Supplementary Table 1. Spontaneous loss of endobacteria and asexual sporulation during serial propagation of *Rm* ATCC 52813 lines 1-1, 1-2, 2-1, 2-2, 3-1, 3-2 via single spores and mycelial fragments. P, present; A, absent.

Generation	**Sporulation in single spore lines**						**Sporulation in mycelial fragment lines**					
	1-1	**1-2**	**2-1**	**2-2**	**3-1**	**3-2**	**1-1**	**1-2**	**2-1**	**2-2**	**3-1**	**3-2**
1	P	P	P	P	P	P	P	P	P	P	P	P
2	P	P	P	P	P	P	P	P	P	P	P	P
3	P	P	P	P	P	P	P	P	P	P	P	P
4	P	P	P	P	P	P	P	P	P	P	P	P
5	**A**	**A**	P	P	P	P	P	P	P	P	P	P
6	**A**	**A**	P	P	P	P	P	P	P	P	P	P
7	**A**	**A**	P	P	P	P	P	P	P	P	P	P
8	**A**	**A**	P	P	P	P	P	P	P	P	P	P
9	**A**	**A**	P	P	P	P	P	P	P	P	P	P
10	**A**	**A**	P	P	P	P	P	P	P	P	P	P
11	**A**	**A**	P	P	P	P	P	P	P	P	P	P
12	**A**	**A**	P	P	P	P	P	P	P	P	P	P
13	**A**	**A**	P	P	P	P	P	P	**A**	P	P	P
14	**A**	**A**	P	P	P	P	P	P	**A**	P	P	P
15	**A**	**A**	P	P	P	P	P	P	**A**	P	P	P

Supplementary Table 2. Effects of various environmental factors on asexual and sexual reproduction in B(+) and B(–) isolates of *Rm* ATCC 52813 and ATCC 52814. +, normal reproduction; ++, exceptionally robust reproduction; ±, reduced reproduction; A, reproduction absent; X, failure to grow vegetatively; (Y), accumulation of β-carotene; (YY), rapid accumulation of β-carotene; NA, data not available. Each observation summarizes three replicate cultures.

Environmental factor	**Wild type fungi harboring endobacteria**				**Fungi cured of endobacteria**			
	ATCC 52813		ATCC 52814		ATCC 52813		ATCC 52814	
level	asexual sporulation	mating	asexual sporulation	mating	asexual sporulation	mating	asexual sporulation	mating
Thiamine	+	+	+	+	A	A	A	A
β-carotene	+	+	+	+	A	A	A	A
NaNO$_3$	+	+	+	+	A	A	A	A
NaNO$_2$	±	+	±	+	A	A	A	A
Mated culture extract	+	+	+	+	A	A	A	A
Humidity								
high	+	+	+	+	A	A	A	A
low	++	+	++	+	A(Y)	A	A(Y)	A
Light								
ambient	+	+	+	+	A	A	A	A
darkness	+	+	+	+	A	A	A	A
Cold treatment								
0 weeks	+	+	+	+	A	A	A	A
1 week	+	+	+	+	A	A	A	A
2 weeks	+	+	+	+	A	A	A	A
4 weeks	+	+	+	+	A	A	A	A
H_2O_2 [mM]								
0.0	+	NA	+	NA	A	NA	A	NA
0.3	±	NA	±	NA	A	NA	A	NA
0.6	±	NA	±	NA	A	NA	A	NA
1.2	±	NA	±	NA	A	NA	A	NA
2.4	±	NA	±	NA	A	NA	A	NA
10.0	X	NA	X	NA	X	NA	X	NA
NaCl [M]								
0.0	+	NA	+	NA	A	NA	A	NA
0.4	+	NA	++	NA	A(YY)	NA	A(YY)	NA
0.8	+	NA	++	NA	A(YY)	NA	A(YY)	NA
1.6	+	NA	++	NA	A(YY)	NA	A(YY)	NA

Supplementary Table 3. Genome assembly statistics for *Rm* ATCC 52813 and comparative organisms from the Mucoromycotina subphylum.

