Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY MATERIAL

Molecular investigations on *Candida glabrata* clinical isolates for pharmacological targeting

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Running Title: Molecular characterization of *Candida glabrata* isolates

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Figure S1: Growth pattern of *C. glabrata* strains. Colony forming units (CFU) mL^{-1} determined from samples collected at specific time intervals from cultures grown at 37 °C. Data expressed as mean ± SD (n = 3).

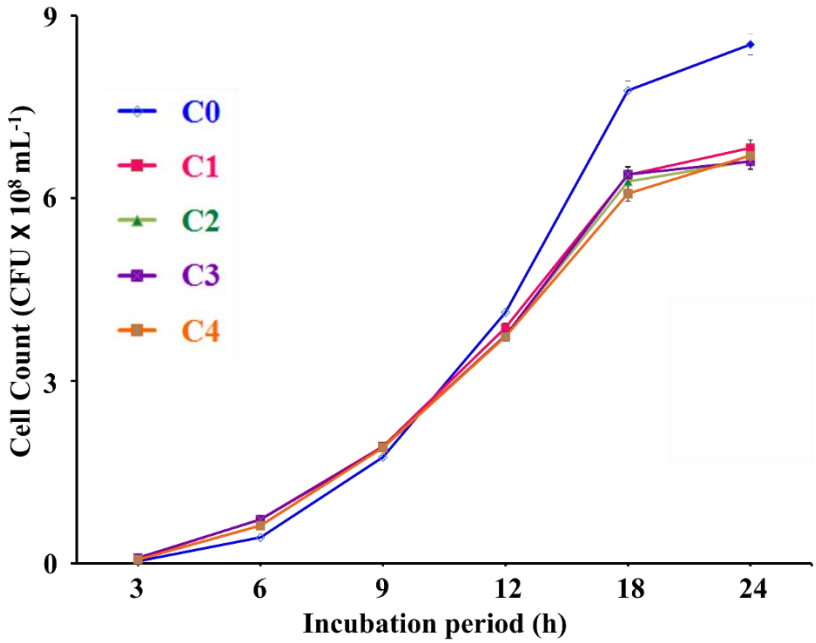

Figure S2: Light microscopy images of all strains captured at 40X (Scale = 50 µm). *C. glabrata* wild-type strain (C0), and clinical isolates (C1-C4).

