Agro-wastes as feedstock for the production of endo-β-xylanase from Cohnella sp. strain AR92

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Online supplementary fig. 1. Maximum-likelihood (**a**) and maximum-parsimony (**b**) phylogenetic trees based on 16S rRNA gene sequences, showing the position of strain AR92 and related taxa. Bootstrap values are shown as percentages of 100 replicates; only values above 70% are shown. The four species of the genus *Paenibacillus* were used as outgroup. Bar, 0.02 substitutions per nucleotide position. Accession numbers are shown after names

Online supplementary fig. 2. Effect of different nitrogen sources on the *Cohnella* sp. AR92 growth (●, biomass at 96 h) and xylanase production at (■) 48 h; (■) 96 h and (■) 192 h. Error bars represent the standard deviation from at least three independent experiments

Online supplementary fig. 3. Response surface and contour plots of the combined effects on xylanase production (**a**) of yeast extract and phosphor concentrations, and (**b**) of yeast extract and carbon (OH-SCB) concentrations

Online supplementary table 1. Plackett-Burman design matrix and the response variables evaluated

Run	Coded values*						Xylanase Activity [IU/ml]	Biomass [CFU/ml]
	C	**N**	**Ca^{2+}**	**Mg^{2+}**	**YE**	**P**		
1	-1	+1	+1	+1	+1	+1	11.2	$7.0 \cdot 10^7$
2	+1	-1	-1	+1	+1	-1	4.3	$1.2 \cdot 10^7$
3	-1	-1	-1	-1	+1	-1	5.2	$9.0 \cdot 10^6$
4	+1	+1	+1	+1	-1	-1	0.9	$3.0 \cdot 10^6$
5	+1	-1	+1	-1	+1	+1	4.9	$4.5 \cdot 10^7$
6	+1	-1	-1	-1	-1	+1	0.9	$5.0 \cdot 10^6$
7	+1	+1	-1	-1	+1	+1	5.3	$2.5 \cdot 10^8$
8	+1	-1	+1	+1	-1	+1	4.8	$2.0 \cdot 10^6$
9	+1	+1	+1	-1	-1	-1	0.5	$2.3 \cdot 10^6$
10	-1	-1	-1	-1	-1	-1	0.8	$7.2 \cdot 10^5$
11	+1	-1	+1	+1	+1	+1	8.1	$7.0 \cdot 10^7$
12	-1	+1	+1	-1	-1	+1	3.3	$2.0 \cdot 10^7$
13	-1	-1	+1	+1	-1	-1	3.1	$8.8 \cdot 10^5$
14	-1	-1	-1	+1	-1	+1	3.5	$2.5 \cdot 10^6$
15	+1	+1	-1	+1	-1	+1	2.5	$7.0 \cdot 10^6$
16	+1	+1	+1	+1	+1	-1	4.4	$2.0 \cdot 10^7$
17	+1	+1	-1	-1	-1	-1	0.2	$7.5 \cdot 10^5$
18	+1	-1	-1	+1	+1	-1	4.1	$1.5 \cdot 10^7$
19	-1	+1	-1	+1	-1	-1	3.7	$8.5 \cdot 10^5$
20	-1	+1	-1	+1	+1	+1	13.7	$5.5 \cdot 10^7$
21	-1	+1	-1	-1	+1	+1	7.9	$2.5 \cdot 10^7$
22	+1	-1	+1	-1	-1	+1	0.9	$9.5 \cdot 10^6$
23	-1	-1	+1	-1	+1	-1	5.4	$7.5 \cdot 10^6$
24	-1	+1	+1	-1	+1	-1	4.2	$6.0 \cdot 10^6$

* Coded value (-1) represented the low levels of the studied factors (g/l): OH-SCB (C) 5.0; $(NH4)_2SO_4$ (N) 0.5; $CaCl_2 \cdot 2H_2O$ (Ca^{2+}) 0.0; $MgCl_2 \cdot 6H_2O$ (Mg^{2+}) 0.0; yeast extract (YE) 0.1 and Na_2HPO_4/KH_2PO_4 (P) 0.35/0.1. Coded value (+1) denoted the high levels of the independent variables: C 10.0; N 1.0; Ca^{2+} 0.1; Mg^{2+} 0.5; YE 1.0 and P 3.5/1.0.