	***Rhizopus microsporus* ATCC 52813**	***Rhizopus microsporus* ATCC 52814**	***Rhizopus microsporus* ATCC 11559**	***Rhizopus chinensis* CCTCCM201021**	***Rhizopus delemar* 99-880**
Assembly length, bp	25,972,395	24,950,816	24,077,254	45,739,792	46,087,117
Number of scaffolds	131	560	595	3,281	81
Scaffold N50	8	8	87	463	6
Scaffold L50, bp	1,118,338	105,542	84,607	30,430	3,104,119
Number of contigs	823	649	653	3,374	389
Contig N50	111	84	96	469	46
Contig L50, bp	69,382	92,626	73,149	30,211	303,660
% GC	37.48	37.42	37.25	36.92	35.6

Supplementary Table 4. Homology support and functional assignment statistics for *Rm* ATCC 52813 gene models.

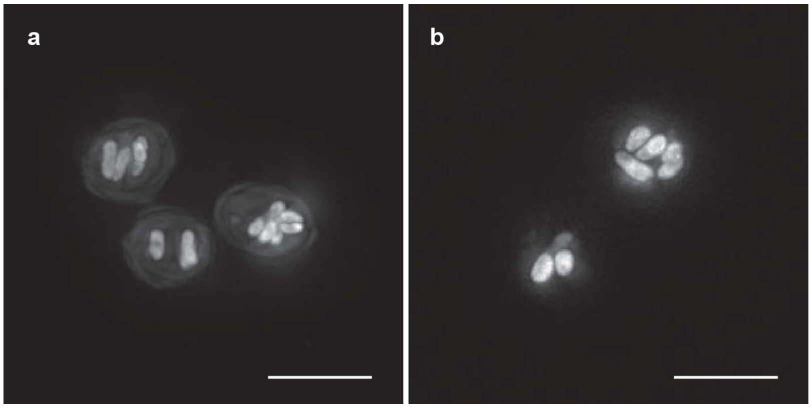
	***Rhizopus microsporus* ATCC 52813**		***Rhizopus chinensis* CCTCCM201021**		***Rhizopus delemar* 99-880**	
Number of genes	10,905	100%	17,676	100%	17,467	100%
Hits to databases:						
KEGG	8,119	74%	13,686	77%	11,456	66%
KOG	7,601	70%	12,878	73%	9,028	52%
Swissprot	7,688	71%	12,964	73%	10,609	61%
nr_b_b	7,835	72%	15,983	90%	10,565	60%
HMMPfam	6,587	60%	11,056	63%	9,030	52%
Number of genes with complete CDS	10,229	94%	16,036	91%	17,412	100%
Proteins with transmembrane helices	1,863	17%	2,934	17%	2,133	12%
Proteins with signal peptide	1,294	12%	1,960	11%	1,546	9%

Supplementary Table 5. Reproductive proteins across fungi. Unless otherwise noted, *A. nidulans* nomenclature is used following Dyer and O'Gorman[1]; [a]*S. cerevisiae* nomenclature; [b]*P. blakesleeanus* nomenclature; [c]*U. maydis* nomenclature.

