

Characterization and optimization of exopolysaccharide extracted from a newly isolated halotolerant cyanobacterium, *Acaryochloris Al-Azhar MNE ON864448.1* with antiviral activity

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Table S1. The effect of fourteen independent variables with coded levels on EPS production from *A. Al-Azhar MNE ON864448.1* using Plackett–Burman experimental design

Std	Run	A: NaHCO ₃	B: NaNO ₃	C: K ₂ HPO ₄	D: MgSO ₄ .7 H ₂ O	E: CaCl ₂	F: Citric acid	G: Ferric ammoniu m citrate	H: EDTA	J: Trace metal	K: NaCl	L: Temperature	M: pH	N: working volume	O: Inoculum size	P: Dummy 1	Q: Dummy 2	R: Dummy 3	S: Dummy 4	T: Dummy 5	Polysaccharide yield (mg/ml)		Residuals
		g/L	g/L	g/L	g/L	g/L	g/L	g/L	g/L	mL	g/L	°C		mL	%	-	-	-	-	-	Actual values	Predicted values	
9	1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	1	1	1	1	-1	1	-1	0.6315	0.6279	0.0036
13	2	1	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	1	1	1	0.6109	0.6138	-0.0029
20	3	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.2245	0.2244	0.0001
10	4	-1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	1	1	1	1	-1	1	0.57368	0.5898	-0.0161
5	5	-1	1	1	-1	1	1	-1	-1	1	1	1	1	-1	1	-1	1	-1	-1	-1	0.4355	0.4347	0.0008
18	6	-1	-1	1	1	1	1	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	1	1	0.434	0.4570	-0.0230
16	7	1	1	1	1	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	0.55761	0.5405	0.0171
7	8	-1	-1	-1	1	1	-1	1	1	-1	-1	1	1	1	1	-1	1	-1	1	-1	0.77523	0.7720	0.0032
15	9	1	1	1	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	1	0.37332	0.3895	-0.0162
1	10	1	1	-1	-1	1	1	1	1	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	0.6066	0.5798	0.0268
2	11	-1	1	1	-1	-1	1	1	1	1	-1	1	-1	1	-1	-1	-1	-1	1	1	0.42572	0.4218	0.0039
19	12	1	-1	-1	1	1	1	1	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	1	0.33309	0.3332	-0.0001
8	13	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	1	1	1	1	-1	1	-1	1	0.753247	0.7720	-0.0188
6	14	-1	-1	1	1	-1	1	1	-1	-1	1	1	1	1	-1	1	-1	1	-1	-1	0.38453	0.3864	-0.0018
4	15	1	1	-1	1	1	-1	-1	1	1	1	1	-1	1	-1	1	-1	-1	-1	-1	0.766379	0.7589	0.0075
17	16	-1	1	1	1	1	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	0.351948	0.3291	0.0228
3	17	1	-1	1	1	-1	-1	1	1	1	1	-1	1	-1	1	-1	-1	-1	-1	1	0.467864	0.4670	0.0009
12	18	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	1	1	1	1	0.513549	0.5092	0.0044
11	19	1	-1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	1	1	1	1	-1	0.241173	0.2427	-0.0015
14	20	1	1	-1	1	-1	1	-1	-1	-1	-1	1	1	-1	1	1	-1	-1	1	1	0.33262	0.3432	-0.0106
Low actual (-1)		0.7	0.5	0.02	0.04	0.016	0.003	0.003	0.0005	0.5	0	22	7	400	3	-	-	-	-	-	-	-	-
High actual (1)		3.4	3	0.06	0.1	0.056	0.009	0.009	0.001	1.5	10	30	10	600	7	-	-	-	-	-	-	-	-

Table S2. Regression statistics and analysis of variance (ANOVA).

