
SUPPLEMENTARY MATERIALS

Antifungal Activity and Biocontrol Potential of *Simplicillium lamellicola* JC-1 against Multiple Fungal Diseases of Oilseed Rape

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SUPPLEMENTARY FIGURES

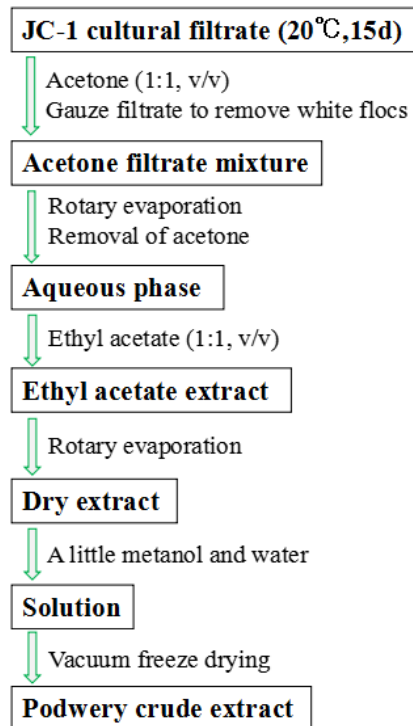


Figure S1. The procedure for obtaining the crude extract from the cultures of *Simplicillium lamellicola* JC-1 in potato dextrose broth.

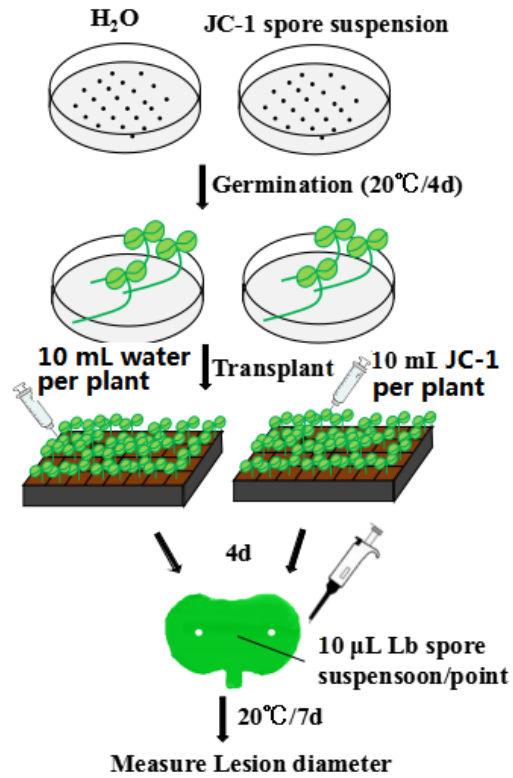


Figure S2. The procedure for determination of the induced systemic resistance of *S. lamellicola* JC-1 against infection by *L. biglobosa* in oilseed rape.