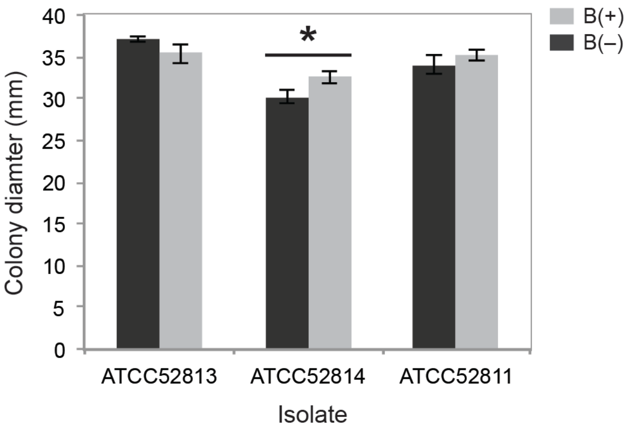
Protein	LocusID	NCBI accession	Role in reproduction	Reference
CpcA	AN3675	CBF75634	Amino acid sensing	2
CpcB	AN4163	CBF74576	Amino acid sensing	2
ImeB	AN6243	CBF69873	Light response, sexual development, secondary metabolism	3
LsdA	AN2330	CBF86620	Osmotic stress sensing	4
LreA	AN3435	CBF82714	Blue light sensing	5
LreB	AN3607	CBF75789	Blue light sensing	5
CryA	AN0387	CBF89585	Blue light and UV sensing	6
FphA	AN9008	CBF84471	Red light sensing	7
VeA/VosA	AN1052/ AN1959	CBF88249/ CBF85898	Light/dark response regulator, secondary metabolite production	8,9
VelB	AN0363	CBF89638	Light/dark response regulator, secondary metabolite production	8
LaeA	AN0807	CBF88745	Light/dark response regulator, secondary metabolite production	8
VelC	AN2059	CBF86097	Light/dark response regulator, secondary metabolite production	10
Pho80	AN5156	CBF80977	Phosphorous sensing cyclin	11
PhoA	AN8261	CBF74234	Phosphorous sensing, interacts with Pho80	12
FhbA	AN7169	CBF78937	Nitrogen metabolism	13
MAT1	AN2755	CBF84081	Transcription factor: regulator of sexual reproduction	14
MAT2	AN4734	CBF76901	HMG box transcription factor: regulator of sexual reproduction	14
SexP[b]	NA	ABX27912	HMG box transcription factor: regulator of sexual reproduction	15
bE1[c]	UM00577	XP_011386823	Homeobox domain transcription factor: regulator of sexual reproduction	16
bW1[c]	UM00578	XP_011386405	Homeobox domain transcription factor: regulator of sexual reproduction	16
Ram1p[a]	YDL090C	NP_010193	Pheromone biogenesis: a-factor prenylation	17
Ste24p[a]	YJR117W	NP_012651	Pheromone biogenesis: a-factor proteolysis	18
Ste14p[a]	YDR410C	NP_010698	Pheromone biogenesis: a-factor carboxyl methylation	19
Ste23p[a]	YLR389C	NP_013493	Pheromone biogenesis: a-factor proteolysis	20
Axl1p[a]	YPR122W	NP_015447	Pheromone biogenesis: a-factor proteolysis	20
Ste6p[a]	YKL209C	NP_012713	Pheromone biogenesis: a-factor transporter	21
Ste13p[a]	YOR219C	NP_014862	Pheromone biogenesis: α-factor processing	22
PpgA	AN5791	CBF81183	Pheromone precursor (α type)	14
PreA/GprB	AN7743	CBF80019	GPCR: a type pheromone receptor	23,24
PreB/GprA	AN2520	CBF87016	GPCR: α type pheromone receptor	23,24
GprD	AN3387	CBF82810	GPCR	25
GprK	AN7795	CBF80123	GPCR	26
TAR1	NA	To be provided upon publication	GPCR: candidate risporic acid receptor	This study