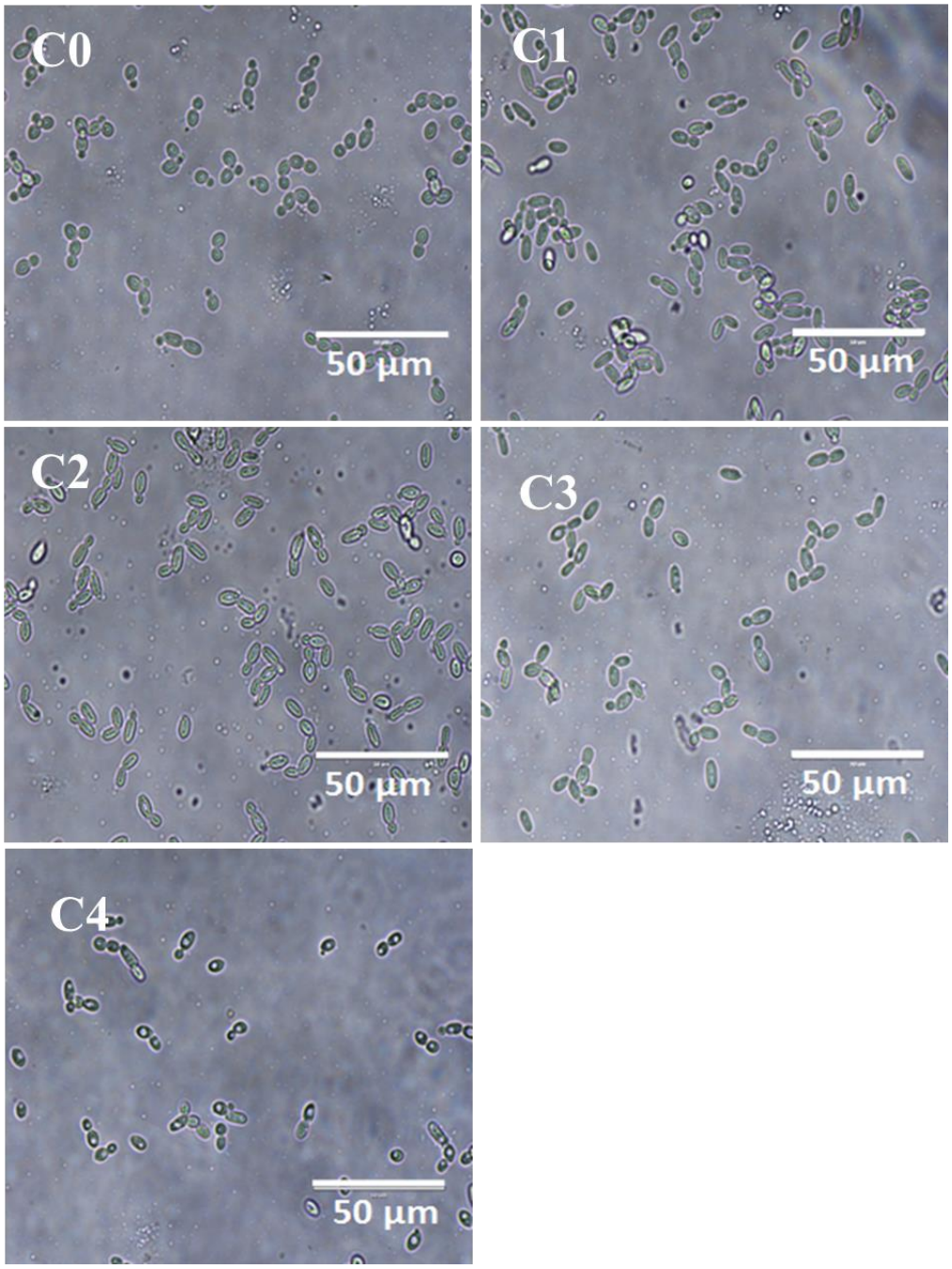

Figure S3: Morphological and biochemical characterization of *C. glabrata*. **A)** Mean size of *C. glabrata* strains as measured along the large ellipsoid axis of budding cells using Image J software. The data is expressed as mean ± SD (n = 100). **B**) Surface charge (zeta potential, mV ± SD, n = 3) analysis of all strains. **C)** Ergosterol content of cell wall expressed as % ergosterol (mean ± SD, n = 3). *C. glabrata* wild-type strain (C0), and clinical isolates (C1-C4).

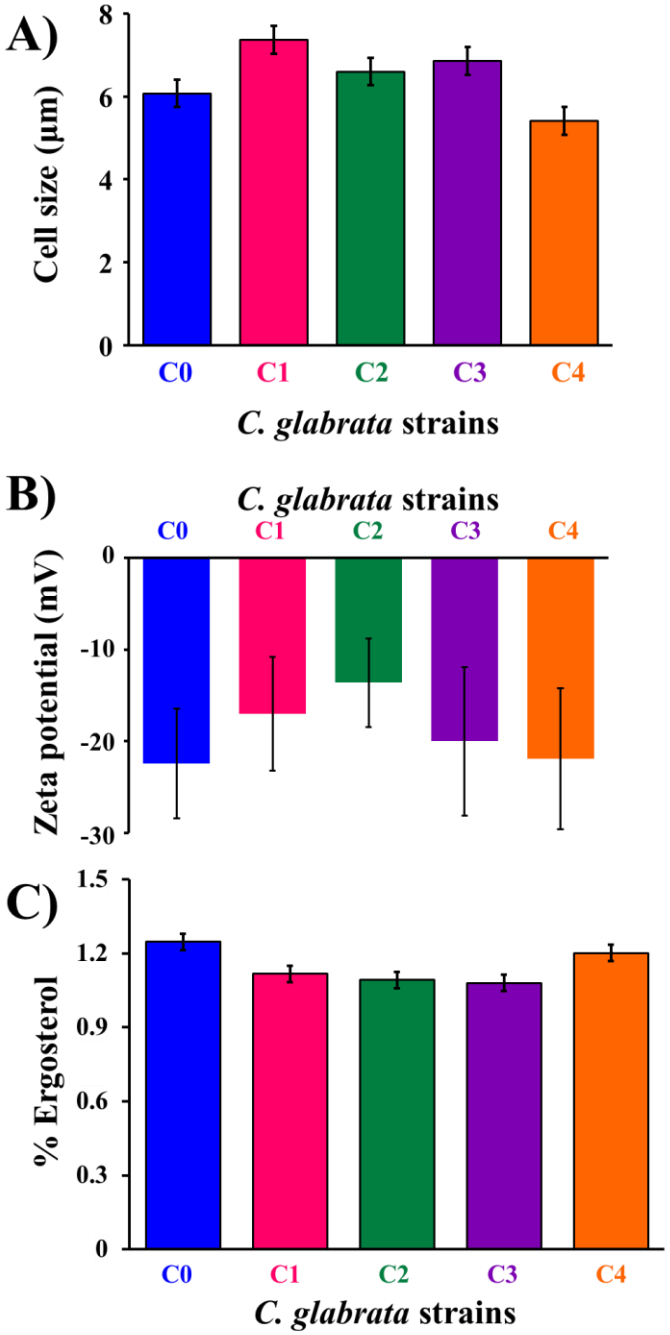

Figure S4: Principal Component Analysis (PCA) scores plot of metabolomics data representing the group discrimination. *C. glabrata* wild-type strain (C0), and clinical isolates (C1-C4).

