

Mémoire

Auteur : Samar Abdelmohsen Elsayed Youssef Talkhan,

Promoteur(s) : Wilmotte, Annick; Christodoulou, Maria

Faculté : Faculté des Sciences

Diplôme : Master en biologie des organismes et écologie, à finalité approfondie

Année académique : 2024-2025

URI/URL : <http://hdl.handle.net/2268.2/23861>

Avertissement à l'attention des usagers :

Tous les documents placés en accès ouvert sur le site le site MatheO sont protégés par le droit d'auteur. Conformément aux principes énoncés par la "Budapest Open Access Initiative"(BOAI, 2002), l'utilisateur du site peut lire, télécharger, copier, transmettre, imprimer, chercher ou faire un lien vers le texte intégral de ces documents, les disséquer pour les indexer, s'en servir de données pour un logiciel, ou s'en servir à toute autre fin légale (ou prévue par la réglementation relative au droit d'auteur). Toute utilisation du document à des fins commerciales est strictement interdite.

Par ailleurs, l'utilisateur s'engage à respecter les droits moraux de l'auteur, principalement le droit à l'intégrité de l'oeuvre et le droit de paternité et ce dans toute utilisation que l'utilisateur entreprend. Ainsi, à titre d'exemple, lorsqu'il reproduira un document par extrait ou dans son intégralité, l'utilisateur citera de manière complète les sources telles que mentionnées ci-dessus. Toute utilisation non explicitement autorisée ci-avant (telle que par exemple, la modification du document ou son résumé) nécessite l'autorisation préalable et expresse des auteurs ou de leurs ayants droit.

Appendix 1: Preparation of BG110 Medium and Trace Metal Mix A5 (Rippka, 1988)

Table 1.

Chemical composition of BG11₀ media

Product/Prepared solution	Concentration of solution stock (g/L)	Final concentration (g/L)	Quantity to take (mL)
K ₂ HPO ₄ ·3H ₂ O	4.0	0.040	10
MgSO ₄ ·7H ₂ O	7.5	0.075	10
CaCl ₂ ·2H ₂ O	3.6	0.036	10
EDTA - Na ₂ ·2H ₂ O	0.1	0.001	10
Citric acid monohydrate	0.6	0.006	10
Ferric ammonium citrate	0.6	0.006	10
Na ₂ CO ₃ · 10H ₂ O	5.4	0.054	10
NaHCO ₃	15.0	0.150	10
Trace metal mix A5	/	/	1
Milli-Q water	/	/	919
pH			7.1

Note. To prepare 1 L of BG110 medium, suspend all ingredients in 500 mL of Milli-Q water, mix well, add Milli-Q water again to reach a final volume of 1 L, adjust the pH to 7.1, and sterilize by autoclaving at 121 °C for 15 minutes.

Table 2.

Chemical composition of the trace metal mix A5 of the BG11₀ culture media

Chemical	Quantity
H ₃ BO ₃	2.86 g
MnCl ₂ ·4H ₂ O	1.81 g
ZnSO ₄ ·7H ₂ O	0.222 g
NaMoO ₄ ·2H ₂ O	0.39 g
CuSO ₄ ·5H ₂ O	0.079 g
Co(NO ₃) ₂ ·6H ₂ O	49.4 mg
Distilled water	1.0 L

Note. Dissolve all components in 1 L of Milli-Q water,, and store in a dark bottle at 4 °C.

Appendix 2: Tris-Borate-EDTA (TBE) 10X preparation (Sambrook, J. & Russell, 2001)

Table 3.

Tris-Borate-EDTA (TBE) 10X

Ingredient	Amount (g/L)
Tris (hydroxymethyl) aminomethane (TRIS, Trometamol)	108
Boric acid	55
EDTA	9.3
pH	8.3

Note. Dissolve the components in 500 mL of distilled water, add water to reach a final volume of 1 L, and adjust the pH to 8.3. To prepare a 1X working solution, dilute 100 mL of the 10X stock with 900 mL of distilled water.