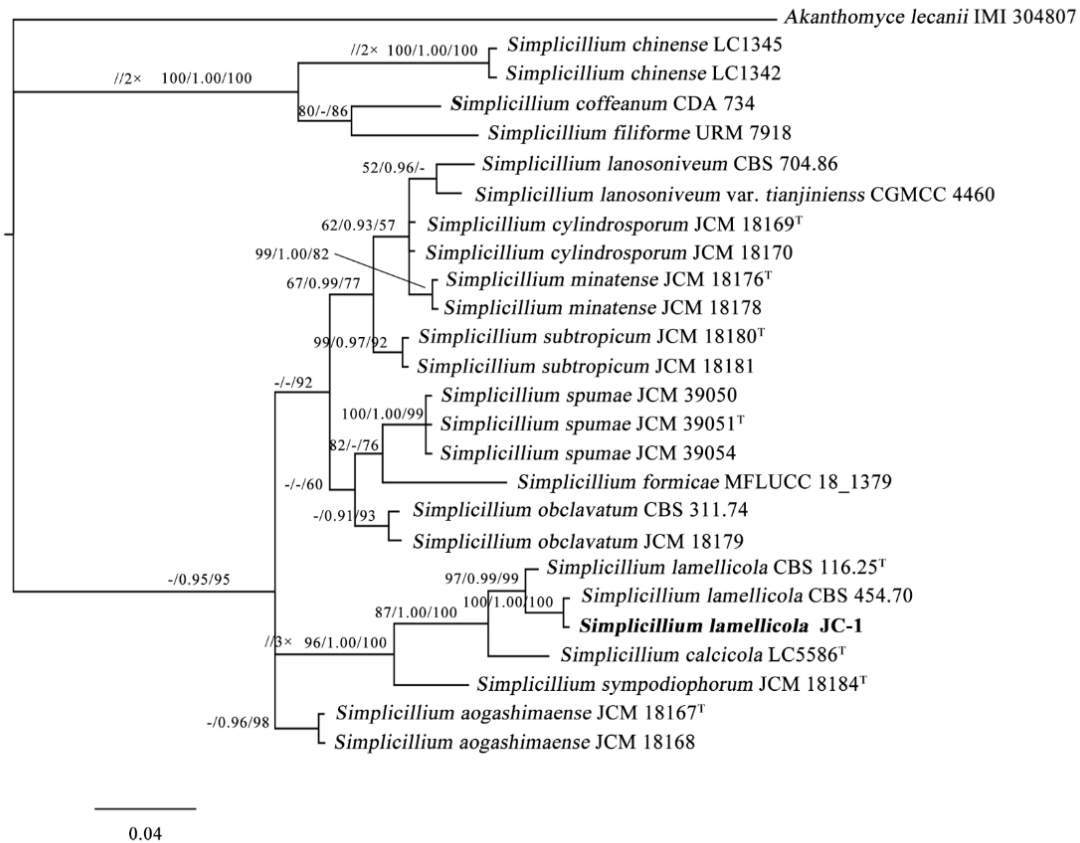


Figure S3. Phylogenetic tree of 26 fungal taxa in the genus *Simplicillium* and *Akanthomyces lecanii* (out-group). The tree was constructed using concatenated sequences of ITS (see Table 1 for GenBank Acc. Nos.). RAxML bootstrap support values (ML) higher than 50, Bayesian posterior probability (PP) higher than 0.90 and maximum parsimony (MP) bootstrap support values higher than 50 were shown at the nodes (ML/PP/MP). Scale bar indicates 0.4% sequence divergence. Type strains labeled with 'T' at the top-right corner.