Online supplementary table 2. Estimated effects for xylanase production and growth of *Cohnella* sp. strain AR92 from the Plackett-Burman design

Factor	Xylanase Activity [IU/ml]			Biomass [CFU/ml]		
	Effect	Standard error	*p*- value	Effect	Standard error	*p*- value
C	-2.482	0.498	0.000	0.292	0.099	0.009
N	0.988	0.498	0.063	0.192	0.099	0.071
P	2.512	0.498	0.000	0.708	0.099	0.000
YE	4.467	0.498	0.000	1.008	0.099	0.000
Mg^{2+}	2.068	0.498	0.001	-0.058	0.099	0.565
Ca^{2+}	-0.012	0.498	0.982	0.108	0.099	0.291

Online supplementary table 3. Box-Behnken matrix for the three selected factors and their experimental and predicted values of xylanase activity
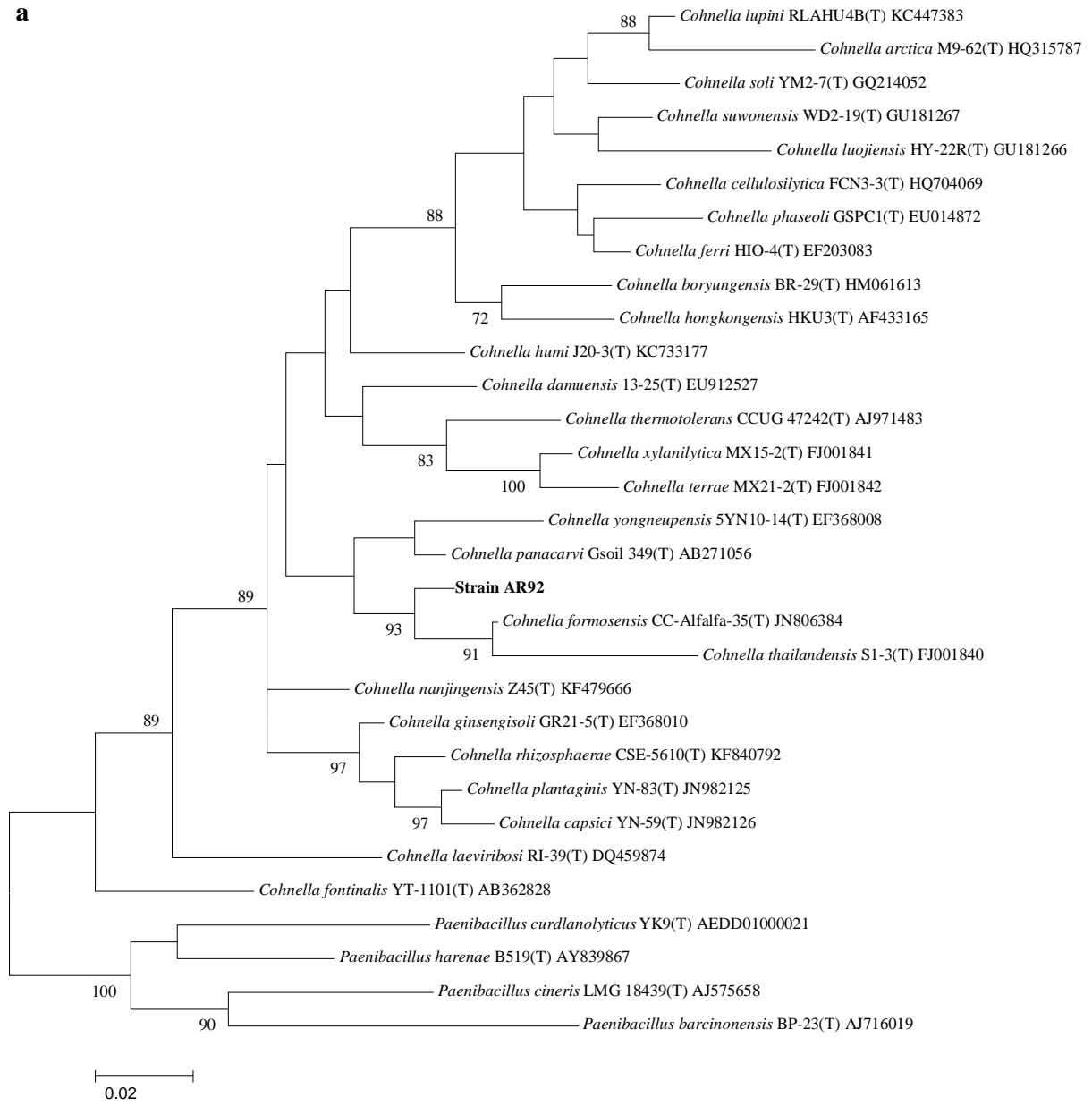
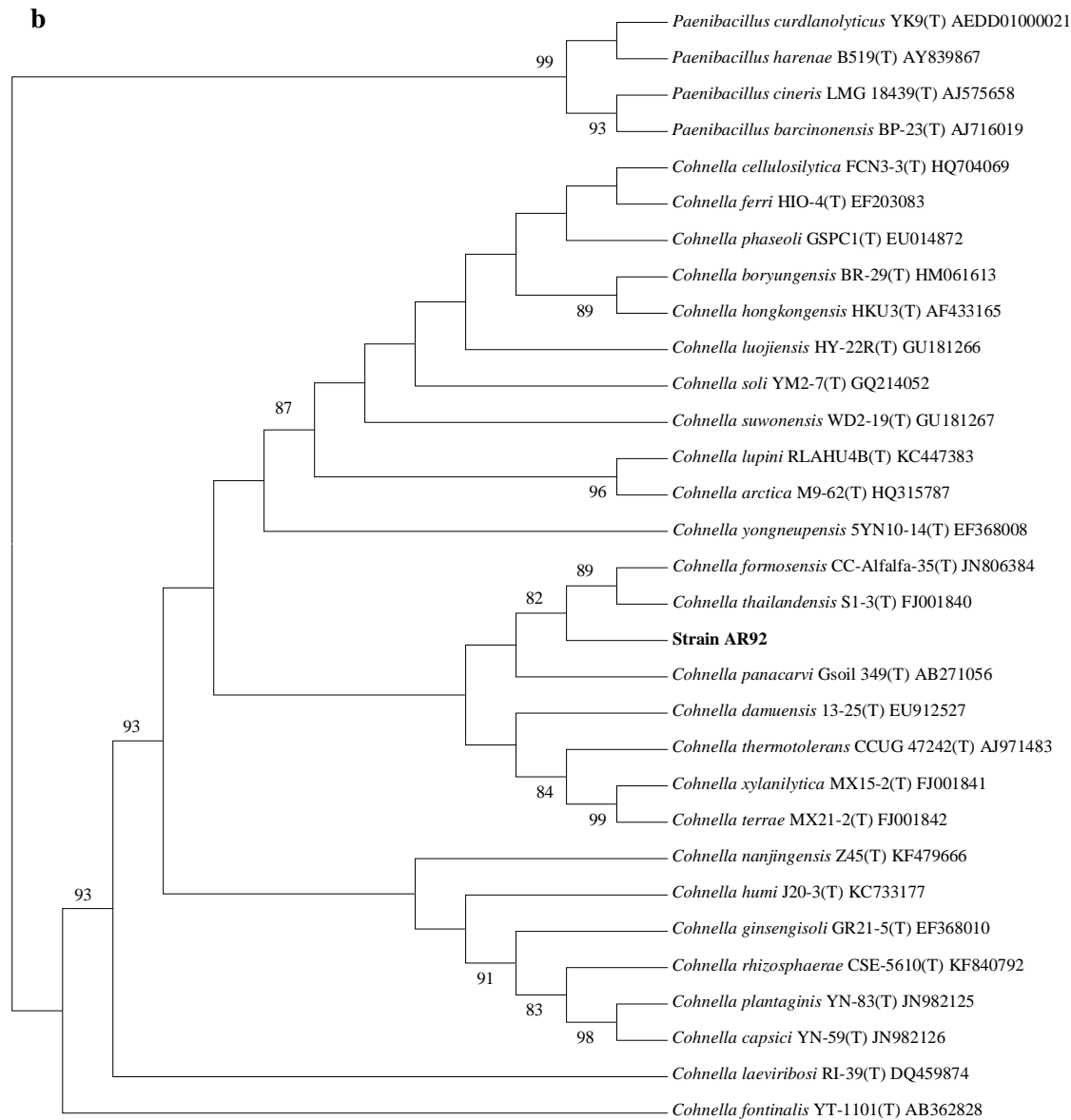| Run	Values (g/l)			Produced EA [IU/ml]	Predicted EA [IU/ml]
	C	**YE**	**P**		
1	6.0	1.5	5.25/1.50	14.2	14.5
2	10.0	1.0	1.75/0.50	12.4	12.2
3	6.0	1.0	3.50/1.00	13.9	14.5
4	6.0	1.5	1.75/0.50	11.4	11.9
5	2.0	0.5	3.50/1.00	6.5	6.3
6	2.0	1.0	1.75/0.50	7.2	7.1
7	2.0	1.0	5.25/1.50	8.1	8.6
8	6.0	1.0	3.50/1.00	14.6	14.5
9	6.0	0.5	1.75/0.50	9.9	9.4
10	10.0	0.5	3.50/1.00	9.7	10.3
11	6.0	1.0	3.50/1.00	14.6	14.5
12	6.0	0.5	5.25/1.50	11.3	10.3
13	10.0	1.5	3.50/1.00	14.7	14.8
14	10.0	1.0	5.25/1.50	14.2	14.1
15	2.0	1.5	3.50/1.00	8.9	8.4
16	6.0	1.0	3.50/1.00	14.5	14.5
17	6.0	1.0	3.50/1.00	14.6	15.0
18	2.0	1.5	3.50/1.00	9.7	8.9
19	6.0	1.5	1.75/0.50	12.4	12.5
20	2.0	0.5	3.50/1.00	6.8	6.7
21	10.0	1.0	1.75/0.50	13.4	12.7
22	2.0	1.0	5.25/1.50	8.7	9.1
23	2.0	1.0	1.75/0.50	6.9	7.6
24	6.0	0.5	1.75/0.50	9.7	9.9
25	10.0	0.5	3.50/1.00	10.2	10.8
26	6.0	1.0	3.50/1.00	15.5	15.0
27	6.0	0.5	5.25/1.50	10.6	10.8
28	10.0	1.5	3.50/1.00	15.2	15.4
29	6.0	1.0	3.50/1.00	15.1	15.0
30	10.0	1.0	5.25/1.50	15.1	14.6
31	6.0	1.0	3.50/1.00	15.4	15.0
32	6.0	1.5	5.25/1.50	15.1	15.0