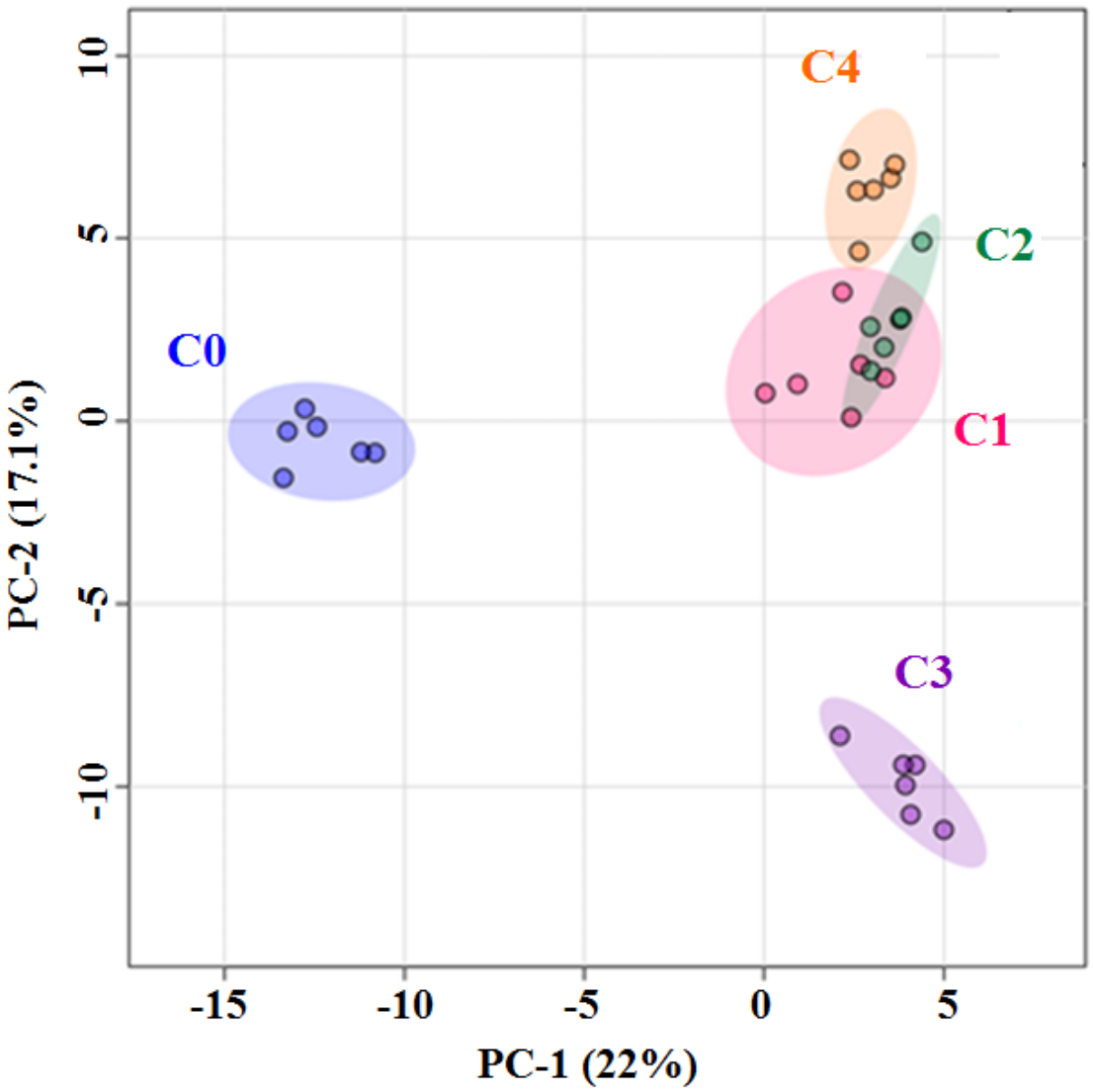

Figure S5: Cross validation of PLS-DA model performance generated from spectral data. The bars represent accuracy, goodness of fit (R^2) and predictability (Q^2) determined during cross validation. The red asterisk mark indicates optimum predictability.