Appendix 3: Agarose gel electrophoresis (2%) for the 16S–23S ITS purified PCR products

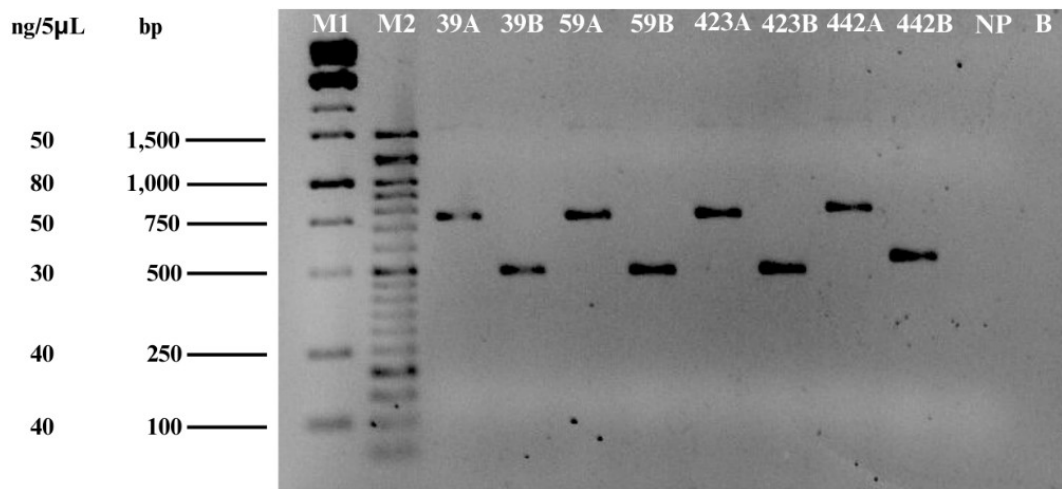


Figure 1.

Agarose gel electrophoresis (2%) stained with Midori Green nucleic acid stain was performed for the 16S–23S ITS purified PCR products. 2–5 μL (approximately 60 ng) of each gel-purified PCR product were loaded per well. Samples 39A, 39B, 59A, 59B, 423A, 423B, 442A, and 442B correspond to the studied strains and their respective ULC numbers, with ‘A’ representing the first, larger band and ‘B’ representing the second, smaller band. NP represents the PCR negative control, B is the blank lane (MQ water + dye), and M1 and M2 are DNA ladders (FastGene 50 bp and FastGene 1 kb, respectively). Electrophoresis was run for 2 h at 75 V.

Appendix 4: Concentration and Purity of 16S rRNA and ITS PCR products

Table 4.

Concentration and Purity of 16S rRNA PCR products

Strain Name	Concentration (ng/μL)	A _{260/280}	A _{260/230}
ULC8	139.50	1.69	1.66
ULC16	124.00	1.72	2.12
ULC38	109.00	1.69	2.00
ULC39	118.00	1.70	1.66
ULC46	154.00	1.70	2.00
ULC50	114.00	1.68	1.86
ULC59	138.00	1.68	2.04
ULC169	146.5	1.69	2.00
ULC179	152.00	1.70	1.80
ULC423	133.50	1.68	2.10
ULC442	130.00	1.70	1.97
ULC619	181.50	1.70	1.40
ULC632	107.00	1.70	2.18

Table 5.

Concentration and Purity of 16S–23S ITS PCR products. A and B designate the high and low molecular weight bands, respectively

Strain Name/Band	Concentration (ng/μL)	A _{260/280}	A _{260/230}
ULC8/A	31.00	1.72	0.51
ULC8/B	10.70	1.60	0.43
ULC16/A	31.50	1.70	0.60
ULC16/B	10.10	1.55	1.09
ULC38/A	25.50	1.62	1.21
ULC38/B	14.10	1.57	0.46
ULC39/B	8.70	1.63	1.30
ULC46/A	43.00	1.87	0.84

Strain Number	Concentration (ng/μL)	A260/280	A260/230
ULC46/B	21.50	1.81	1.80
ULC50/A	12.20	1.45	0.92
ULC50/B	21.50	1.61	0.36
ULC59/A	34.50	1.77	2.34
ULC59/B	8.70	1.88	0.25
ULC169/A	14.40	1.60	0.48
ULC169/B	45.00	1.63	0.90
ULC179/A	53.00	1.80	1.23

Appendix 5: Percentage 16S rRNA gene sequence similarity matrix

Table 6.