Online supplementary table 4. Estimated effects and statistical analysis for xylanase production from the results of the Box-Behnken design

	Estimated effects			ANOVA		
Factor	Effect	Standard error	Sum of squares	Degree of freedom	F-value	p-value
C	5.418	0.262	106.245	1	428.56	0.000
YE	3.194	0.262	36.926	1	148.95	0.000
P	1.735	0.249	12.041	1	48.57	0.000
CC	-5.797	0.361	63.849	1	257.55	0.000
C:YE	1.471	0.387	3.5772	1	14.43	0.001
C:P	0.210	0.352	0.088	1	0.36	0.558
YE:YE	-3.667	0.361	25.548	1	103.06	0.000
YE:P	0.845	0.352	1.428	1	5.76	0.026
P:P	-2.280	0.361	9.881	1	39.86	0.000

Online supplementary figure 1

a

- 88 — *Cohnella lupini* RLAHU4B(T) KC447383
- *Cohnella arctica* M9-62(T) HQ315787
- *Cohnella soli* YM2-7(T) GQ214052
- *Cohnella suwonensis* WD2-19(T) GU181267
- *Cohnella luojiensis* HY-22R(T) GU181266
- *Cohnella cellulosilytica* FCN3-3(T) HQ704069
- *Cohnella phaseoli* GSPC1(T) EU014872
- *Cohnella ferri* HIO-4(T) EF203083
- *Cohnella boryungensis* BR-29(T) HM061613
- 72 — *Cohnella hongkongensis* HKU3(T) AF433165
- *Cohnella humi* J20-3(T) KC733177
- *Cohnella damuensis* 13-25(T) EU912527
- *Cohnella thermotolerans* CCUG 47242(T) AJ971483
- 83 — *Cohnella xylanilytica* MX15-2(T) FJ001841