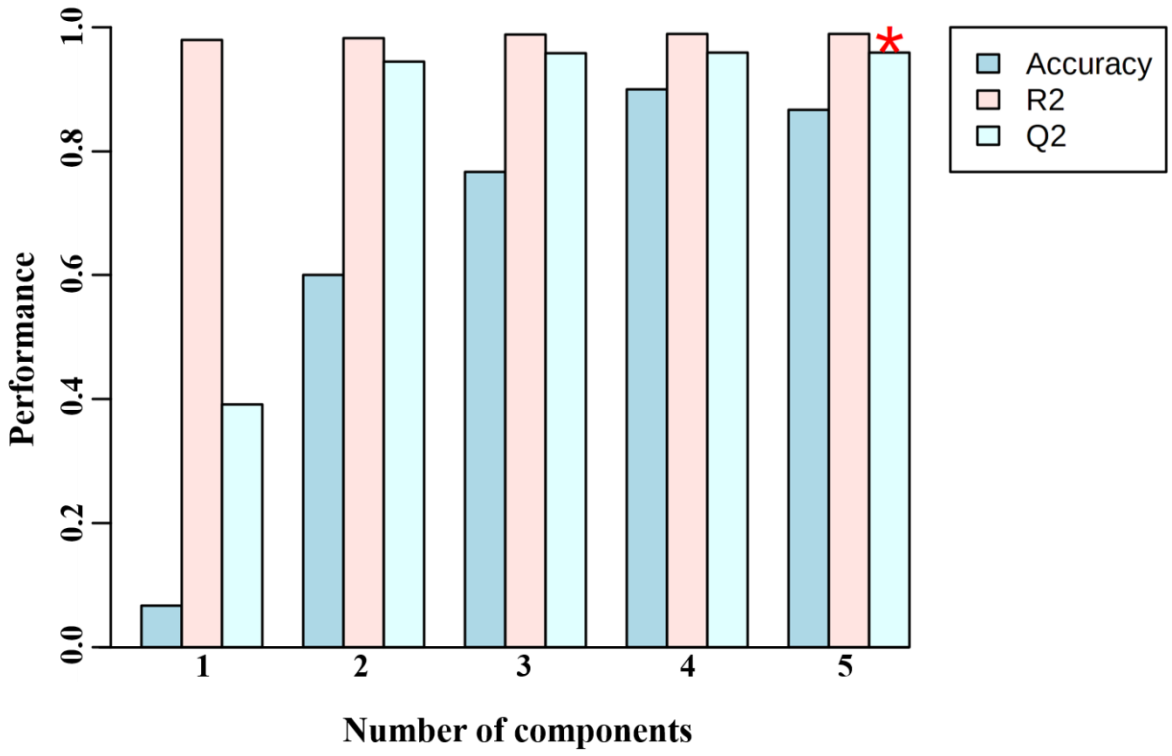

Figure S6: Heatmap showing the pattern of distribution of top 18 significant metabolites (as per VIP score), and involved in group discrimination among the chosen *C. glabrata* groups. *C. glabrata* wild-type strain (C0), and clinical isolates (C1-C4). The dark maroon and dark blue colours represent highest up and down fold change in the metabolites respectively as described in the scale bar.