Percentage 16S rRNA gene sequence similarity matrix among studied strains and closely related taxa

	08	16	38	39	46	50	59	179	423	442	632
08	–										
16	100.00	–									
38	100.00	100.00	–								
39	100.00	100.00	100.00	–							
46	98.41	98.41	98.41	98.41	–						
50	98.79	98.79	98.79	98.79	98.60	–					
59	100.00	100.00	100.00	100.00	98.41	98.79	–				
179	100.00	100.00	100.00	100.00	98.40	98.79	100.00	–			
423	98.79	98.79	98.79	98.79	98.60	100.00	98.79	98.79	–		
442	98.51	98.51	98.51	98.51	97.83	98.32	98.51	98.50	98.32	–	
632	99.54	99.54	99.54	99.54	98.22	99.07	99.54	99.53	99.07	98.70	–
HQ877826_Nostoc_cf_commune_257_16	98.41	98.41	98.41	98.41	98.12	97.94	98.41	98.41	97.94	98.13	98.41
MH427701_Nostoc_commune_EV1_KK1_clone_1	99.07	99.07	99.07	99.07	98.21	98.04	99.07	99.07	98.03	99.07	98.79
MH427702_Nostoc_commune_EV1_KK1_clone_2	99.07	99.07	99.07	99.07	98.21	98.04	99.07	99.07	98.03	99.07	98.79
NR_114430_Nostoc_punctiforme_strain_PCC73102	98.60	98.60	98.60	98.60	97.93	98.51	98.60	98.60	98.51	99.44	99.07
MW649141_Nostoc_mirabile_CHAB_5756	97.18	97.17	97.18	97.18	96.19	96.50	97.18	97.17	96.50	96.01	97.08
NR_176570_Nostoc_mirabile_strain_CHAB_5784	97.46	97.46	97.46	97.46	96.29	96.60	97.46	97.45	96.59	96.31	97.37
MW649809_Nostoc_favosum_CHAB_5709	98.32	98.32	98.32	98.32	98.03	97.56	98.32	98.31	97.56	97.75	97.94
AY577535_Nostoc_lichenoides_CNP_AK1	98.32	98.32	98.32	98.32	97.93	97.94	98.32	98.31	97.94	98.60	98.51
EU178143_Nostoc_flagelliforme_IMGA0408	98.88	98.88	98.88	98.88	98.31	98.60	98.88	98.88	98.60	99.35	98.79
MH427659_Nostoc_oromo_ETH_2_4_M_5	97.83	97.83	97.83	97.83	97.44	97.64	97.83	97.83	97.64	98.12	98.03
MH427691_Nostoc_desertorum_CM1_VF14	97.18	97.17	97.18	97.18	97.06	97.37	97.18	97.17	97.36	97.94	97.17
MH427692_Nostoc_indistinguendum_CM1_VF10	97.84	97.84	97.84	97.84	98.31	98.50	97.84	97.83	98.50	98.41	97.75
MH427693_Nostoc_indistinguendum_FI5_VF4	97.27	97.27	97.27	97.27	98.02	97.65	97.27	97.26	97.65	96.88	97.17
AJ630449_Nostoc_edaphicum_X	99.16	99.16	99.16	99.16	97.83	98.98	99.16	99.16	98.98	98.98	99.54
CP054698_Nostoc_edaphicum_CCNP1411	98.41	98.41	98.41	98.41	97.83	98.41	98.41	98.41	98.41	99.81	98.60
NR_189726_Nostoc_tlalocii	97.94	97.94	97.94	97.94	98.78	98.13	97.94	97.93	98.13	97.27	97.75
NR_189727_Nostoc_tlalocii	97.94	97.94	97.94	97.94	98.78	98.13	97.94	97.93	98.13	97.27	97.75
OP688055_Nostoc_tlalocii_clone_1_4	97.94	97.94	97.94	97.94	98.78	98.13	97.94	97.93	98.13	97.27	97.75
OP688056_Nostoc_tlalocii_clone_1_5	97.94	97.94	97.94	97.94	98.78	98.13	97.94	97.93	98.13	97.27	97.75
OP688060_Nostoc_montejanii_clone_46_3	97.85	97.84	97.85	97.85	97.64	97.18	97.85	97.84	97.17	97.46	98.13
OR149082_Nostoc_montejanii_clone_46_4	97.85	97.84	97.85	97.85	97.64	97.18	97.85	97.84	97.17	97.46	98.13

