

Plant growth-promoting potential of *Diaporthe osmanthi* COFS1, an endophyte of *Coleus forskohlii*

Riya Dutta¹, Debdulal Banerjee^{1,2}

¹Centre for Life Sciences, Vidyasagar University, Midnapore, India.

²Microbiology and Microbial Biotechnology Laboratory, Department of Botany and Forestry, Vidyasagar University, Midnapore, India.

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SUPPLEMENTARY MATERIAL

Supplementary table 1: Colonization rate of endophytic isolates

Host	<i>C. forskohlii</i>	
No. of segments plated (No. of segments colonized by EF)	Root	25(6)
	Stem	25(10)
	Leaf	25(8)
	Total	75(24)
No. of EF isolated	Root	3
	Stem	5
	Leaf	5
	Total	13
CR(%)	Root	24%
	Stem	42%
	Leaf	32%
	Total	32%

N.B: EF-Endophytic fungi

Supplementary table 2: IAA producing ability of endophytic isolates

Plant parts	Endophytic isolates	GenBank Accession No.	IAA production ($\mu\text{g mL}^{-1}$)	
			Without L-tryptophan	With L-tryptophan
Root	COFR1 (Sterile mycelia 1)	-	-	-
	COFR2 (<i>Fusarium</i> sp.)	OR297623	7.681 $\pm$ 0.12a	18.545 $\pm$ 0.03a ₁
	COFR3 (<i>Cochliobolus</i> sp.)	-	-	-
Stem	COFS1 (<i>Diaporthe osmanthi</i>)	ON927178	12.032$\pm$1.72b	31.72$\pm$0.04b₁
	COFS2 (<i>Fusarium</i> sp.)	-	-	0.3636 $\pm$ 0.14c ₁
	COFS3 (Sterile mycelia 2)	-	-	-
	COFS4 (Sterile mycelia 3)	-	-	2.1818 $\pm$ 0.03d ₁
	COFS5 (Sterile mycelia 4)	-	-	0.1818 $\pm$ 0.11e ₁
Leaf	COFL1 (<i>Helminthosporium</i> sp.)	-	0.212 $\pm$ 0.06c	0.450 $\pm$ 0.21e ₁
	COFL2 (<i>Fusarium</i> sp.)	-	0.454 $\pm$ 0.18c	9.1364 $\pm$ 0.01f ₁
	COFL3 (<i>Nigrospora</i> sp.)	-	4.363 $\pm$ 0.40d	8.0455 $\pm$ 0.02g ₁
	COFL4 (<i>Cladosporium</i> sp.)	-	3.984 $\pm$ 0.06d	15.546 $\pm$ 0.09h ₁
	COFL5 (<i>Chrysosporium</i> sp.)	-	-	4.929 $\pm$ 0.05i ₁

Supplementary table 3: Production of IAA by *Diaporthe osmanthi* COFS1 under different growth parameters

Parameters	Effectors	Percentage added (g L^{-1})	Dry weight (g)	IAA concentration ($\mu\text{g mL}^{-1}$)
Incubation time (days)	1	-	0.09 $\pm$ 0.028a	1.01 $\pm$ 0.069a ₁
	2	-	0.11 $\pm$ 0.057a	4.30 $\pm$ 0.109b ₁
	3	-	0.21 $\pm$ 0.011ab	6.54 $\pm$ 0.658c ₁
	4	-	0.48 $\pm$ 0.017b	14.32 $\pm$ 0.144d ₁
	5	-	0.64 $\pm$ 0.007bc	16.44 $\pm$ 0.121e ₁
	6	-	0.65 $\pm$ 0.028c	29.54 $\pm$ 0.121f ₁
	7	-	0.93$\pm$0.057c	31.72$\pm$0.184f₁
	8	-	1.22 $\pm$ 0.132cd	31.68 $\pm$ 0.028f ₁
	9	-	1.45 $\pm$ 0.080d	31.62 $\pm$ 0.589f ₁
	10	-	0.86 $\pm$ 0.086c	31.70 $\pm$ 0.046f ₁
pH	4	-	0.14 $\pm$ 0.017a	1.12 $\pm$ 0.104a ₁
	4.5	-	0.24 $\pm$ 0.011a	1.03 $\pm$ 0.300a ₁
	5	-	0.61 $\pm$ 0.040b	3.32 $\pm$ 0.196b ₁
	5.5	-	0.66 $\pm$ 0.104b	6.89 $\pm$ 0.069c ₁