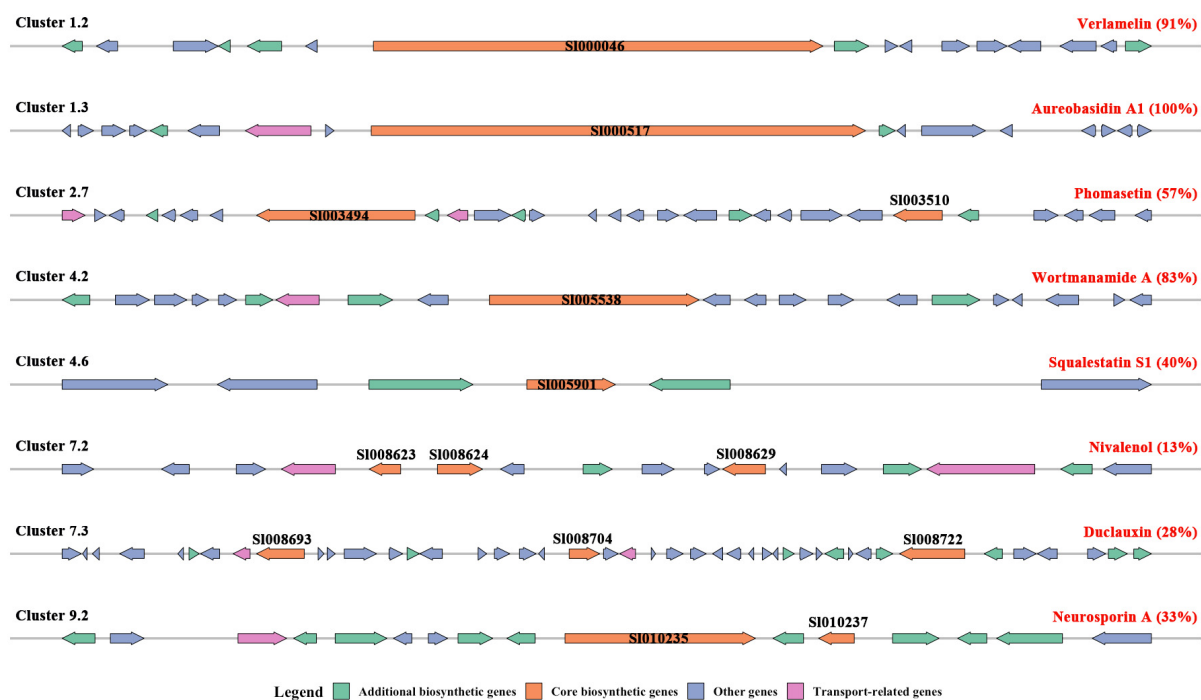


Figure S4. Diagram showing composition of the eight biosynthetic gene clusters for known metabolites in *Simplicillium lamellicola* JC-1 based on antiSMASH.

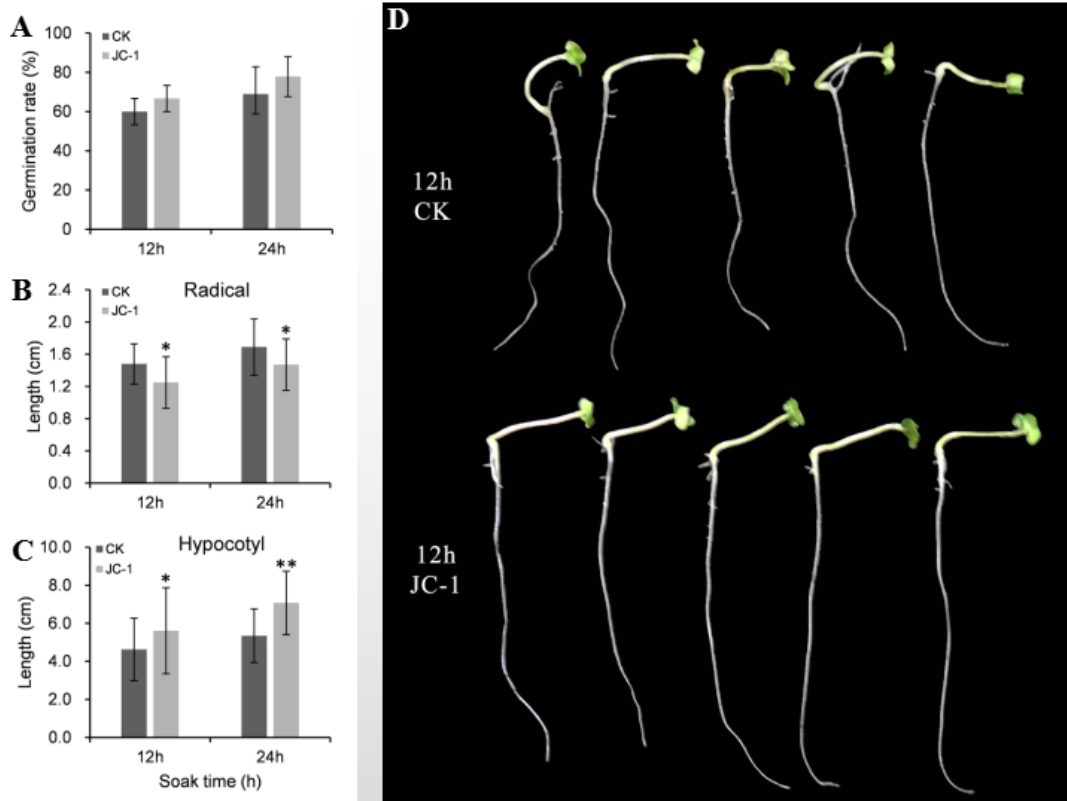


Figure S5. Effect of treatment of seeds of oilseed rape with the conidia of *S. lamellicola* JC-1 on seed germination rate and elongation of the radicles and hypocotyls of the resulting seedlings (20°C, 4 d).