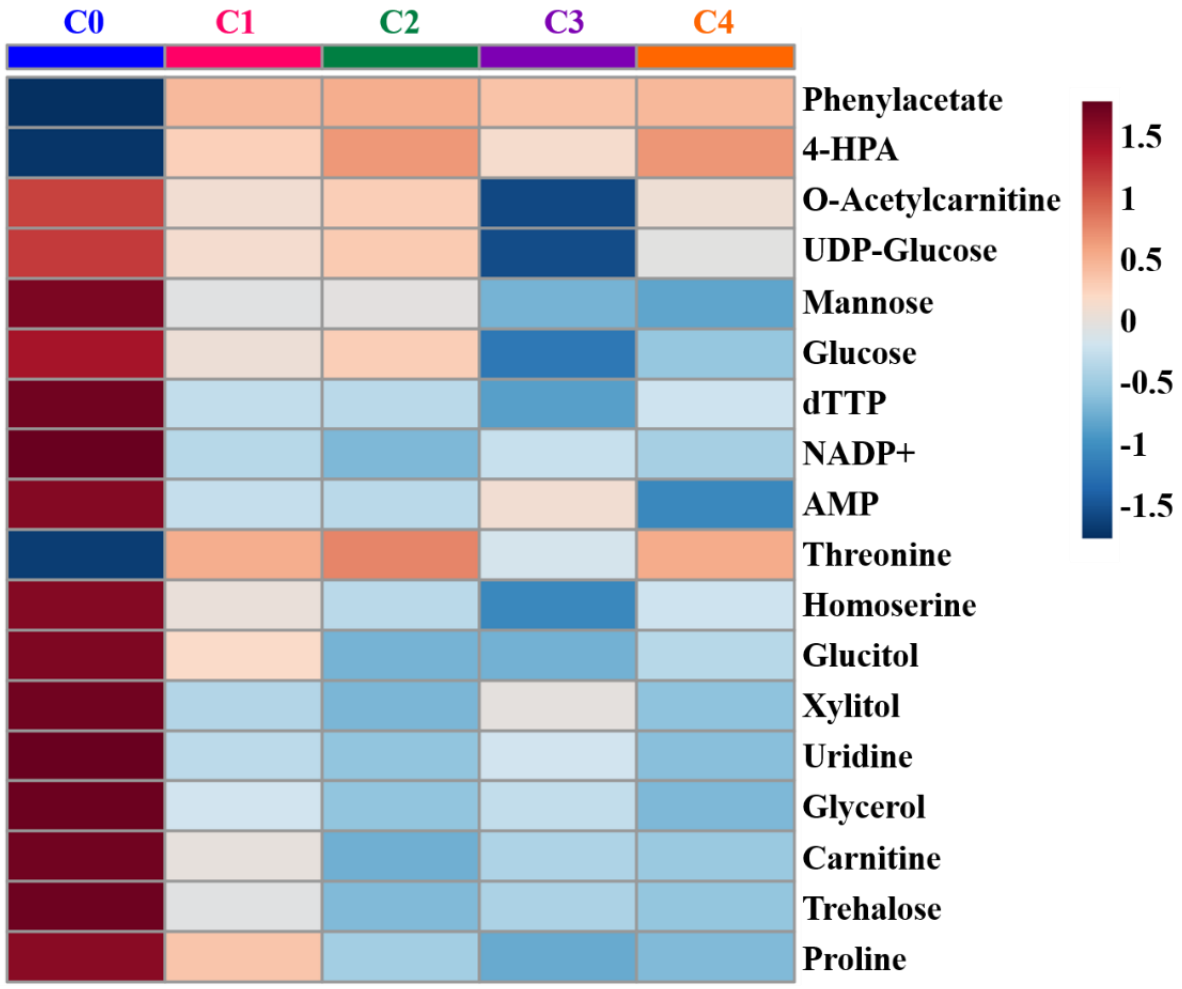

Table S1: Sequence of Primers used for RT-PCR analysis

S No	Enzyme	Code	Gene	Sequence 5'-3'	Amplicon size (bp)
1	Phosphatidylserine decarboxylase	CAGL0I08745g	PSD2 (F)	TGCCCAGTAGTGATGAAGTTATG	102
			PSD2 (R)	ATGTGGACTTCCGCTCTTAATC	
2	Lysophospholipidacyltransferase	CAGL0L04642g	ALE1 (F)	GTCTAGCTACCAGAAGTCGAAAG	100
			ALE1 (R)	ACTTGGGCCTGTTAGGATTG	
3	Acetyl-CoA carboxylase	CAGL0L10780g	HFA1(F)	CCTCGTGGTAGACAGTTTGTT	117
			HFA1 (R)	GCCTCTCTTTCTGGCATAGTT	
4	Fatty acid elongase 3	CAGL0G04851g	SUR4 (F)	CTTGACCTCCATCTCTTTCATACT	94
			SUR4 (R)	GGAACAGATAGCCCAGAACAA	
5	Hydroxymethylglutaryl-CoA synthase	CAGL0H04081g	ERG13 (F)	AACTACAGGGCAAGCGTATC	101
			ERG13 (R)	GCTGACTATGTCGCTGATGT	
6	Phosphoglycerate kinase	CAGL0L07722g	PGK1 (F)	GCTTTGCCAACCATCAAGTATG	117
			PGK1 (R)	CAACTGGAGCCAAGGAGTATTT	
7	Glucose-6-phosphate isomerase	CAGL0H05445g	PGI1 (F)	GGGTTCCTTGATTGCCTACTAC	106
			PGI1 (R)	AGCCAAGACCTTACCCAATTC	
8	1,4-alpha-glucan branching enzyme	CAGL0M03377g	GLC3 (F)	TCATTCACAGTCACGCATCTAA	97
			GLC3 (R)	CCTCTACCAGAAGTCAAGGAATG	
9	Trehalose 6-phosphate synthase complex regulatory subunit	CAGL0J06468g	TSL1 (F)	CCGCAGAGGATTTCTTCACTAC	100
			TSL1 (R)	GCATTGATTTCCGGTGCTATTG	
10	Trehalose 6-phosphate synthase/phosphatase	CAGL0G05335g	TPS2 (F)	TGGTCAACTCCATCAACTCAAA	108
			TPS2 (R)	CAGCAACTCTCAGCAAGGATAG	
11	Glucoamylase	CAGL0G02717g	SGA1 (F)	AGCGCTCGGGTTTAGATATTG	101
			SGA1 (R)	GCGGTTGTCCACATGAAATAAG	
12	Argininosuccinatesynthase	CAGL0C05115g	ARG1 (F)	CCCACCAAAGGATATGTGGAAG	126
			ARG1 (R)	CCAGTAGCGTTGTCCTTGTATG	
13	Argininosuccinatelyase	CAGL0I08987g	ARG4 (F)	GAGGACATCCACACTGCTAAT	106
			ARG4 (R)	CCGTGACAACTTGGTCATTTC	
14	Arginase	CAGL0J07062g	CAR1 (F)	GGTGAAGCCACTGAACTTATCT	123
			CAR1 (R)	CTTGTCCAAAGCAGCAGAAAC	
15	Asparagine synthase (glutamine-hydrolysing)	CAGL0I10648g	ASN2 (F)	TCTATCGCTGCTCGTGAAAC	127
			ASN2 (R)	GAGACCAACCAGCAGTATGTAG	
16	ATP-binding cassette, subfamily B (MDR/TAP), member 1	CAGL0K00363g	STE6 (F)	CTGTGGATATGCGTCAGAGAAT	107
			STE6 (R)	CGTGTTACGGATGAACTCTAGG	
17	β-actin/housekeeping gene	CAGL0K12694g	ACT1 (F)	TTACCAACTGGGATGACATGGA	100
			ACT1 (R)	GGAGCCTCGGTCAACAAGAC	

Table S2: List of metabolites identified from the 1D ^{1}H-NMR spectra recorded from intracellular metabolite extract of all the *C. glabrata* strains. (Peak type: s = singlet, d = doublet, t = triplet, d = quadrate, m = multiplet). *C. glabrata* wild-type strain (C0), and clinical isolates (C1-C4).