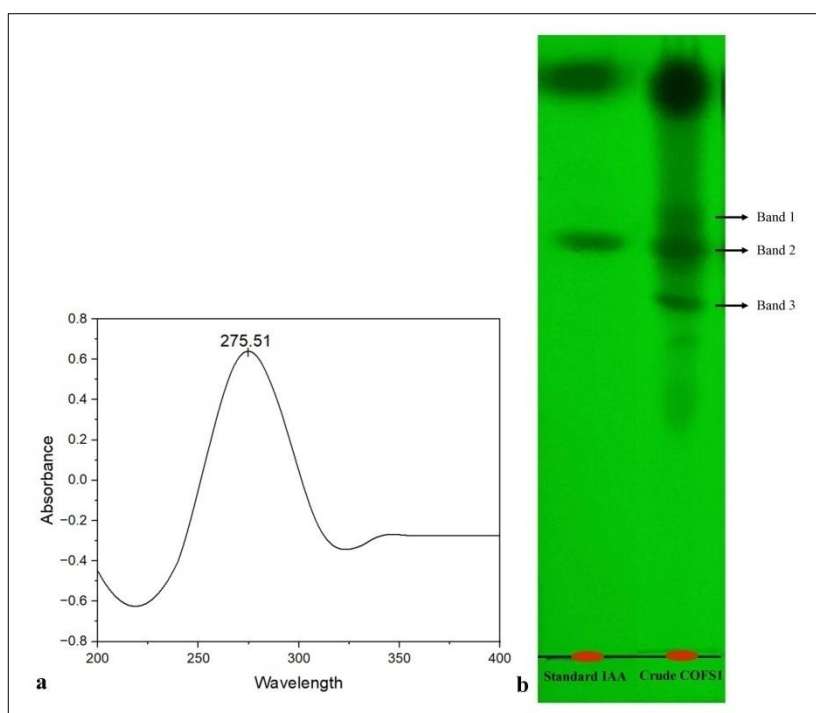
	6	-	0.88±0.034b	10.54±0.017d ₁
	6.5	-	0.98±0.028b	32.02±0.046e₁
	7	-	0.94±0.011b	30.45±0.092f ₁
	7.5	-	1.02±0.063bc	24.65±0.080g ₁
	8	-	0.67±0.015b	10.56±0.069d ₁
	8.5	-	0.32±0.132a	10.03±0.063d ₁
Temperature (°C)	20	-	0.84±0.086a	11.45±0.011a ₁
	25	-	1.05±0.017ab	21.05±0.011b ₁
	30	-	1.23±0.023b	32.45±0.009c₁
	35	-	0.94±0.011a	27.01±0.005d ₁
	40	-	0.56±0.069c	8.14±0.017e ₁
L-tryptophan Conc. (mg mL⁻¹)	-	0	0.62±0.034a	12.03±0.017a ₁
	-	0.1	0.84±0.017b	30.48±0.028b ₁
	-	0.2	0.78±0.017b	31.99±0.069b ₁
	-	0.3	1.06±0.011c	32.05±0.104b ₁ c ₁
	-	0.4	1.25±0.005d	32.77±0.606b ₁ c ₁
	-	0.5	1.66±0.005e	35.23±0.647c₁
	-	0.6	1.54±0.005f	35.08±0.046c ₁
	-	0.7	1.58±0.005f	32.74±0.109b ₁ c ₁
Carbon source	Dextrose	10	1.23±0.011a	17.36±0.075a ₁
	Fructose	10	0.52±0.011b	6.27±0.011b ₁
	Sucrose	10	1.52±0.086c	22.72±0.017c₁
	Lactose	10	0.23±0.028d	0.00±0.000d ₁
	Maltose	10	0.34±0.023bd	0.23±0.242d ₁
Nitrogen source	NH ₄ Cl	1	0.54±0.011a	1.27±0.011a ₁
	peptone	1	0.33±0.063a	0.00±0.000a ₁
	KNO ₃	1	0.45±0.132a	7.77±0.757b ₁
	NaNO₃	1	1.11±0.156b	19.22±0.046c₁
Sucrose concentration	-	26	1.02±0.104a	23.86±0.011a ₁
	-	28	1.35±0.069ab	30.40±0.005b ₁
	-	30	1.32±0.005ab	35.63±0.005c₁
	-	32	1.11±0.046a	34.27±0.023d ₁
	-	34	0.45±0.011b	4.54±0.069e ₁
NaNO₃ Concentration	-	1	0.78±0.080a	17.00±0.017a ₁
	-	1.5	0.75±0.144a	24.27±0.086b ₁
	-	2.0	0.93±0.017ab	28.63±0.063c₁
	-	2.5	1.17±0.028b	28.31±0.011d ₁
	-	3.0	0.35±0.011c	4.77±0.069e ₁

