE S1. Accession Numbers for proteins used in CESA/CSL phylogenetic tree (Fig. 1)

NCBI Accession/ Gene Model ^a	Description	Organism
ABT16312.1	hypothetical protein	Acanthocystis turfacea Chlorella virus 1
ABT16551.1	hypothetical protein	Acanthocystis turfacea Chlorella virus 1
ABT16957.1	hypothetical protein	Acanthocystis turfacea Chlorella virus 1
AAD20440.2	putative beta 13 glucan synthase catalytic subunit	Agrobacterium sp. ATCC 31749
ABA21191.1	cellulose synthase catalytic subunit	Anabaena variabilis ATCC 29413
NP_180821.1	cellulose synthase-like protein B2	Arabidopsis thaliana
NP_180869.1	cellulose synthase-like protein D1	Arabidopsis thaliana
NP_195645.1	cellulose synthase A catalytic subunit 2 UDP-forming	Arabidopsis thaliana
NP_194887.1	cellulose-synthase-like C5	Arabidopsis thaliana
NP_194967.1	cellulose synthase A catalytic subunit 1 UDP-forming	Arabidopsis thaliana
NP_197193.1	cellulose synthase-like protein D2	Arabidopsis thaliana
NP_197666.1	glucomannan 4-beta-mannosyltransferase 2	Arabidopsis thaliana
NP_566835.1	xyloglucan glycosyltransferase 4	Arabidopsis thaliana
NP_175981.2	cellulose synthase-like protein E1	Arabidopsis thaliana
NP_567692.2	cellulose synthase-like protein G2	Arabidopsis thaliana
NP_180820.2	cellulose synthase-like protein B1	Arabidopsis thaliana
NP_197123.3	cellulose synthase like A11	Arabidopsis thaliana
NP_194132.3	cellulose synthase-like protein G1	Arabidopsis thaliana
EAQ91514.1	conserved hypothetical protein	Chaetomium globosum CBS 148.51
XP_005715589.1	Cellulose synthase (UDP-forming) family GT2	Chondrus crispus
XP_005711952.1	Cellulose synthase (UDP-forming) family GT2	Chondrus crispus
Esi0004_0105	CESA	Ectocarpus salacius
Esi0120_0014	CESA	Ectocarpus salacius
Esi0231_0017	CESA	Ectocarpus salacius
Esi0231_0020	CESA	Ectocarpus salacius
Esi0097_0013	CSL	Ectocarpus salacius
Esi0097_0016	CSL	Ectocarpus salacius
Esi0097_0024	CSL	Ectocarpus salacius
Esi0185_0053	CSL	Ectocarpus salacius
Esi0200_0018	CSL	Ectocarpus salacius
WP_001413335.1	cellulose synthase	Escherichia coli
ZP_01440894.1	beta 13 glucan synthase catalytic subunit	Fulvimarina pelagi HTCC2506
ACI89427.1	cellulose synthase subunit A	Gluconacetobacter xylinus
EDX74293.1	Cellulose synthase superfamily	Microcoleus chthonoplastes PCC 7420
JU963065	CESA1	Nannochloropsis gaditana CCMP526
JU963444	CESA2	Nannochloropsis gaditana CCMP526
JU981242	CESA3	Nannochloropsis gaditana CCMP526
JU963368	CESA4	Nannochloropsis gaditana CCMP526
NannoCCMP-1779_7935-mRNA-1	CESA1	Nannochloropsis oceanica CCMP1779

NannoCCMP-1779_5780-mRNA-1	CESA2	Nannochloropsis oceanica CCMP1779
NannoCCMP-1779_6440-mRNA-1	CESA3	Nannochloropsis oceanica CCMP1779
EAA30080.3	hypothetical protein NCU08226	Neurospora crassa OR74A
ACC80169.1	cellulose synthase	Nostoc punctiforme PCC 73102
AAL25130.1	cellulose synthase-like protein OsCslE2	Oryza sativa
DAA01753.1	TPA exp: cellulose synthase-like D2	Oryza sativa Japonica Group
BAD33978.1	putative cellulose synthase-like protein OsCslE1	Oryza sativa Japonica Group
BAA82375.1	Similar to glycogenin glucosyltransferase	Oryza sativa Japonica Group
AAC39333.1	RSW1-like cellulose synthase catalytic subunit	Oryza sativa Japonica Group
AAL25131.1	cellulose synthase-like protein OsCslF1	Oryza sativa Japonica Group
AAL25132.1	cellulose synthase-like protein OsCslF2	Oryza sativa Japonica Group
AAL38525.1	CSLA2	Oryza sativa Japonica Group
BAC10759.1	putative CSLC9	Oryza sativa Japonica Group
AAP21426.1	putative cellulose synthase catalytic subunit	Oryza sativa Japonica Group
DAA01752.1	TPA exp: cellulose synthase-like D1	Oryza sativa Japonica Group
BAC98512.1	putative glucosyltransferase	Oryza sativa Japonica Group
BAF26298.1	Os10g0341700	Oryza sativa Japonica Group
BAF14725.2	Os04g0429500	Oryza sativa Japonica Group
CAL57811.1	unnamed protein product	Ostreococcus tauri OTTH0595
ABT13740.1	hypothetical protein	Paramecium bursaria chlorella virus MT325
ABP96902.1	cellulose synthase 1	Phytophthora infestans
ABP96903.1	cellulose synthase 2	Phytophthora infestans
ABP96904.1	cellulose synthase 3	Phytophthora infestans
ABP96905.1	cellulose synthase 4	Phytophthora infestans
XP_001907680.1	hypothetical protein	Podospora anserina
ABX71734.1	cellulose synthase	Porphyra yezoensis
ACX56230.1	cellulose synthase 2	Saprolegnia monoica
ACX56231.1	cellulose synthase 3	Saprolegnia monoica
ACX56232.1	cellulose synthase 4	Saprolegnia monoica
EDY42701.1	cellulose synthase	Streptomyces sp. SPB74
ACB00100.1	cellulose synthase catalytic subunit	Synechococcus sp. PCC 7002
WP_016266338.1	cellulose synthase catalytic subunit partial	Yersinia enterocolitica