S. No	Metabolite Name	Assignment	Chemical shifts (δ) in ppm with peak type
Amino Acids (24)			
1	Alanine	C6H3 C4H	1.45*(d) 3.76(q)
2	Alloisoleucine	C9H3 C8H3 C7H2 C6H C4H	0.92 (d) 0.95 (q) 1.31/1.42 (m) 2.05 (m) 3.73 (d)
3	Arginine	N9H N11H, N12H2 C4H C8H2 C6H2 C7H2	7.22 (s) 6.66 (s) 3.76 (q) 3.23 (m) 1.92/1.88 (m) 1.71/1.63 (m)
4	Asparagine	C6H2 C4H N9H2 N5H2	2.84/2.94 (m) 4.00 (m) 6.90 (s) 7.62 (s)
5	Betaine	C3H2 C5H3&C7H3& C8H3	3.87(s) 3.24(s)
6	Carnitine	β-CH γ-CH2 N^{+} 3 (CH3) α-CH2	4.56 (m) 3.44 & 3.38 (m) 3.20 (t) 2.43 (q) & 2.40 (q)
7	Citrulline	N3H C7H C4H2 C6H2 C5H2	6.37 (q) 3.74(q) 3.14/3.12 (m) 1.88/1.84 (m) 1.59/ 1.52 (m)
8	Cysteine	C3H C2H2	3.97 (q) 3.09/ 3.02 (q)
9	Cystine	C4H/C7H C3H2/C6H2	4.10 (q) 3.39/ 3.17 (q)
10	Glutamate	C4H C7H2 C6H2	3.75(m) 2.34(m),2.30(m) 2.12(m),2.04(m)
11	Glutamine	N10H2 N5H2 C4H C7H2 C6H2	7.58 (s) 6.87 (s) 3.76 (q) 2.46/ 2.40 (m) 2.14/ 2.10 (m)
12	Glutathione	N4H N13H C3H C14H2 C11H	8.49 (d) 8.24 (q) 4.58 (m) 3.78/ 3.77 (q) 3.75 (q)

		C2H2 C9H2 C10H2	2.97/ 2.92 (q) 2.56/ 2.52 (m) 2.16/ 2.13 (m)
13	Glycine	C4H2	3.54 (s)
14	Homoserine	C4H2 C2H2 C3H2	3.84 (q) 3.75 (m) 2.12 (m) &2.01 (m)
15	Isoleucine	C4H C6H C7H2 C9H3 C8H3	3.66 (d) 1.96 (m) 1.45/1.24 (m) 0.99 (d) 0.92 (q)
16	Leucine	C4H C6H2 C7H C8H3 C9H3	3.72 (q) 1.73 & 1.66 (m) 1.69 (m) 0.95 (d) 0.94 (d)
17	Methionine	C5H C3H C2H3 C4H2	3.85 (q) 2.64 (m) 2.12 (s) 2.10/2.19 (m)
18	O-Acetylcarnitine	C5H C9H2 C11/13/14-H3 C4H2 C12H3	5.58 (m) 3.83, 3.59 (m) 3.17 (t) 2.63, 2.49 (q) 2.13 (s)
19	Ornithine	C4H C8H2 C6H2 C7H2	3.77 (m) 3.05 (q) 1.93 (m) 1.82/1.73 (m)
20	Proline	C5H C2H2 C3H2 C4H2	4.10 (q) 3.41/3.31 (m) 1.97/2.02 (m) 2.05/2.32 (m)
21	Serine	C3H C2H2	3.83 (dd) 3.94 (q)/3.97 (q)
22	Taurine	C5H2 C6H2	3.41 (m) 3.25 (m)
23	trans-4-Hydroxy-L-proline	C5H C3H C2H2 C4H2	4.66 (m) 4.33 (m) 3.49/3.36 (m) 2.42/2.14 (m)
24	Valine	C4H C6H C7H3 C8H3	3.60 (d) 2.26 (m) 1.01 (d) 0.96 (d)
Nitrogen bases (15)			
25	Adenine	C2H C6H	8.21 (s) 8.17 (s)
26	Adenosine	C7H C12H C2H C3H C4H C5H C17H2	8.34 (s) 8.24 (s) 6.06 (d) 4.79 (q) 4.42 (q) 4.30 (m) 3.83/3.91 (dd)