Supplementary table 4: Analysis of Variance for response surface model of COFS1

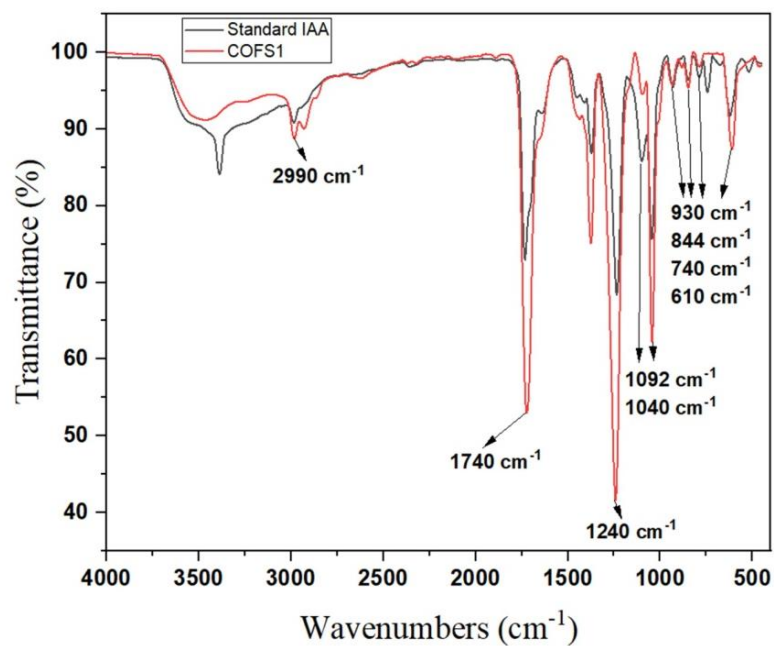
Source	Sum of squares	Degree of freedom	Mean square	F-value	<i>p</i> -value
Model	1140.89	14	81.492	8756.37	<0.001
Linear	50.82	4	12.706	1365.24	<0.001
A (Incubation time)	7.89	1	7.889	847.72	<0.001
B (pH)	0.95	1	0.946	101.69	<0.001
C (L-tryptophan concentration)	29.36	1	29.359	3154.68	<0.001
D (Sucrose concentration)	12.63	1	12.628	1356.88	<0.001
A ²	809.31	4	809.309	86960.53	<0.001
B ²	257.45	1	257.447	27662.79	<0.001
C ²	278.07	1	278.073	29878.99	<0.001
D ²	254.39	1	254.392	27334.45	<0.001
AB	0.12	1	0.492	13.16	0.003
AC	1.02	6	0.122	109.61	<0.001
AD	1.22	1	1.020	131.20	<0.001
BC	0.08	1	1.221	8.13	0.013
BD	0.03	1	0.076	2.75	0.119
CD	0.49	1	0.026	52.65	<0.001
Error	0.13	14	0.009	-	-
Lack-of-Fit	0.08	10	0.008	0.61	0.759
Pure Error	0.05	4	0.013	-	-
Total	0.26	28	-	-	-

Supplementary table 5: Different metabolites synthesized by endophytic fungi COFS1 presented along with retention time and area percentage