TAR2	NA	To be provided upon publication	GPCR: candidate trisporic acid receptor	This study
TAR3	NA	To be provided upon publication	GPCR: candidate trisporic acid receptor	This study
Gpa3[c]	UM04474	P87034	*U. maydis* G protein α subunit: signaling to pheromone MAP kinase and cAMP cascades	27
FadA	AN0651	CBF89057	G protein α subunit: signaling to pheromone MAP kinase cascade	28
SfaD	AN0081	CBF90238	G protein β subunit: signaling to pheromone MAP kinase cascade	28
GpgA	AN2742	CBF84107	G protein γ subunit: signaling to pheromone MAP kinase cascade	29
PhnA	AN0082	CBF90234	Regulator of GpgA, SfaD and FlbA	30
FlbA	AN5893	CBF70620	Regulator of G-protein signaling	31
Sql2[c]	UM01516	XP_011387982	Regulator of Ras2	32
RasA	AN0182	CBF90024	Ras signaling protein involved in asexual development	2
Ras2[c]	UM01643	XP_011387629	Ras signaling protein involved in reproductive development, cAMP regulation, and filamentous growth	33
Msn5p[a]	YDR335W	NP_010622	Nuclear import and export of sex-related transcription factors	34
Uac1[c]	UM05232	XP_761379	Adenylate cyclase	35
Uka1-1/1-2[c]	UM01124	XP_757271	cAMP dependent protein kinase catalytic subunit	36
Ubc1[c]	UM06450	XP_762597	cAMP dependent protein kinase regulatory subunit	35
Far1p[a]	YJL157C	NP_012378	Pheromone regulation	37
Cdc42p[a]	YLR229C	NP_013330	Rho-GTPase, cell polarity	38
Cdc24p[a]	YAL041W	NP_009359	Guanyl nucleotide exchange factor for Cdc42, cell polarity	39
Cdc28p[a]	YBR160W	NP_009718	Cyclin-dependent kinase catalytic subunit	40
Ste20p[a]	YHL007C	NP_011856	Pheromone response	41
SteD	AN7252	CBF78763	Pheromone MAP kinase cascade regulator	14
Ste5p[a]	YDR103W	NP_010388	Pheromone MAP kinase cascade scaffold	42
Ste20	AN5674	CBF81427	MAP kinase, kinase, kinase, kinase (pheromone cascade)	1
SteC	AN2269	CBF86503	MAP kinase, kinase, kinase (pheromone cascade)	43
Ste7	AN3422	CBF82742	MAP kinase, kinase (pheromone cascade)	14
MpkB	AN3719	CBF75537	Map kinase (pheromone cascade)	14
CandA-C	AN2458	CBF86897	Regulate reproductive mode in response to light	44
CandA-N	AN10306	CBF86906	Regulate reproductive mode in response to light	44
Dig1p[a]	YPL049C	NP_015276	Regulation of mating-specific genes	45
Dig2p[a]	YDR480W	NP_010768	Regulation of mating-specific genes	45
Msg5p[a]	YNL053W	NP_014345	Regulation of pheromone MAP kinase cascade	46
CsnA	AN1491	CBF84973	Regulation of sexual development	47
CsnB	AN4783	CBF76790	Regulation of sexual development	47
CsnD	AN1539	CBF85069	Regulation of sexual development	47
CsnE	AN2129	CBF86237	Regulation of sexual development	47