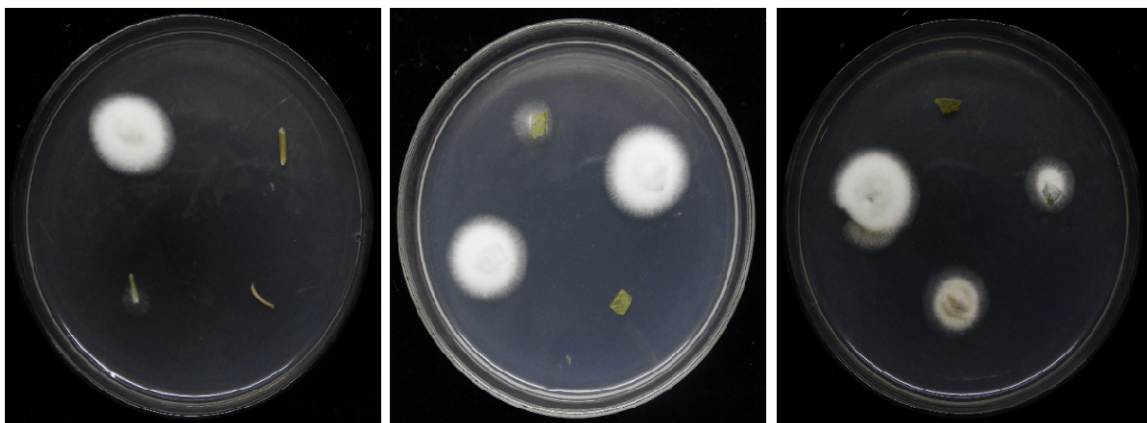


Figure S6. Detection of *S. lamellicola* JC-1 in stems (right dish) and leaves (middle and left dishes) of oilseed rape. Note the whitish colonies of *S. lamellicola*.

SUPPLEMENTARY TABLES

Table S1 PCR primers and their oligonucleotide sequences

Locus ¹	Oligonucleotide Sequence (5' to 3')	Annealing Temperature	Reference ²
ITS	ITS4: TCCTCCGCTTATTGATATGC	49°C	White <i>et al.</i> , 1990
	ITS5: GGAAGTAAAAGTCGTAACAAGG		
SSU	NS1: GTAGTCATATGCTTGTCTC	45°C	White <i>et al.</i> , 1990
	NS4: CTTCCGTCAATTCCTTTAAG		
LSU	LR0R: GTACCCGCTGAACTTAAGC	49°C	Vilgalys & Hester, 1990
	LR5: ATCCTGAGGGAACTTC		
<i>TEF1α</i>	EF1-983F: GCYCCYGGHCAYCGTGAYTTYAT	52°C	Rehner & Buckley, 2005
	EF1-2218R: ATGACACCRACRGCACRGTYTG		
<i>ACT7</i>	ACT7F: GCTGACCGTATGAGCAAAG	49°C	
	ACT7R: AAGATGGATGGACCCGAC		
<i>CHI</i>	CHI : FG TAGTCATATGCTTGTCTC	47°C	Peng <i>et al.</i> , 2020
	CHIR: CTTCCGTCAATTCCTTTAAG		
<i>NCED3</i>	NCED3F: CGATTTGCCTTACCAAGTCAG	49°C	
	NCED3R: TTTATCCCTTCCGGTGAGAA		
<i>PR-1</i>	PR-1F: CATCCCTCGAAAGCTCAAGAC	53°C	
	PR-1R: CCACTGCACGGGACCTAC		

¹ITS = Internal Transcribed Spacer (ITS1-5.8S rDNA-ITS2), SSU = Small Subunit ribosomal RNA gene, LSU = Large Subunit ribosomal RNA gene, *TEF1α* = translation elongation factor 1α, *ACT7* codes for actin, *CHI* codes for chalcone isomerase, *NCED3* codes for 9-cis-epoxycarotenoid dioxygenase, *PR-1* codes for pathogenesis-related protein 1.

²Reference: (1) Peng G *et al. Can J Plant Pathol* 2020, 42: 480–492; (2) Rehner SA, and Buckley E. *Mycologia*, 2005, 97: 84–98; (3) Vilgalys R and Hester M. *J. Bacteriol.* 1990, 172: 4238–4246; (4) White TJ *et al. In: PCR Protocols: A Guide to Methods and Applications* (Innis MA *et al.* eds) Academic Press, San Diego, CA, USA, 1990, pp315–322.

