

**Phyco-nanobionics biohybrid system for increased carotenoids
accumulation in *C. sorokiniana* UUIND6**

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FIGURE CAPTIONS

Fig S1. Standard calibration curve of H₂O₂

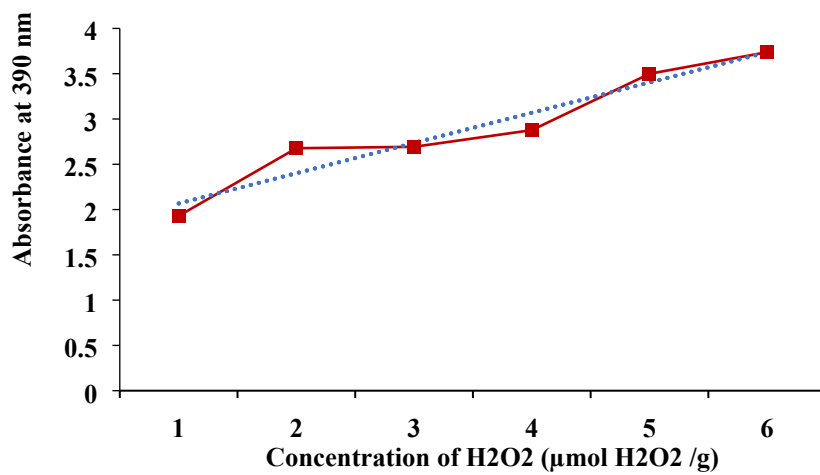


Fig S1.

TABLE CAPTIONS

Table S1. Potential functional groups within the MB associated with AgNPs using FTIR analysis

Table S2. One-way ANOVA on Chl-a yield of microalgae

Table S3. One-way ANOVA on Chl-b yield of microalgae

Table S4. One-way ANOVA on Chl-a/Chl-b ratio of microalgae

Table S5. One-way ANOVA on carotenoid yield of microalgae

Table S6. Metabolites detected in the $^1\text{H-NMR}$ spectra of organic extracts from microalgae, and their respective chemical shifts (in ppm). The ^1H chemical shifts in bold identify the signal that was integrated for quantitative comparisons. Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets.

Table S7. $^1\text{H-NMR}$ spectra data of pure carotenoids isolated from microalgae (MB-CARs) and standard β -carotene.

Table S1

Wavenumber (cm ⁻¹)	Bond	Functional groups	MB-Ag-0 (Control)	MB-Ag-1	MB-Ag-2.5	MB-Ag-5
3200-3500	O-H stretch, intermolecular H-bonded	alcohols, phenols	3421	-	-	-
2850-3100	C-H stretch =C-H	alkenes, alkanes, aromatics	2929 2851	-	-	-
1640-1710	C=O stretch -C=C- stretch	α , β -unsaturated aldehydes and ketones, alkenes	1654	-	-	-
1475-1600	C-C stretch N-O stretch	aromatics, nitro compound	1530	-	-	-
1350-1450	C-H bend C-H rock	Alkanes	1408	-	-	-
1250-1335	C-N stretch	aromatic amines	1318	-	-	-
1020-1250	C-N stretch	aliphatic amines	1239 1161 1106 1049	-	-	-
700-1000	C-N/ R-O-C/ R-O- CH ₃ stretching/ aromatic C-H	protein constituents	982 848 702	-	-	-
500-700	C-C stretching	Aliphatic groups	-	557	557	557

Table S2

Groups	Count	Sum	Average	Variance	S.D
Control	3	11.45	3.82	0.000933	0.03
HMBE-AgNP (1 ppm)	3	14.28	4.76	0.0031	0.05
HMBE-AgNP (2.5 ppm)	3	16.36	5.45	0.002533	0.05
HMBE-AgNP (5 ppm)	3	24.62	8.21	0.002633	0.05
HMBE-AgNP (10 ppm)	3	15.21	5.07	0.0049	0.07

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	32.66057	4	8.165143	2895.441	2.86E-15	3.47805
Within Groups	0.0282	10	0.00282			
Total	32.68877	14				