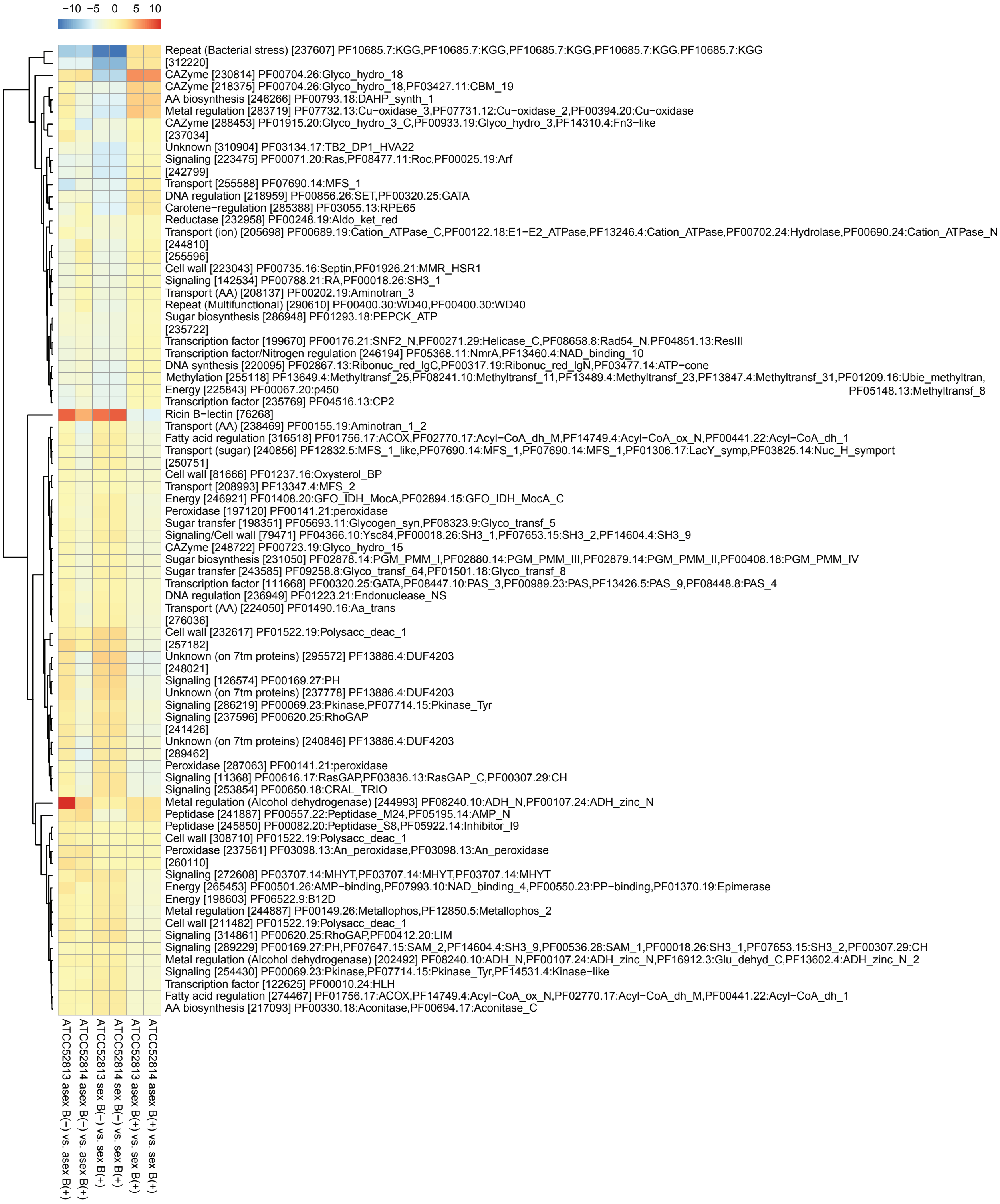
CsnG/AcoB	AN3623	CBF75752	Regulation of sexual development	47
StrA	AN8071	CBF73824	Sexual and asexual development: striatin scaffolding and Ca signaling	48
EsdC	AN9121	CBF82506	Sexual development	49
RcoA	AN6505	CBF70872	Sexual vs asexual development, secondary metabolism	50
PpoA/PpoC	AN1967/ AN5028	CBF85912/ CBF76249	Sexual vs asexual development, secondary metabolism (oxylipin)	51
PpoB	AN6320	CBF69709	Sexual vs asexual development, secondary metabolism (oxylipin)	51
Sir3p[a]	YLR442C	NP_013547	Silencing MAT loci	52
Sir4p[a]	YDR227W	NP_010513	Silencing MAT loci	53
HogA	AN1017	CBF88327	Stress response	54
AtfA	AN2911	CBF83765	Stress response	55
MsnA	AN1652	CBF85299	Transcription factor	1
StuA	AN5836	CBF70741	Transcription factor	56
DopA	AN2094	CBF86166	Transcription factor	57
MedA	AN6230	CBF69898	Transcription factor	58
FhpA	AN4521	CBF77337	Transcription factor	59
DevR	AN7553	CBF79619	Transcription factor	60
NosA	AN1848	CBF85681	Transcription factor	61
FlbE	AN0721	CBF88918	Transcription factor	62
FlbC	AN2421	CBF86815	Transcription factor	63
NsdC	AN4263	CBF74364	Transcription factor	64
NsdD	AN3152	CBF83303	Transcription factor	31
RosA	AN5170	CBF81007	Transcription factor	65
SteA	AN2290	CBF86542	Transcription factor activated by pheromone MAP kinase cascade	66
SilG	AN0709	CBF88942	Transcription factor: light response	1
SilA	AN1893	CBF85772	Transcription factor: light response	1
TrxA	AN0170	CBF90049	Redox regulation	67
NoxA	AN5457	CBF81876	Redox regulation	68
SidC	AN0607	CBF89140	Iron regulation (NRPS)	69
HxtA	AN6923	CBF71745	Hexose transport	70
MutA	AN7349	CBF78568	Carbohydrate catabolism, mutanase activity	71
SamB	AN0078	CBF90245	Cell polarity	72
GrrA	AN10516	CBF74496	Meiotic structure development	73
TubB	AN0316	CBF89739	Microtubule assembly	74