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Confidence level (%)
Model	0.5214	14	0.0372	81.20	< 0.0001	>99.99
A-NaHCO ₃	0.0001	1	0.0001	0.2634	0.6297	37.03
B-NaNO ₃	0.0003	1	0.0003	0.7133	0.4369	56.31
C-K ₂ HPO ₄	0.0754	1	0.0754	164.34	< 0.0001	>99.99
D-MgSO ₄ .7H ₂ O	0.0001	1	0.0001	0.1804	0.6887	31.13
E-CaCl ₂	0.0591	1	0.0591	128.91	< 0.0001	>99.99
F-Citric acid	8.512E-07	1	8.512E-07	0.0019	0.9673	3.27
G-Ferric ammonium citrate	0.0001	1	0.0001	0.3179	0.5972	40.28
H-EDTA	0.0994	1	0.0994	216.73	< 0.0001	>99.99
J-Trace metal	0.0001	1	0.0001	0.2406	0.6445	35.55
K-NaCl	0.0558	1	0.0558	121.57	0.0001	99.99
L-Temperature	0.0001	1	0.0001	0.1360	0.7274	27.26
M-pH	0.0000	1	0.0000	0.0720	0.7992	20.08
N-working volume	0.1604	1	0.1604	349.67	< 0.0001	>99.99
O- Inoculum size	0.0705	1	0.0705	153.65	< 0.0001	>99.99
Residual	0.0023	5	0.0005			
Cor. Total	0.5237	19				
Std. Dev.	0.0214		R²	0.9956		
Mean	0.4896		Adjusted R²	0.9834		
C.V. %	4.37		Predicted R²	0.9299		
PRESS	0.03669		Adeq Precision	29.7989		

Table S3. FCCD showing cyanobacterial polysaccharide production by the selected strain as influenced by the most significant variables.

Std	Run	X1: Working volume	X2: EDTA	X3: Inoculum size	X4: CaCl ₂	X5: NaCl	Polysaccharide yield (mg/ml)		Residuals
		mL	g/L	%	g/L	g/L	Actual values	Predicted values	
23	1	0	0	0	-2	0	3.84706	3.73	0.1139
13	2	-1	-1	1	1	1	7.48235	7.52	-0.0351
31	3	0	0	0	0	0	3.94706	4.52	-0.5683
32	4	0	0	0	0	0	4.79324	4.52	0.2778
19	5	0	-2	0	0	0	4.3529	4.33	0.0251
5	6	-1	-1	1	-1	-1	4.74706	4.79	-0.0399
18	7	2	0	0	0	0	5.26471	5.15	0.1177
9	8	-1	-1	-1	1	-1	5.4706	5.50	-0.0287
25	9	0	0	0	0	-2	2.82353	2.71	0.1183
14	10	1	-1	1	1	-1	4.24882	4.29	-0.0418
27	11	0	0	0	0	0	4.37059	4.52	-0.1448
3	12	-1	1	-1	-1	-1	4.1992	4.27	-0.0730
8	13	1	1	1	-1	-1	3.17529	3.26	-0.0862
28	14	0	0	0	0	0	4.38824	4.52	-0.1272
7	15	-1	1	1	-1	1	5.63541	5.71	-0.0795
11	16	-1	1	-1	1	1	5.97059	6.04	-0.0683

4	17	1	1	-1	-1	1	2.81	2.88	-0.0727
6	18	1	-1	1	-1	1	4.46	4.50	-0.0396
29	19	0	0	0	0	0	4.37059	4.52	-0.1448
12	20	1	1	-1	1	-1	2.836	2.91	-0.0749
20	21	0	2	0	0	0	3.46353	3.27	0.1975
21	22	0	0	-2	0	0	3.6118	3.52	0.0915
2	23	1	-1	-1	-1	-1	3.28	3.31	-0.0331
26	24	0	0	0	0	2	4.3647	4.26	0.1044
1	25	-1	-1	-1	-1	1	5.63529	5.66	-0.0265
15	26	-1	1	1	1	-1	5.6529	5.73	-0.0817
22	27	0	0	2	0	0	5.08471	4.95	0.1312
24	28	0	0	0	2	0	5.23529	5.13	0.1088
30	29	0	0	0	0	0	5	4.52	0.4846
17	30	-2	0	0	0	0	9.2753	9.17	0.1050
16	31	1	1	1	1	1	4.1588	4.24	-0.0814
10	32	1	-1	-1	1	1	3.70588	3.73	-0.0283

Variable actual levels

X1: Working volume					X2: EDTA					X3: Inoculum size					X4: CaCl ₂					X5: NaCl				
-2	-1	0	1	2	-2	-1	0	1	2	-2	-1	0	1	2	-2	-1	0	1	2	-2	-1	0	1	2
300	400	500	600	700	0.0005	0.001	0.002	0.003	0.004	3%	5%	7%	9%	11%	0.026	0.036	0.046	0.056	0.066	10	15	20	25	30

Table S4. FCCD regression statistics, regression coefficients of second order polynomial model for cyanobacterial polysaccharide production.

