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Research article

Nocardiopsis synnemataformans NBRM9, an extremophilic actinomycete producing extremozyme cellulase, using lignocellulosic agro-wastes and its biotechnological applications

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Supplementary

Table S1. Geographical locations of sampling sites and properties of the collected soils.

Location	Site No.	GPS		Sample code	Number of samples	Sample characteristics
		Latitude (N)	Longitude (E)			
Rafha	1	43°31'30’N	35°36'29'E	R-S1	4	Yellow, sandy, dry, soft, and rough soil
		43°29'10’N	34°39'29'E	R-S2	4	
		43°32'52’N	08°40'29'E	R-S3	4	
Al-Markooz	2	42°38'02’N	35°07'30'E	M-S1	3	Yellowish black, sandy, dry, soft soil
		42°38'10’N	18°08'30'E	M-S2	2	
		42°37'33’N	14°08'30'E	M-S3	2	
Al-Owiqela	3	42°15'10’N	56°19'30'E	O-S1	3	Yellow, sandy, dry, rough soil
		42°15'26’N	25°22'30'E	O-S2	2	
		42°12'33’N	44°22'30'E	O-S3	3	
Al-Kasb	4	41°55'37’N	33°30'30'E	K-S1	4	Yellow, sandy, dry, rough soil
		41°55'42’N	05°30'30'E	K-S2	3	
		41°56'31’N	56°29'30'E	K-S3	2	

Table S2. Cultures of isolated actinomycetes and their cellulase activity on solid CMC media.

Location/ Site No	Isolate code	Colony morphology*				Cellulase activity*
		Color	Form	Consistency	Pigment	
1–S1	NBRR1	Medium gray	Irregular	Rough	ND	0.0
1–S2	NBRR2	Creamy	Irregular	Smooth	Brown	0.0
1–S3	NBRR3	White	Circular	Smooth	ND	0.55 ± 1.15
	NBRR4	Gray	Circular	Rough	ND	0.0
	NBRR5	Light gray	Circular	Rough	ND	0.0
	NBRR6	White	Circular	Smooth	ND	0.0
2–S1	NBRM7	Light gray	Circular	Rough	Red	0.91 ± 0.5
	NBRM8	Gray	Irregular	Smooth	ND	0.0
	NBRM9	White	Circular	Smooth	ND	3.26 ± 0.66
	NBRM10	Gray	Punctiform	Smooth	Orange	2.60 ± 0.32
	NBRM11	Gray	Punctiform	Rough	ND	0.0
2–S2	NBRM12	Light orange	Irregular	Smooth	ND	1.00 ± 0.47
	NBRM13	White	Irregular	Smooth	ND	0.87 ± 0.37
2–S3	NBRM14	Medium gray	Circular	Smooth	ND	0.0
	NBRM15	Creamy	Irregular	Rhizoid	Orange	1.59 ± 0.32
	NBRM16	Creamy	Circular	Rhizoid	Brown	1.03 ± 0.43
	NBRM16	Light orange	Circular	Smooth	Violet	1.90 ± 1.13
3–S2	NBRO18	White	Circular	Rough	ND	0.0
	NBRO19	Light gray	Circular	Rough	ND	0.0
	NBRO20	Light gray	Circular	Rough	Blue	0.0
	NBRO21	Purple	Circular	Smooth	Red	0.0
4–S1	NBRK22	Medium gray	Punctiform	Smooth	Orange	1.03 ± 0.73
	NBRK23	Medium gray	Punctiform	Smooth	Brown	0.0
	NBRK24	White	Irregular	Rough	Red	0.0
4–S2	NBRK25	Medium gray	Circular	Smooth	Red	0.85 ± 0.41
	NBRK26	White	Irregular	Smooth	ND	0.0
	NBRK27	White	Punctiform	Smooth	ND	0.0
	NBRK28	White	Irregular	Smooth	Violet	1.73 ± 0.95
4–S3	NBRK29	Light gray	Circular	Rough	ND	0.0
	NBRK30	Light gray	Circular	Rough	Brown	0.0
	NBRK31	Medium gray	Irregular	Smooth	ND	0.0
Incidence of activity			**Total isolates**		**31**	**12**
			%		**100**	**38.70**

* The presented morphological features were recorded from the growth on isolation media (ISP–4).

*Average index of cellulase enzyme activity.

Table S3. Cultural characteristics of the actinomycete isolate NBRM9 allowed to grow on 7-ISP and other growth media.