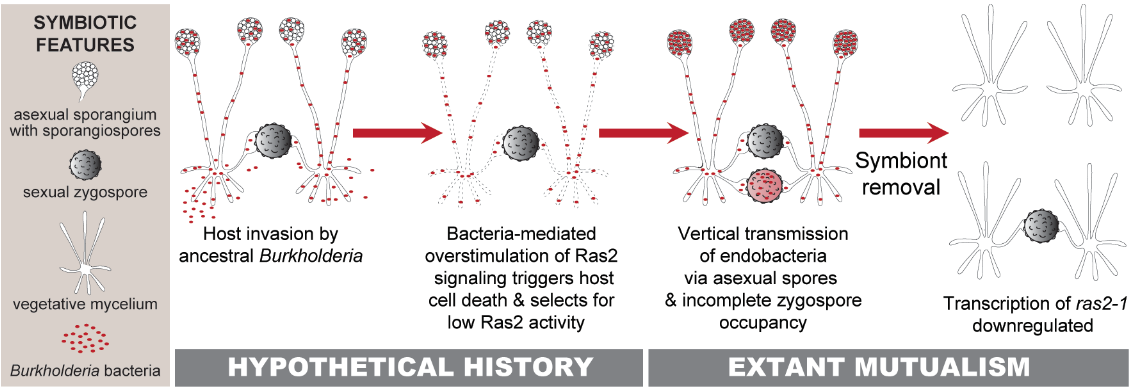
Supplementary Figure 1. *Burkholderia* endobacteria in sporangiospores of *Rm*. (a) ATCC 52813. **(b)** ATCC 52814. For visualization, bacteria expressed mCherry fluorescent protein in ATCC 52813 and YFP in ATCC 52814. Scale bars, 5 µm.



Supplementary Figure 2. Impact of *Burkholderia* endobacteria on *Rm* colony expansion. Colony diameter was measured after one day of growth. *, *t* test $0.05 \geq P > 0.01$. Error bars represent s.e.m.



Supplementary Figure 3. Log2 fold change in expression of *Rm* genes displaying significant differences across all experimental comparisons. Broad categories (Signaling, Cell wall, etc) as well as all Pfam annotations are shown for each gene. *Rm* ATCC 52813 Protein IDs are shown in brackets.



Supplementary Figure 4. Hypothetical evolutionary trajectory leading to the present-day *Rm-Burkholderia* mutualism.

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