- 100 — *Cohnella terrae* MX21-2(T) FJ001842
- *Cohnella yongneupensis* 5YN10-14(T) EF368008
- *Cohnella panacarvi* Gsoil 349(T) AB271056
- **Strain AR92**
- 93 — *Cohnella formosensis* CC-Alfalfa-35(T) JN806384
- 91 — *Cohnella thailandensis* S1-3(T) FJ001840
- *Cohnella nanjingensis* Z45(T) KF479666
- *Cohnella ginsengisoli* GR21-5(T) EF368010
- 97 — *Cohnella rhizosphaerae* CSE-5610(T) KF840792
- *Cohnella plantaginis* YN-83(T) JN982125
- 97 — *Cohnella capsici* YN-59(T) JN982126
- *Cohnella laeviribosi* RI-39(T) DQ459874
- *Cohnella fontinalis* YT-1101(T) AB362828
- *Paenibacillus curdlanolyticus* YK9(T) AEDD01000021
- *Paenibacillus harenae* B519(T) AY839867
- *Paenibacillus cineris* LMG 18439(T) AJ575658
- 100 / 90 — *Paenibacillus barcinonensis* BP-23(T) AJ716019
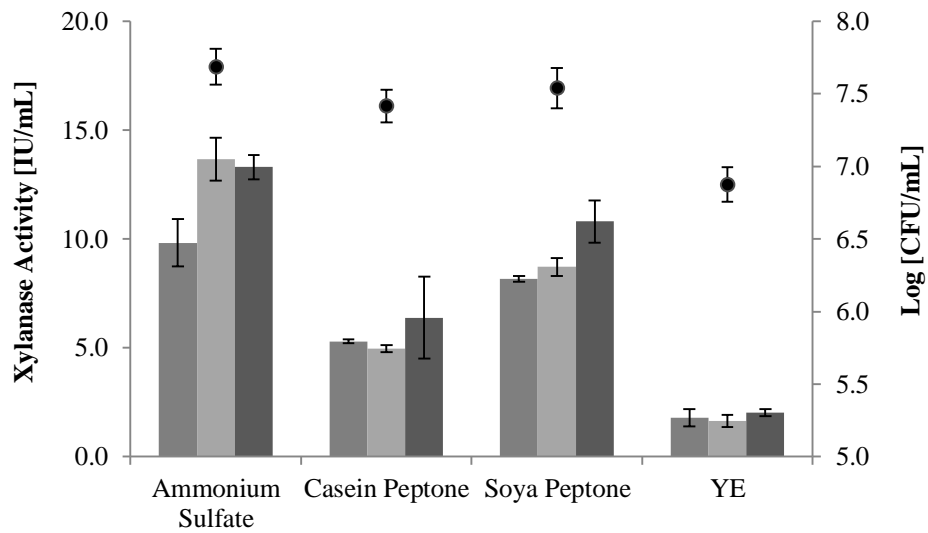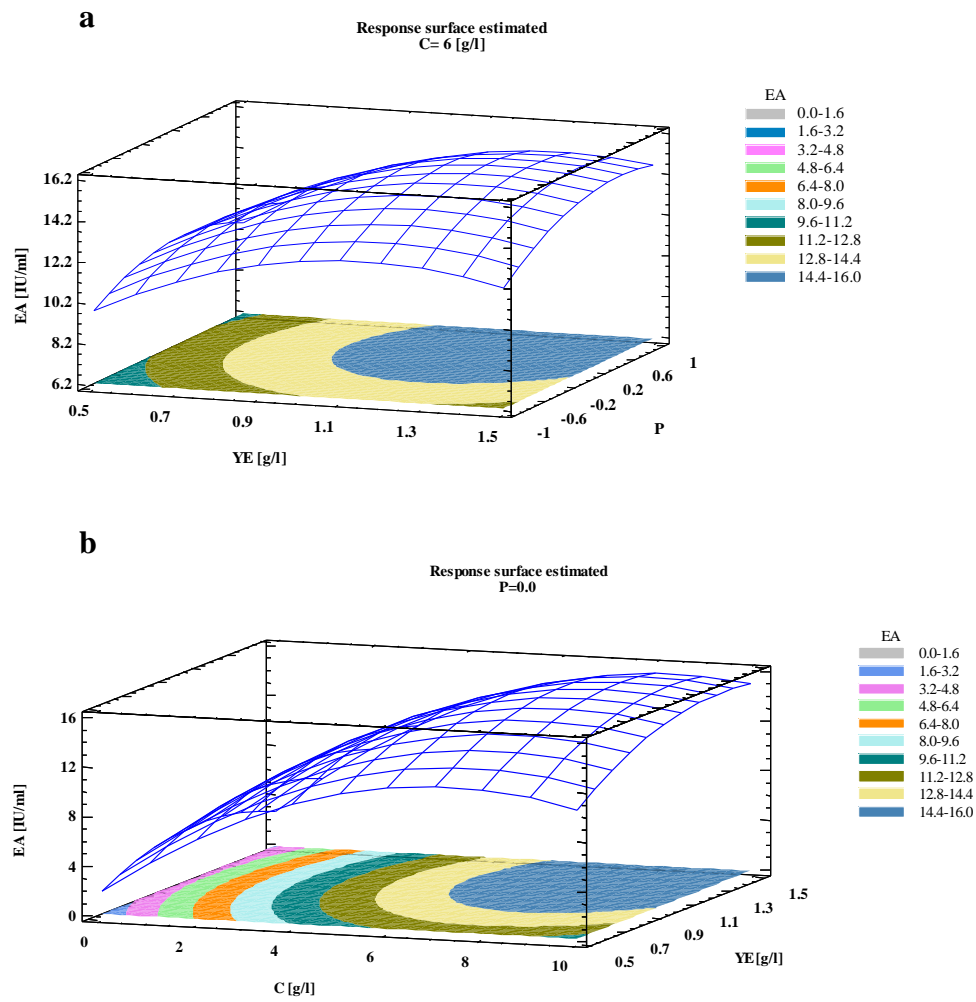
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Online supplementary figure 2

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a

b

Online supplementary fig. 3. Response surface and contour plots of the combined effects on xylanase production **(a)** of yeast extract and phosphor concentrations, and **(b)** of yeast extract and carbon (OH-SCB) concentrations.