Table S2. Reagents for PCR amplification of ITS, LSU, SSU and *TEF1a*

Reagent	Concentration	Dosage
Forward primer	20 µmol/L	1.0 µL
Reverse primer	20 µmol/L	1.0 µL
DNA template	50 ng/µL	1.0 µL
Master Mix	-	12.5 µL
ddH ₂ O	-	9.5 µL
Total volume	-	25.0 µL

Table S3. Thermal program for PCR amplification of ITS, SSU, LSU and *TEF1a*

Stage	Temperature	Time	Cycle
Pre-denaturation	95°C	3 min	1 cycle
Denaturation	95°C	30 s	32 cycles
Annealing	-	30 s	
Extension	72°C	1 min	
Final extension	72°C	10 min	1 cycle
Temperature dropping	16°C	5 min	Not applicable

Table S4. Reaction system for DNA elimination in the RNA extracts.

Reagent	Dosage	Temperature	Time
RNA	2 µg		
10×DNaseIBuffer	2 µL	37°C	30 min
Recombinant DNaseI	1 µL		
Stop solution	1 µL	65°C	10 min

Table S5. Reagents for RT-PCR to detect expression of defense-related genes.

Reagent	Concentration	Dosage
Forward primer	20 µmol/L	1.0 µL
Reverse primer	20 µmol/L	1.0 µL
cDNA template	50 ng/µL	1.0 µL
Master Mix	-	12.5 µL
ddH ₂ O	-	9.5 µL
Total volume	-	25.0 µL

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Table S6. Thermal program for quantitative RT-PCR.

Temperature	Time	Cycle
95°C	3 min	1
95°C	10 s	
60°C	30 s	39
72°C	30 s	
95°C	1 min	1
55°C	1 min	1
Melt Curve 55°C to 95°C, increment with 0.5°C	10 s	1

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141 **Table S7.** Genes clusters for biosynthesis of secondary metabolites (SMs) in *Simplicillium*
 142 *lamellicola* strain JC-1.

Cluster	Type	Position (nt)	Backbone Gene	Putative Protein	Secondary Metabolite	MIBiG Acc. No.	Similarity
1.1	T1PKS NRPS	33680-82407	SI000016	Putative lovastatin nonaketide synthase	Unknown	— ^c	—
1.2	NRPS	131433-196258	SI000046	Non-ribosomal peptide synthetase	Verlamelin	BGC0000456	100%
1.3	NRPS	1433867-1503267	SI000517	Aureobasidin A1 biosynthesis complex	Aureobasidin A1	BGC0000307	100%
1.4	NRPS-like	1970795-2013061	SI000693	NRPS-like enzyme	Unknown	—	—
1.5	Terpene	2109867-2131080	SI000745	Isoprenoid synthase	Unknown	—	—
1.6	NRPS	3773786-3821937	SI001334	Non-ribosomal peptide synthase	Unknown	—	—
1.7	NRPS T1PKS	3935356-3987606	SI001394	Putative hybrid NRPS/PKS enzyme	Unknown	—	—
2.1	Betalactone	1033569-1058485	SI002201 SI002202	Hydroxymethylglutaryl-lyase, Acetoacetyl-synthase	Unknown	—	—
2.2	NRPS	1435270-1534754	SI002347 SI002349	Non-ribosomal peptide synthetase, Nonribosomal peptide synthase	Unknown	—	—
2.3	NRPS-like	2397011-2439229	SI002661	Oxygen-dependent choline dehydrogenase	Unknown	—	—
2.4	T1PKS	2680421-2725247	SI002765	Putative polyketide synthase	Unknown	—	—
2.5	NRPS-like	3093698-3134818	SI002932	Putative peroxisomal-coenzyme A	Unknown	—	—