Media	Growth rate	Age (days)	Mycelium color		Soluble pigments
			Aerial mycelium	Substrate mycelium	
ISP medium 1 (Liquid tryptone yeast extract)	Abundant	7	White (ISCC-NBS 263)	White (ISCC-NBS 263)	None
		14	Pale yellow (ISCC-NBS 89)	Pale greenish yellow (ISCC-NBS 104)	None
		21	Pale yellow (ISCC-NBS 89)	Pale orange yellow (ISCC-NBS 73)	None
ISP medium 2 (Yeast extract-malt extract agar)	Abundant	7	White (ISCC-NBS 263)	Pale yellow (ISCC-NBS 89)	None
		14	White (ISCC-NBS 263)	Pale orange yellow (ISCC-NBS 73)	None
		21	Yellowish white (ISCC-NBS 92)	Pale orange yellow (ISCC-NBS 73)	None
ISP medium 3 (Oatmeal agar)	No growth	7-21	-	-	-
ISP medium 4 (Inorganic salts-starch agar)	Abundant	7	White (ISCC-NBS 263)	White (ISCC-NBS 263)	None
		14	Yellowish white (ISCC-NBS 92)	Yellowish gray (ISCC-NBS 93)	None
		21	Yellowish white (ISCC-NBS 92)	Pale greenish yellow (ISCC-NBS 104)	None
ISP medium 5 (Glycerol asparagine agar)	Moderate	7	Pale yellow (ISCC-NBS 89)	Yellowish white (ISCC-NBS 92)	None
		14	Pale yellow (ISCC-NBS 89)	Light yellow (ISCC-NBS 86)	None
		21	Light orange yellow (ISCC-NBS 70)	Deep orange yellow (ISCC-NBS 69)	None
ISP medium 6 (Peptone yeast extract iron agar)	Moderate	7	Yellowish white (ISCC-NBS 92)	Vivid yellow (ISCC-NBS 82)	None
		14	Yellowish white (ISCC-NBS 92)	Pale yellow (ISCC-NBS 89)	None
		21	Yellowish gray (ISCC-NBS 93)	Strong orange yellow (ISCC-NBS 68)	None
ISP medium 7 (Tyrosine agar)	Moderate	7	White (ISCC-NBS 263)	Dark yellow (ISCC-NBS 88)	None
		14	White (ISCC-NBS 263)	Dark yellow (ISCC-NBS 88)	None
		21	White (ISCC-NBS 263)	Light yellowish brown (ISCC-NBS 76)	None
Starch-nitrate agar	Abundant	7	White (ISCC-NBS 263)	White (ISCC-NBS 263)	None
		14	Pale greenish yellow (ISCC-NBS 104)	Light orange yellow (ISCC-NBS 70)	None
		21	Pale orange yellow (ISCC-NBS 73)	Light orange yellow (ISCC-NBS 70)	None

Table S4. Box–Behnken design levels of independent factors.

No.	Factor	Variables	Units	Range		
				Minimum	Maximum	Mean
1	A	Temperature	°C	30	50	40
2	B	pH	-	5	9	7
3	C	Incubation time	Day	3	7	5
4	D	Substrate concentration	g	1	5	3

Table S5. Box–Behnken design of optimization variables with experimental and predicted cellulase activity of *N. synnemataformans* NBRM9.

Run No.	Variables				Cellulase activity (U/mL)	
	A	B	C	D	Exp. response	Pred. response
1	40	7	7	3	11.77	12.07
2	40	7	3	3	10.84	10.85
3	40	5	7	2	11.00	10.83
4	40	7	7	1	9.97	10.14
5	40	5	5	1	8.94	8.96
6	50	7	5	1	8.68	8.54
7	40	7	3	1	10.87	10.76
8	40	5	5	3	12.16	12.14
9	30	9	5	2	12.08	12.25
10	40	9	3	2	11.24	11.02
11	30	7	3	2	12.25	12.35
12	50	7	3	2	9.82	9.87
13	30	7	5	1	11.56	11.40
14	40	7	5	2	10.88	10.86
15	40	7	5	2	10.87	10.86
16	30	7	7	2	12.51	12.67
17	50	5	5	2	9.25	9.26
18	50	9	5	2	10.24	10.44
19	50	7	7	2	10.04	10.15
20	40	7	5	2	10.84	10.86
21	40	9	5	3	10.28	10.46
22	40	9	5	1	11.40	11.62
23	30	5	5	2	12.45	12.44
24	50	7	5	3	10.13	9.91
25	30	7	5	3	12.29	12.04
26	40	9	7	2	13.20	12.63
27	40	5	3	2	11.67	11.84

Exp. (experimental); Pred. (predicted).

Table S6. Estimated regression coefficients for the quadratic polynomial model and analysis of variance for the experimental results of cellulase production by *N. synnemataformans* NBRM9.

Source	DF	Sum of squares	Mean of squares	F value	*p* value	Prob > F
Model	14.00	33.6197	2.4014	31.56	0.000	Significant
Linear	4.00	22.7774	5.6944	74.83	0.000	
A	1.00	18.7143	18.7143	245.92	0.000	
B	1.00	0.7323	0.7323	9.62	0.009	
C	1.00	0.2737	0.2737	3.60	0.082	
D	1.00	3.0571	3.0571	40.17	0.000	
Square	4.00	3.0030	0.7507	9.87	0.001	
A^2	1.00	0.0097	0.0097	0.13	0.727	
B^2	1.00	0.4211	0.4211	5.53	0.037	
C^2	1.00	1.0333	1.0333	13.58	0.003	
D^2	1.00	0.6364	0.6364	8.36	0.014	
2-Way Interaction	6.00	7.8393	1.3066	17.17	0.000	
AB	1.00	0.4670	0.4670	6.14	0.029	
AC	1.00	0.0004	0.0004	0.00	0.946	
AD	1.00	0.1303	0.1303	1.71	0.215	
BC	1.00	1.7134	1.7134	22.51	0.000	
BD	1.00	4.6897	4.6897	61.62	0.000	
CD	1.00	0.8387	0.8387	11.02	0.006	
Error	12.00	0.9132	0.0761			
Lack-of-Fit	10.00	0.9124	0.0912	227.29	0.004	
Pure Error	2.00	0.0008	0.0004			
Total	26.00	34.5330				Not significant

R^2 (0.9736), adjusted R^2 (0.9427), predicted R^2 (0.8478), df (degree of freedom), highly significant ($p \leq 0.0001$), significant ($p \leq 0.05$), and nonsignificant ($p > 0.05$).