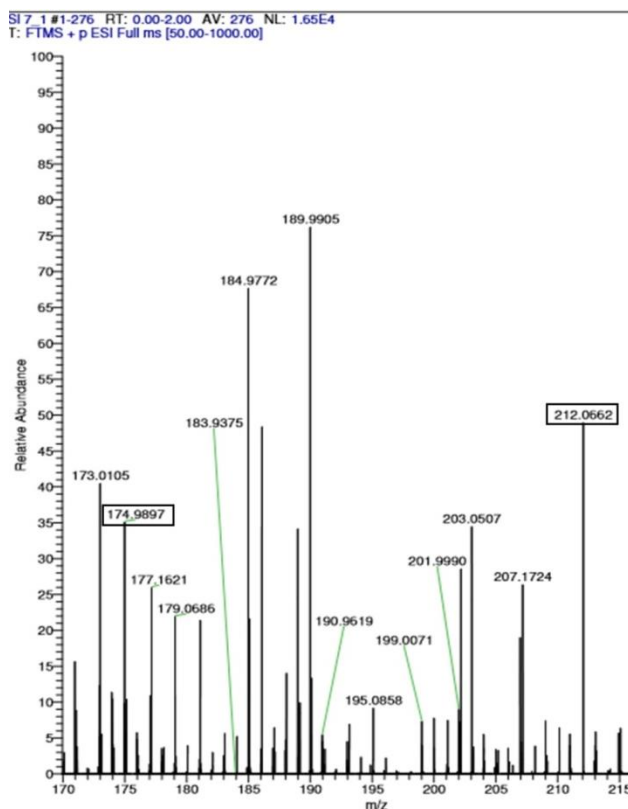
Sl. No.	Compounds with Molecular formula	Retention time	Molecular Weight (g mol ⁻¹)	Area percentage
1.	Phenylethyl Alcohol (C ₈ H ₁₀ O)	9.42	122	5.4
2.	1-Methylene-1H-indene (C ₁₀ H ₈)	10.52	128	18.46
3.	1H-Indole, 4-methyl (C ₉ H ₉ N)	13.35	131	20.27
4.	Indole-3-acetic acid, methyl ester (C ₁₁ H ₁₁ NO ₂)	14.50	189	40.99
5.	2-Naphthol (C ₁₁ H ₁₀ O ₂)	16.76	174	2.52
6.	Tryptophol (C ₁₀ H ₁₁ NO)	17.88	161	9.45
7.	Indole-6-carboxaldehyde (C ₉ H ₇ NO)	18.52	145	1.35
8.	2H-1-Benzopyran-2-one (C ₉ H ₆ O ₄)	18.67	178	1.8
9.	Dibutyl Phthalate (C ₁₆ H ₂₂ O ₄)	20.29	278	8.4



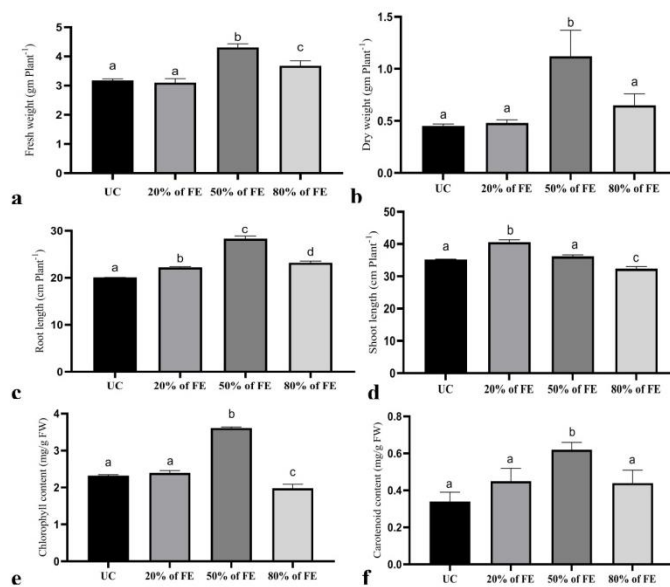
Supplementary figure 1: (a) UV-VIS spectral scan of COFS1; (b) Thin layer chromatography of standard IAA and crude COFS1



Supplementary figure 2: Comparison of FTIR spectra of standard IAA and crude extract of COFS1



Supplementary figure 3: HR-MS analysis of a methanolic extract of band-2 of crude COFS1 extract



Supplementary figure 4: Effect of endophytic culture filtrate of COFS1 on the growth parameters of wheat. (a-f). The error bar represents the standard deviation of means from replicates. Different letters indicate differences in plant growth parameters compared to the control at $P < 0.05$