				synthetase-like			
2.6	NRPS	3816434-3867433	SI003210	Nonribosomal peptide synthase	Unknown	—	—
2.7	NRPS T1PKS NRPS-like	4644938-4738920	SI003494 SI003510	Putative equisetin synthetase, Putative Linear gramicidin synthase subunit D	Phomasetin	BGC0001738	57%
2.8	T1PKS	4820789-4868697	SI003552	Polyketide synthase	Unknown	—	—
3.1	T1PKS	1641267-1679314	SI004278	Methylphloroacetophenone synthase	Unknown	—	—
3.2	Terpene	1,740,519-1,761,688	SI004310	Geranylgeranyl pyrophosphate synthase	Unknown	—	—
3.3	NRPS NRPS-like	3,601,494-3,656,816	SI004994, SI004998	Non-ribosomal peptide synthetase, Nrps-like enzyme	Unknown	—	—
3.4	T1PKS	3674131-3722214	SI005026	Polyketide synthase	Unknown	—	—
4.1	Terpene	642917-661016	SI005401	Squalene cyclase	Unknown	—	—
4.2	T1PKS	969343-1014595	SI005538	Polyketide synthase	Wortmanamide A	BGC0001954	83%
4.3	T1PKS	1184816-1229761	SI005624	Polyketide synthase	Unknown	—	—
4.4	T1PKS	1365775-1403953	SI005676	Polyketide synthase	Unknown	—	—
4.5	NRPS T1PKS	1505312-1565249	SI005729 SI005730	Nonribosomal peptide synthetase-like Lovastatin nonaketide synthase-like	Unknown	—	—
4.6	Terpene	2065671-2087169	SI005901	Squalene synthetase	Squalestatin S1	BGC0001839	40%
4.7	NRPS	2482414-2543614	SI006048	Non-ribosomal peptide synthetase	Unknown	—	—
5.1	NRPS	635243-679402	SI006597	Nonribosomal peptide synthase	Unknown	—	—
5.2	NRPS T1PKS	2612716-2664965	SI007294	Polyketide synthase	Unknown	—	—
7.1	NAPAA T1PKS	18904-94369	SI008578 SI008589	Non-ribosomal peptide synthetase, Putative acyl carrier	Unknown	—	—
7.2	Fungal-RiPP Terpene	128776-171501	SI008623 SI008624 SI008629	Trichodiene synthase, Alpha-1,3-mannosyltransferase Putative Gamma-glutamyltranspeptidase	Nivalenol	BGC0001277	13%
7.3	NRPS-like T1PKS	303687-415883	SI008693 SI008704 SI008722	Transferase family, Acetyl-CoA synthetase-like, Polyketide synthase Putative polyketide synthase,	Duclauxin	BGC0001578	28%
8.1	T1PKS NRPS-like, Indole	330549-393056	SI009359 SI009365 SI009366	Aromatic prenyltransferase, Acetyl-CoA synthetase-like	Unknown	—	—
8.2	T1PKS	766436-814788	SI009509	Polyketide synthase	Unknown	—	—
8.3	NRPS-like	1725699-1771470	SI009893	Amino adipate reductase enzyme	Unknown	—	—

9.1	NRPS-like	59244-102327	SI009957	Acetyl-CoA synthetase-like protein	Unknown	–	–
9.2	T1PKS Indole	738964-786458	SI010235 SI010237	Polyketide synthase, Prenyltransferase PT1	Neurosporin A	BGC0002729	33%
10.1	NRPS-like	381851-425168	SI010404	Nonribosomal peptide synthetase	Unknown	–	–

^aThe codes for the gene clusters were named after their positions in the contigs for the genome of JC-1;

^bT1PKS, Type I polyketide synthase; NRPS, Non-ribosomal peptide synthetase; Terpene, Terpene cluster; Betalactone, beta-lactone containing protease inhibitor; NAPAA, non-alpha poly-amino acids like e-Polylysine; Fungal-RiPP, Fungal RiPP with POP or UstH peptidase types and a modification; Indole, Indole cluster.

^c–, Not applicable.

Table S8. Putative genes of the verlamelin biosynthesis gene cluster in *S. lamellicola* JC-1.

Gene in JC-1	Predicted function	Homolog in <i>Lecanicillium</i> HF627 (GenBank Acc. No.)	Identity	
			nt	aa
SI000046	Non-ribosomal peptide synthase	<i>vImS</i> (AB862312)	90%	92%
SI000045	Thioesterase	<i>vImB</i> (AB862314)	92%	96%
SI000044	AMP-dependent ligase	<i>vImC</i> (AB862315)	90%	96%
SI005221	Fatty acid hydroxylase	<i>vImA</i> (AB862313)	51%	47%

Note: Three genes (SI000044, SI000045 and SI000046) are located in Contig No. 01, whereas the gene SI005221 is located in Contig No. 4.