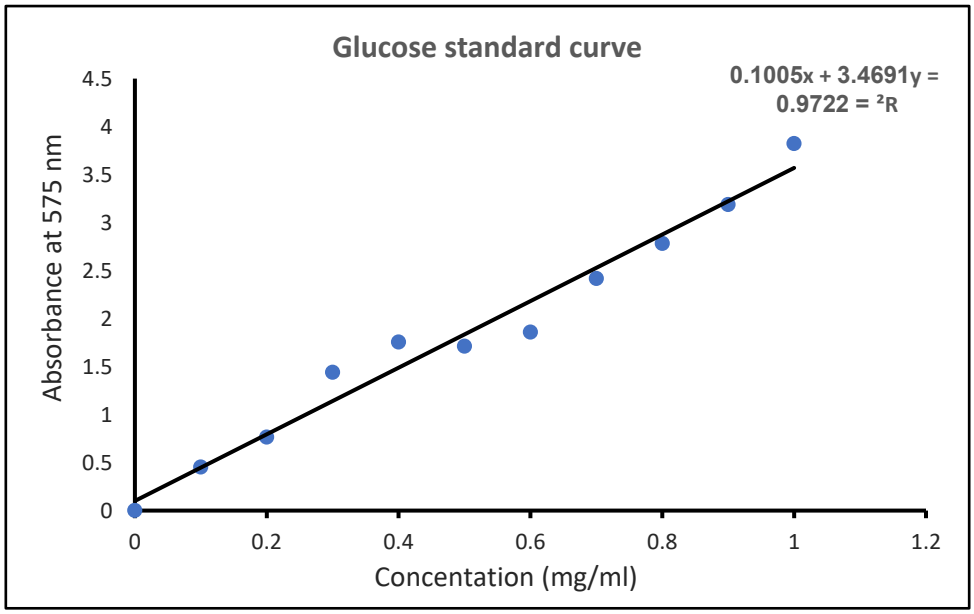

Figure S1. Glucose calibration curve.

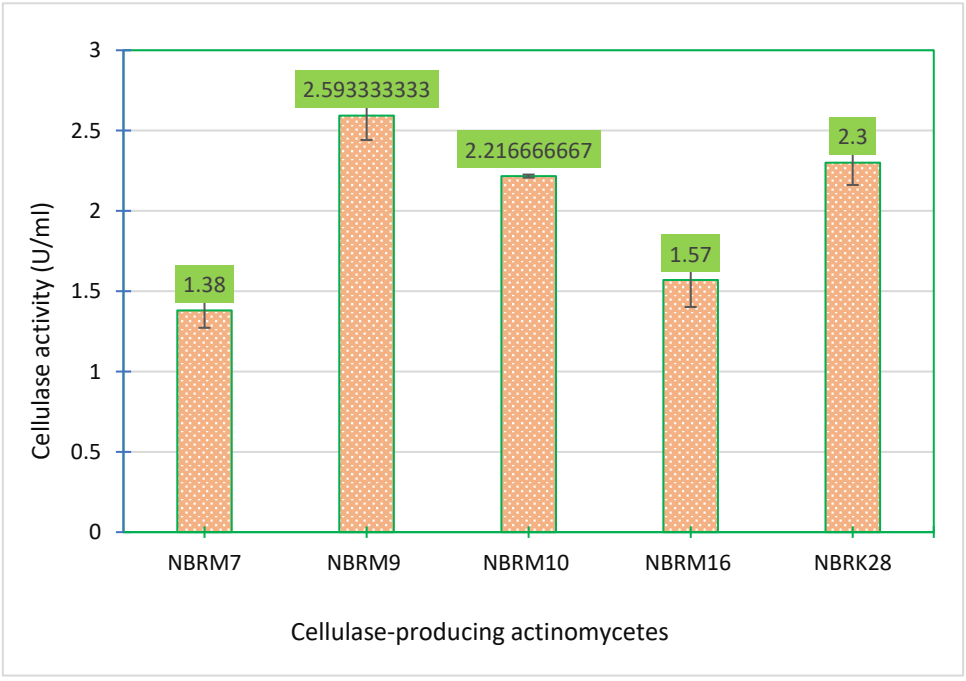

Figure S2. Cellulase activity of actinomycetes using CMC as the sole carbon source in liquid media.

Figure S3. Cellulase production activities of the most effective actinomycete NBRM9 using the different agro-industrial wastes under SmF.