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High
Intercept	4.52	0.1135	4.27	4.77
A-Working volume	-1.01	0.0581	-1.13	-0.8780
B-EDTA	-0.2654	0.0581	-0.3933	-0.1376
C-Inoculum size	0.3583	0.0581	0.2305	0.4861
D-CaCl ₂	0.3483	0.0581	0.2205	0.4762
E-NaCl	0.3888	0.0581	0.2610	0.5166
AB	-0.0523	0.0711	-0.2089	0.1042
AC	0.0731	0.0711	-0.0835	0.2296
AD	-0.1960	0.0711	-0.3525	-0.0394
AE	-0.1912	0.0711	-0.3478	-0.0347
BC	-0.0025	0.0711	-0.1590	0.1541
BD	0.0008	0.0711	-0.1557	0.1574
BE	-0.0516	0.0711	-0.2082	0.1050
CD	0.0917	0.0711	-0.0649	0.2482
CE	0.0985	0.0711	-0.0580	0.2551
DE	-0.0019	0.0711	-0.1584	0.1547
A ²	0.6608	0.0525	0.5452	0.7764
B ²	-0.1796	0.0525	-0.2953	-0.0640
C ²	-0.0696	0.0525	-0.1852	0.0460
D ²	-0.0214	0.0525	-0.1370	0.0942
E ²	-0.2582	0.0525	-0.3738	-0.1425
Std. Dev.	0.2845		R²	0.9839
Mean	4.61		Adjusted R²	0.9547
C.V. %	6.17		Predicted R²	0.9060
PRESS	5.76		Adeq Precision	28.0502

Table S5. Analysis of variance (ANOVA) for the quadratic regression model obtained from FCCD for cyanobacterial polysaccharide production.

Source	Sum of Squares	Df ¹	Mean Square	F-value ²	p-value ³
Model	54.53	20	2.73	33.68	< 0.0001
A-Working volume	24.28	1	24.28	299.95	< 0.0001
B-EDTA	1.69	1	1.69	20.89	0.0008
C-Inoculum size	3.08	1	3.08	38.06	< 0.0001
D-CaCl ₂	2.91	1	2.91	35.98	< 0.0001
E-NaCl	3.63	1	3.63	44.81	< 0.0001
AB	0.0438	1	0.0438	0.5414	0.4772
AC	0.0854	1	0.0854	1.06	0.3264
AD	0.6144	1	0.6144	7.59	0.0187
AE	0.5850	1	0.5850	7.23	0.0211
BC	0.0001	1	0.0001	0.0012	0.9727
BD	0.0000	1	0.0000	0.0001	0.9910
BE	0.0426	1	0.0426	0.5263	0.4833
CD	0.1344	1	0.1344	1.66	0.2240
CE	0.1553	1	0.1553	1.92	0.1934
DE	0.0001	1	0.0001	0.0007	0.9795
A ²	12.81	1	12.81	158.24	< 0.0001
B ²	0.9465	1	0.9465	11.69	0.0057
C ²	0.1422	1	0.1422	1.76	0.2120
D ²	0.0134	1	0.0134	0.1658	0.6917
E ²	1.95	1	1.95	24.15	0.0005
Residual	0.8904	11	0.0809		
Lack of Fit	0.2055	6	0.0343	0.2501	0.9392
Pure Error	0.6849	5	0.1370		
Cor. Total	55.42	31			

(1) Df: Degree of freedom, (2) F: Fishers's function, (3) P: Level of significance

Table S6. Displayed the fit summary of FCCD for cyanobacterial EPS production.

Sequential model Sum of Squares					
Source	Sum of Squares	Df	Mean Square	F-value	p-value
Mean vs Total	681.37	1	681.37		
Linear vs Mean	35.59	5	7.12	9.34	< 0.0001
2FI vs Linear	1.66	10	0.1661	0.1463	0.9980
Quadratic vs 2FI	17.27	5	3.45	42.68	< 0.0001
Cubic vs Quadratic	0.0238	5	0.0048	0.0329	0.9991
Residual	0.8667	6	0.1444		
Lack of Fit Tests					
Source	Sum of Squares	Df	Mean Square	F-value	p-value
Linear	19.14	21	0.9114	6.65	0.0223
2FI	17.48	11	1.59	11.60	0.0071
Quadratic	0.2055	6	0.0343	0.2501	0.9392
Cubic	0.1818	1	0.1818	1.33	0.3014
Pure Error	0.6849	5	0.1370		
Model Summary Statistics					
Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS
Linear	0.8732	0.6423	0.5735	0.4456	30.72
2FI	1.07	0.6722	0.3649	0.4821	28.70
Quadratic	0.2845	0.9839	0.9547	0.9060	5.76