	8	16	38	39	46	50	59	179	423	442	632
OQ785341_ <i>Nostoc_jammuense</i> _2JNA_PS	98.79	98.79	98.79	98.79	98.12	98.32	98.79	98.79	98.32	99.26	99.07
OQ785342_ <i>Nostoc_jammuense</i> _21A_PS	98.88	98.88	98.88	98.88	98.21	98.41	98.88	98.88	98.41	99.35	99.16
NR_199295_ <i>Nostoc_globosum</i> _strain_4CHA_PS	98.88	98.88	98.88	98.88	97.84	98.51	98.88	98.88	98.51	98.13	98.88
OQ785343_ <i>Nostoc_globosum</i> _4CHA_PS	98.88	98.88	98.88	98.88	97.84	98.51	98.88	98.88	98.51	98.13	98.88
OQ785344_ <i>Nostoc_breve</i> _BG11_PS	98.70	98.69	98.70	98.70	98.03	98.04	98.70	98.69	98.03	98.79	98.79
OQ785345_ <i>Nostoc_coriaceum</i> _34TNA_PS	99.26	99.26	99.26	99.26	97.84	98.60	99.26	99.25	98.60	98.41	99.35
PP454078_ <i>Nostoc_sikkimense</i> _RESHI_1B_PS	99.54	99.54	99.54	99.54	98.02	98.88	99.54	99.53	98.88	98.50	99.63
PP849349_ <i>Nostocales_cyanobacterium</i> _Esc15_4	98.32	98.32	98.32	98.32	97.93	98.13	98.32	98.31	98.13	99.54	98.32
HF678493_ <i>Nostoc_sp</i> _CCAP_1453_28	98.41	98.41	98.41	98.41	97.83	98.22	98.41	98.41	98.22	99.44	98.60
AM711528_ <i>Nostoc_sp</i> _Lukesova_40_93	98.04	98.03	98.04	98.04	98.59	99.26	98.04	98.03	99.26	98.41	98.32
619	96.60	96.59	96.60	96.60	95.70	96.40	96.60	96.58	96.40	96.69	96.98
OK161253_2_ <i>Nostocales_cyanobacterium</i> _2_7	97.85	97.84	97.85	97.85	98.40	98.98	97.85	97.84	98.98	98.88	98.04

Appendix 6: ITS length

Table 7.
Length (nucleotide) of the 16S-23S ITS region for studied *Nostoc* strains and described taxa