^a NCBI accession numbers are listed for all organisms except for *Ectocarpus salacius* and *Nannochloropsis oceanica*. NCBI TSA accession listed for *Nannochloropsis gaditana*.

TABLE S2 Enzymatic release of monomeric sugars from the *N. gaditana* cell wall.

Enzyme	% Monomeric Carb ^a	% as Glucose ^b	Other Monomers Detected
Cellulase #1	27.9 ± 4.5	99.8	Fuc, Man, Rha
Lyticase	6.5 ± 0.1	100	none
Zymolyase	1.2 ± 0.4	100	none

^a Enzymatically released monomeric carbohydrates are expressed as a percentage of total carbohydrates (n = 3 ± 1 SD) using HPAEC-PAD.

^b TFA digestion of the enzyme hydrolysate was used to calculate the percent of the carbohydrate that was glucose (n = 3, standard deviations were all < 0.05%).

TABLE S3 Pairwise sequence identity of *Nannochloropsis* CESA.^a

		<i>N. gaditana</i>				<i>N. oceanica</i>			
		CESA1	CESA2	CESA3	CESA4	CESA1	CESA2	CESA3 ^b	CESA4
<i>N. oceanica</i>	CESA1	80.3	42.1	17.7	15.4	100	41.3	NA	14.8
	CESA2	41.3	86	16.4	14.4		100	NA	12.9
	CESA3 ^b	NA	NA	NA	NA			NA	NA
	CESA4	14.9	13.9	29.7	73.3				100
<i>N. gaditana</i>	CESA1	100							
	CESA2	42.5	100						
	CESA3	15.4	16.1	100					
	CESA4	16.2	15.4	31.3	100				

^a Values given as percent of residues identical between given proteins. Light grey indicates a comparison between clades, Clade 1: CESA1 and CESA2; Clade 2: CESA3 and CESA4.

^b CESA3 from *N. oceanica* sequence was not available.

TABLE S4 ICP-AES analysis of *N. gaditana* cell wall material.^a

Analyte ^b	f/2 #1	f/2 #2	ASW #1 ^c	ASW #1 ^c	ASW #2
Ag 328.068	BDL	BDL	BDL	BDL	BDL
Al 308.215	0.010 ^d	0.011 ^d	0.001 ^d	0.010 ^d	0.005 ^d
As 188.979	0.001	0.001	BDL	BDL	BDL
B 249.772	0.017	0.016	BDL	BDL	0.003
Ba 233.527	0.043	0.044	0.010	0.047	0.005
Be 313.107	0.000	0.000	BDL	BDL	BDL
Ca 315.887	0.078	0.049	0.187	0.441	0.165
Cd 214.440	0.002	0.009	BDL	BDL	0.004
Co 228.616	BDL	BDL	BDL	BDL	0.004
Cr 205.560	0.003	0.003	0.000	0.000	0.000
Cu 324.752	0.002	0.001	0.000	0.001	0.000
Fe 238.204	0.023	0.014	0.028	0.059	0.030
K 766.490	0.065	0.060	0.020	0.083	0.011
Li 670.784	0.001	0.000	BDL	BDL	BDL
Mg 279.553	0.021	0.013	0.070	0.100	0.132
Mn 257.610	0.003	0.002	0.000	0.001	0.001
Na 589.592	0.197	0.171	0.054	0.268	0.672
Ni 231.604	0.007	0.001	0.000	0.000	0.004
P 177.434	0.011	0.009	0.053	0.082	0.042
Pb 220.353	0.002	0.001	BDL	BDL	BDL
S 180.669	0.273	0.199	0.328	0.509	0.845
Se 196.026	0.002	0.004	BDL	BDL	BDL
Si 251.611	BDL	BDL	BDL	BDL	0.001
Sr 407.771	0.001	0.001	0.002	0.003	0.000
Tl 190.801	0.001	0.001	BDL	BDL	BDL
V 292.402	0.001	0.000	BDL	BDL	BDL
Zn 213.857	0.032	0.031	0.009	0.039	0.011
Sn 189.927	BDL	BDL	BDL	BDL	BDL
Mo 202.031	0.002	0.001	BDL	BDL	BDL
Sb 217.582	BDL	BDL	BDL	BDL	BDL
Ti 334.940	0.000	0.002	BDL	BDL	BDL
Total	0.797	0.641	0.762	1.643	1.936
Average	1.144 ± 0.578				

^a All values are presented as % m/m of the wall prep.^b Numbers after element names refer to emission wavelengths.^c Two technical replicates are provided for the ASW #1 prep. Error expressed as ± 1 SD.^d Aluminum numbers likely reflect aluminum foil contamination during processing.