27	ADP	C7H C12H C2H C3H C4H C5H C17H2	8.50 (s) 8.25 (s) 6.13 (d) 4.77 (q) 4.56 (q) 4.37 (m) 4.24/4.20 (m)
28	AMP	C7H C12H C2H2 C3H C4H C5H C17H2	8.58 (s) 8.24 (s) 6.11 (d) 4.77 (q) 4.48 (q) 4.35 (m) 4.04/4.01 (m)
29	ATP	C7H C12H C2H C3H C4H C5H C17H2	8.50 (s) 8.24 (s) 6.12 (d) 4.76 (q) 4.56 (q) 4.39 (m) 4.27/4.23 (m)
30	Cytidine	C11H C10H C2H C3H C4H C5H C14H2	7.84 (d) 6.05 (q) 5.90 (q) 4.29 (q) 4.19 (q) 4.11 (m) 3.91/3.80 (q)
31	Cytosine	C4H C5H	7.50 (d) 5.96 (d)
32	dTTP	C11H C2H C4H C5H C13H2 C3H2 C19H3	7.68 (q) 6.31 (q) 4.73 (m) 4.61 (m) 4.22/4.18 (m) 2.35/2.38 (m) 1.91 (d)
33	GTP	C7H N22H2 C2H C3H C4H C5H C17H2	8.14 (s) 6.39 (s) 5.93 (d) 4.72 (q) 4.54 (q) 4.35 (m) 4.25/4.22 (m)
34	Inosine	C7H C12H C2H C3H C4H C5H C17H2	8.33 (s) 8.22 (s) 6.08 (d) 4.76 (q) 4.43 (q) 4.26 (m) 3.90/3.83 (q/q)
35	Thymidine	C11H C2H2 C4H2 C5H C13H2 C3H2 C17H3	7.63 (q) 6.28 (q) 4.46 (m) 4.01 (m) 3.83/3.75 (q) 2.36/2.36 (q) 1.88 (d)
36	Thymine	C6H C9H3	7.35 (q) 1.86 (d)
37	Uracil	C6H C5H	7.51 (d) 5.77 (d)

38	Uridine	C11H C2H C10H C3H C4H C5H C14H2	7.84 (d) 5.90 (d) 5.89 (d) 4.34 (q) 4.21 (q) 4.12 (m) 3.90 (q)/3.79 (q)
39	Xanthine	C2H	7.92 (s)
Organic Acids (22)			
40	2-Aminobutyrate	$C7H_3$ C6H2 C4H	0.98 (q) 1.88 (m) 3.70 (q)
41	2-Hydroxybutyrate	C4H3 C3H2 C2H	0.91 (q) 1.65 (m)&1.72 (m) 3.96 (q)
42	2-Oxoglutarate	$C4H_2$ & $C5H_2$	2.42 (t) & 2.99 (t)
43	4-Aminobutyrate	$C5H_2$ $C4H_2$ $C6H_2$	1.88 (m) 2.28 (m) 3.00 (m)
44	4-Hydroxyphenylacetate	C3H/C5H C2H/C6H C8H2	7.16 (m) 6.86 (m) 3.44 (q)
45	Acetate	C4H3	1.88(s)
46	Aspartate	C4H C6H2	3.89 (q) 2.78/2.64 (q)
47	Citrate	C2H2 & C5H2	2.52 (d) &2.67 (d)
48	Formate	C2H	8.42 (s)
49	Fumarate	C4aH&C5aH	6.48 (s)
50	Glycolate	C2H2	3.93 (s)
51	Isocitrate	C2H C5H C9H2	4.03 (d) 2.98 (m) 2.54/2.49 (q)
52	Isovalerate	C4H2 C5H C6H3 & C7H3	2.04 (d) 1.94 (m) 0.90 (d)
53	Lactate	C2H C3H3	4.10 (q) 1.30 (d)
54	Malate	C2H C5H2	4.30 (q) 2.67 (q) & 2.36 (q)
55	Malonate	C4H2	3.10 (s)
56	Methylmalonate	C4H C5H3	3.16 (q) 1.21 (d)
57	Phenylacetate	C3H&C5H C4H C2H&C6H C7H2	7.36 (m) 7.29 (m) 7.28 (m) 3.52 (s)
58	Propionate	C4H2 C5H3	2.15 (q) 1.02 (t)
59	Pyruvate	C6H3	2.37 (s)
60	Succinate	C4H2, C5H2	2.40 (s)
61	Tropate	C2H, C3H C4H, C5H C1H C6H2 C8H	7.39 (m) 7.35 (m) 7.31 (m) 4.06 (q)/3.85 (q) 3.68 (q)