GenBank Accession number	Taxon name		D1 stem	spacer + D2	spacer+D3 + spacer	tRNA Ile	spacer + V2 + spacer	tRNA Ala	post tRNA spacer	Box B	post box B spacer	Box A	D4 + spacer	V3	spacer + D5 + end	Total length of ITS
ULC8	<i>Nostoc</i> sp.	ANT.LPR.1	67	40	16	74	85	73	43	28	16	13	25	39	28	547
ULC16	<i>Nostoc</i> sp.	ANT.L52.5	67	29	16	74	85	73	43	28	16	13	25	39	11	519
ULC38	<i>Nostoc</i> sp.	ANT.L52B.5	67	40	16	74	85	73	43	28	16	13	25	39	28	547
ULC39	<i>Nostoc</i> sp.	ANT.L52B.6	67	40	16	74	85	73	43	28	16	13	25	39	28	547
ULC46	<i>Nostoc</i> sp.	ANT.LG2.6	67	40	16	74	60	73	43	27	16	13	25	39	28	521
ULC50	<i>Nostoc</i> sp.	ANT.L34.1	67	40	17	74	88	73	54	31	16	13	25	39	27	564
ULC59	<i>Nostoc</i> sp.	ANT.L52B.8	67	40	16	74	85	73	43	28	16	13	25	39	28	547
ULC179	<i>Nostoc</i> sp.	OTC control	67	40	16	74	85	73	43	29	16	13	25	39	28	548
ULC423	<i>Nostoc</i> sp.	ANT.L36.1	67	40	17	74	88	73	54	31	16	13	25	39	27	564
ULC442	<i>Nostoc</i> sp.	214	67	33	16	74	88	73	39	28	16	13	25	105	26	603
ULC619	<i>Nostoc</i> sp.	SV009	67	33	22	74	80	73	42	27	16	13	27	25	20	519
ULC632	<i>Nostoc</i> sp.	V21	67	40	16	74	88	73	39	28	16	13	25	105	26	610
CP001037.1:2022977-2023620	<i>Nostoc punctiforme</i>	PCC 73102=ATCC 29133	67	40	17	74	82	73	39	33	16	13	25	106	28	613
MW652794.1	<i>Nostoc mirabile</i>	CHAB5756	67	40	18	74	55	73	42	34	16	13	26	40	29	527
MW652791.1	<i>Nostoc favosum</i>	CHAB5709	69	39	16	74	80	73	45	28	16	13	26	38	28	545
AY579899.1	<i>Nostoc desertorum</i>	CM1-VF14	67	39	16	74	79	73	39	31	16	13	25	39	27	538
MH427692.1	<i>Nostoc indistinguishendum</i>	CM1-VF10	67	41	16	74	83	73	43	31	16	13	25	39	28	549
MH427659.1	<i>Nostoc oromo</i>	ETH. 2.4. M.5	67	38	17	74	76	73	39	28	16	13	25	35	28	529
AY579894.1	<i>Nostoc lichenoides</i>	CNP-AK1	68	40	18	74	88	73	40	31	16	13	25	99	29	614
HQ877826.1	<i>Nostoc commune</i>	257-16	68	39	16	74	80	73	38	28	16	13	25	39	23	532
OQ785342.1	<i>Nostoc jammuese</i>	21A-PS	68	38	17	74	80	73	40	30	16	13	24	39	27	539
OQ785343.1	<i>Nostoc globosum</i>	4CHA-PS	67	39	16	75	89	73	41	28	16	13	25	39	26	547

GenBank Accession number	Taxon name		D1 stem	spacer + D2	spacer+D3 + spacer	tRNA Ile	spacer + V2 + spacer	tRNA Ala	post tRNA spacer	Box B	post box B spacer	Box A	D4 + spacer	V3	spacer + D5 + end	Total length of ITS
OQ785344.1	<i>Nostoc breve</i>	BG11-PS	67	33	16	74	42	73	38	28	16	13	25	39	-	-
OQ785345.1	<i>Nostoc coriaceum</i>	34TNA-PS	71	39	16	74	66	73	39	28	16	13	25	36	-	-
OR149082.1	<i>Nostoc montejanii</i> clone	46.4	67	40	16	74	74	73	41	28	16	13	25	40	26	533
OP688055.1	<i>Nostoc tlalocii</i> clone	1.4	68	38	18	74	73	73	42	28	16	13	25	35	28	531
PP454078	<i>Nostoc sikkimense</i>	RESHI-1B-PS	67	40	17	74	80	73	41	31	16	13	24	105	26	607
CP054698	<i>Nostoc edaphicum</i>	CCNP1411	67	33	16	74	88	73	40	30	16	13	25	100	26	601
JQ083655.1	<i>Spirirestis rafaensis</i>	WJT71-NPBG6	66	32	17	74	59	73	27	29	18	13	25	37	66	32
JQ083656.1	<i>Spirirestis rafaensis</i>	WJT71-NPBG7	66	32	17	74	59	73	27	29	18	13	25	37	66	32
KJ843312.1	<i>Halotia branconii</i>	CENA392	65	38	18	74	88	73	22	37	15	13	25	44	65	38
KY508610.1	<i>Kryptousia macronema</i>	CENA338	63	41	16	74	86	71	15	30	18	13	21	42	63	41
KF417425.1	<i>Goleter apudmare</i>	HA4340-LM2	65	32	16	74	80	73	15	26	17	13	26	35	65	32