Table S3

Groups	Count	Sum	Average	Variance	S.D
Control	3	7.28	2.43	0.000933	0.03
HMBE-AgNP (1 ppm)	3	8.8	2.93	0.000933	0.03
HMBE-AgNP (2.5 ppm)	3	9.15	3.05	0.0016	0.04
HMBE-AgNP (5 ppm)	3	10.1	3.37	0.001733	0.04
HMBE-AgNP (10 ppm)	3	10.57	3.52	0.003033	0.06

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	2.177933	4	0.544483	330.6579	1.42E-10	3.47805
Within Groups	0.016467	10	0.001647			
Total	2.1944	14				

Table S4

Groups	Count	Sum	Average	Variance	S.D
Control	3	4.7	1.57	3.33333E-05	0.01
HMBE-AgNP (1 ppm)	3	4.85	1.62	0.000533333	0.02
HMBE-AgNP (2.5 ppm)	3	5.35	1.78	0.000133333	0.01
HMBE-AgNP (5 ppm)	3	7.3	2.43	0.000433333	0.02
HMBE-AgNP (10 ppm)	3	4.3	1.43	0.000133333	0.01

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	1.855	4	0.46375	1830.592105	2.83E-14	3.47805
Within Groups	0.002533333	10	0.000253333			

Table S5

Groups	Count	Sum	Average	Variance	S.D
Control	3	662.92	221.0	18.98613	4.36
HMBE-AgNP (1 ppm)	3	908.5	302.8	17.72333	4.21
HMBE-AgNP (2.5 ppm)	3	1073.35	357.8	11.12583	3.34
HMBE-AgNP (5 ppm)	3	1372.6	457.5	6.253333	2.5
HMBE-AgNP (10 ppm)	3	1084.8	361.6	16.87	4.11

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	90436.49	4	22609.12	1593.12	5.66E-14	3.47805
Within Groups	141.9173	10	14.19173			
Total	90578.4	14				

Table S6

Compound	δ ¹ H (multiplicity)
Carotenoid 1	6.16 (s), 6.33, 6.40, 6.71
Carotenoid 2	6.67, 6.97
Lutein	1.00(s), 1.40(dd), 1.60(dd), 1.68(s), 1.69(dd), 1.99(s), 2.26(dd), 3.90(m), 6.33(d), 0.98(s), 1.05(s), 1.44(dd), 1.67(s), 1.95(dd), 3.57(m), 3.64(m), 6.31(d), 1.21(s), 1.63(m), 1.86(s), 2.22(m), 6.63(d)
Violaxanthin	1.98 (s), 1.97 (s), 1.74 (s), 1.08 (s)
Zeaxanthin	1.98 (s), 1.94 (s), 1.82 (t), 1.33 (s)
Astaxanthin	0.71 (d), 0.74 (d), 0.80 (d), 1.70 (d), 3.57 (s), 3.78 (s), 6.01 119.60, 6.20 119.60, 6.22 (s) 64.60, 8.11 (dd), 8.46 (s) 92.60, 9.25 (s) 99.20, 9.61 (s) 107.10 0.78-0.88 13.60-15.90, 1.16-1.35 21.80-33.50 (total FA), 1.44-1.54 24.20-28.10, 1.90-1.99 26.30 (MUFA), 1.99-2.06 26.30, 2.22-2.33 33.30, 2.71-2.80 24.90 (PUFA), 5.25-5.38 126.80-131.00
Chlorophyll a	
Fatty acyl (FA) chains	
Glycolipids – galactosyl moieties	3.25, 3.59, 4.09, 4.63
Glycolipids – glucosyl moieties	3.22, 3.24, 3.53, 3.56, 4.81, 4.87
Glycolipids – glyceryl moieties	3.62, 3.81, 4.12, 4.32, 5.10
Linoleic acid	1.28, 2.01, 2.73, 5.32
Linolenic acid	0.92 (t), 1.28, 2.02, 2.76, 5.33

Table S7

Chemical shift (ppm)	Chemical Assignment	Standard β-Carotene	MB-CARs
1.0-1.4	Other Methylenes	+	+
1.5-1.8	Methylenes β to the carbonyl	+	+
1.9-2.1	Allylic methylenes	+	+
5.2-5.5	Glyceryl and olefinic methine	+	+

+ sign indicates: presence, - sign indicates: absence