Sugars and derivatives (13)			
62	Glucitol	C3H C9H C2H2 C5H C11H2 C7H	3.81 (m) 3.82 (m) 3.83/3.65 (q) 3.77 (m) 3.73/3.62 (m) 3.64 (q)
63	Gluconate	C9H C7H C5H C3H C2H2	4.12 (d) 4.03 (q) 3.77 (m) 3.75 (m) 3.65/3.81 (m)
64	Glucose	C2H C3H C4H C5H C6H C11H2	5.21(d)&4.63(d) 3.23(dd)&3.52(dd) 3.70(m)&3.48(m) 3.40(m)&3.39(m) 3.82(m)&3.45(m) 3.89(dd)&3.83(dd)&3.75(dd)& 3.71(dd)
65	Glucose-1-phosphate	C2H C6H C13H2 C4H C3H C5H	5.45 (m) 3.90 (m) 3.86/3.74 (m) 3.77 (m) 3.48 (m) 3.39 (m)
66	Glycerate	C3H C2H2	4.08 (q) 3.81/ 3.72 (dd)
67	Glycerol	C2H C5H2 & C3H2	3.78 (m) 3.64 & 3.54 (m)
68	Mannitol	C2H2/C11H2 C3H/C9H C5H/C7H	3.86/3.65 (q) 3.74 (m) 3.81 (d)
69	Mannose	C2H C3H C4H C5H C6H C11H2	5.17/4.89 (d) 3.93/3.92 (q) 3.84/3.65 (q) 3.65/3.56 (q) 3.80/3.37 (m) 3.90/3.86/3.75/3.72 (q)
70	myo-Inositol	C5H C1H/C3H C4H/C6H C2H	4.07 (t) 3.60 (q) 3.51 (q) 3.28 (t)
71	Threonine	C6H C4H2 C8H3	4.23 (m) 3.58 (d) 1.30 (d)
72	Trehalose	C2H/C8H C4H/C12H C6H/C10H C3H/C13H C5H/C11H C17H2/C19H2	5.17 (q) 3.84 (q) 3.81 (m) 3.62 (q) 3.42 (q) 3.85/3.75 (m)
73	UDP-glucose	C32H C22H C33H C2H C23H C22H C19H	7.92 (d) 5.97 (d) 5.96 (d) 5.57 (q) 4.37 (q) 4.35 (q) 4.28 (m)

		C18H2 C6H C13H C4H C3H C5H	4.18/4.24 (m) 3.88 (m) 3.85/3.77 (q) 3.76 (q) 3.53 (m) 3.46 (q)
74	Xylitol	C7H/C3H C9H2/C2H2 C2H2/C5H2/C9H2	3.81 (dt) 3.72 (m) 3.64 (m)
Vitamins and coenzymes (4)			
75	NAD+	NiC2H NiC6H NiC4H AdC8H NiC5H AdC2H NiC1'H AdC1'H Ad C2'H NiC4'H AdC3'H NiC2'H NiC3'H AdC4'H NiC5'H AdC5'H	9.32 (m) 9.12 (m) 8.85 (m) 8.41 (s) 8.19 (m) 8.16 (s) 6.08 (d) 6.03 (d) 4.75 (q) 4.53 (m) 4.49 (q) 4.47 (q) 4.42 (q) 4.36 (m) 4.35/4.22m 4.25/4.19m
76	NADP+	C39H C41H C43H C7H C42H C12H C32H C2H C3H C4H C30H C33H C34H C5H C29H2 C19H2	9.30 (m) 9.11 (m) 8.80 (m) 8.39 (s) 8.17 (m) 8.14 (s) 6.09 (m) 6.05 (d) 4.99 (m) 4.60 (q) 4.49 (m) 4.45 (q) 4.39 (q) 4.36 (m) 4.31/4.20 (m) 4.27/4.18 (m)
77	NADPH	AdC8H AdC2H NiC2H NiNH2 AdC1'H NiC6H AdC2'H NiC5H NiC1'H AdC3'H AdC4'H AdC5'H NiC3'H NiC2'H NiC4'H NiC5'H NiC4H	8.48 (s) 8.23 (s) 6.94 (m) 6.61 (s) 6.20 (d) 5.96 (m) 4.97 (m) 4.81 (m) 4.74 (d) 4.59 (q) 4.37 (m) 4.26/4.18 (m) 4.20 (q) 4.15 (q) 4.04 (m) 4.04/4.01 (m) 2.85/2.76 (m)
78	Pyridoxine	C6H C9H2 C11H2 C7H3	7.65 (s) 4.81 (s) 4.73 (s) 2.45 (s)

Choline derivatives (2)			
79	O-Phosphocholine	C3H2 C4H2 C3H3/C6H3/C10H3	4.15 (m) 3.58 (m) 3.20 (t)
80	sn-Glycero-3-phosphocholine	C3H2 C9H C8H2 C4H2 C15H2 C6H3, C13H3, C14H3	4.30 (m) 3.90 (m) 3.94/3.86 (m) 3.65 (m) 3.61/3.67 (q) 3.20 (t)
Alcohols (2)			
81	Ethylene glycol	C1H2&C2H2	3.64 (s)
82	Isopropanol	C2H C3H3 & C4H3	4.01 (m) 1.16 (d)
Amide (1)			
83	Acetamide	NH2 CH3	7.53/6.79 (t) 2.00 (d)