Supplementary Data - Table S1

Table S1 – Primers used in this study

Primer Name	Sequence (5' – 3')	Annealing / Extension	Purpose	
RHAFw	CCTTGTTCTCGGTGGCTATTT	55ºC / 90 s	*RHA* forward common primer	Confirm mating type specificity of *RHA* genes.
RHA1Rev	GGCGGATGGATTGGTTGAGC		*RHA1*, *RHA2*, *RHA3* reverse specific primers	
RHA2Rev	CTTGGCCCGCCCTATGTCTG			
RHA3Rev	CTCCGCTGTCGTGTACTGATG			
RHA2Up	GCAGACGAACAAACGATCATGGAACA	65ºC / 3 min	Amplify the upstream region of the *RHA2* gene (PCR with Genome-walker libraries of strain PYCC 4416, *MAT A1*)	
AP1[a]	GTAATACGACTCACTATAGGGC			
RHA2Up2	AAGGGCAGACGACAAGAACAAAGGCC	65ºC / 3 min	Amplify the upstream region of the *RHA2* gene (nested PCR). **[fragment 1 in Fig. 1]**	
AP2[a]	ACTATAGGGCACGCGTGGT			
RHA3Up2	GAGAACCACGGACTGTCGAGGTCGAG	63ºC / 3 min	Amplify the region between the *RHA2* and *RHA3* genes. **[fragment 2 in Fig. 1]**	
SCP2Up	GGCTGCGGAAGACAACGACTG			
STE20CRFw	ACAGACATCATCGACAGCAAC	55ºC / 1 min	Amplify the conserved regions of the *RtSTE20* alleles (*MAT A1* and *MAT A2* strains) **[fragments 3 and 4 in Fig.1]**	
STE20CRRev	CGTACTCCTTCTGCTTGACGA			
NCARFw	CGRGAAGACCTGAARCCGTT	59ºC / 90 s		
NCARRev	CATGWACTCCATGACGACCCA			
STE20DwA2 (AP1)[a]	CGAGTGCTGACAGTCGCATCTTTCCG	67ºC / 3 min	Amplify the downstream region of the *RHA2.A2* gene (PCR with Genome-walking libraries of strain PYCC 4661, MAT A2)	
STE20Dw2A2 (AP2) [a]	CGAAGCTGACCGACCAGAAGTCGAAG	65ºC / 3 min	Amplify the downstream region of the *RHA2.A2* gene (nested PCR). **[fragment 5 in Fig. 1]**	
STE20UpA2 (AP1) [a]	ACCGCAAGGCGAGGTAGAAGGTTCGA	67ºC / 3 min	Amplify the upstream region of the *RtSTE20.A2* gene (PCR with Genome-walking libraries of strain PYCC 4661, *MAT A2*).	
STE20Up2A2 (AP2) [a]	AGGTAGAAGGTTCGAGCCGGTGTCGT	65ºC / 3 min	Amplify the upstream region of the *RtSTE20.A2* gene (nested PCR). **[fragment 6 in fig. 1]**	
NCAR3Fw	TGGCATTGGCGAGCMCGMAA	55ºC / 90 s	Amplify part of the *STE3* homologue in *R. kratochvilovae* (strain PYCC 4818, *MAT A1*)	
NCAR4Rev	GRCCRAAGAAGASGAARAAGA			
NCAR5STE3Fw	CATCTTCTGGTGTKTGGTCAMCGT	60ºC / 1 min	Amplify part of the of the *STE3* homologue in *R. toruloides* (strain PYCC 4416, *MAT A1*).	
NCAR6STE3Rev	GGGASGSTGTAGGCGATTTG			
(STE20Dw2A2) MC074	GGGTCCACGAGATTTCGGGGTACTTG	66ºC / 90 s	Amplify a *MAT A1*-specific region (3' end of *RtSTE20* gene to the CDS of *RHA2)*.	
(STE20Dw2A2) MC071	GGTGCATGCCATGTACGAGGTGACA	63ºC / 90 s	Amplify a *MAT A2*-specific region (3' end of *RtSTE20* gene to the CDS of *RHA2.A2)*.	

[a] primers supplied in the Universal Genome-Walker kit (BD Biosciences Clontech). All the others were obtained from MWG Biotech AG, Germany.

Supplementary Data – Table S2

Trace Archive Sequences of *S. salmonicolor* (IAM 12258):

***SsSTE20* homologue**
>gnl|ti|1243964000 name:BAUG19266.b1 mate:1243962176
>gnl|ti|1243931275 name:BAUG14477.g1 mate:1243936267
>gnl|ti|1244008391 name:BAUG52617.b1 mate:1244005607
>gnl|ti|1244012976 name:BAUG51538.g1 mate:1244013840
>gnl|ti|1244008568 name:BAUG52794.b1 mate:1244005784
>gnl|ti|1243932804 name:BAUG11782.g1 mate:1243930020
>gnl|ti|1243983780 name:BAUG45862.b1 mate:1243984260
>gnl|ti|1243949766 name:BAUG28456.b1 mate:1243948710
>gnl|ti|1244033153 name:BAUG61059.b1 mate:1244033249
>gnl|ti|1243947537 name:BAUG24979.g1 mate:1243947825
***SsRHA1* homologue**
>gnl|ti|1244034102 name:BAUG63928.g1 mate:1244034006
>gnl|ti|1244025649 name:BAUG59699.b1 mate:1244026609
>gnl|ti|1244026866 name:BAUG59956.b1 mate:1244025906
>gnl|ti|1243949840 name:BAUG28530.b1 mate:1243949936
***SsRHA2* homologue**
>gnl|ti|1244013519 name:BAUG53905.g1 mate:1244012751
>gnl|ti|1244014847 name:BAUG53217.b1 mate:1244019455
>gnl|ti|1243996949 name:BAUG45015.g1 mate:1243996565
>gnl|ti|1243918143 name:BAUG5948.y1 mate:1243917764
>gnl|ti|1244015954 name:BAUG56436.g1 mate:1244017874
***SsRHA3* homologue**
>gnl|ti|1244010230 name:BAUG51096.g1 mate:1244010134
>gnl|ti|1243911835 name:BAUG389.y1 mate:1243911451
>gnl|ti|1243919179 name:BAUG3533.b1 mate:1243920619
>gnl|ti|1244012751 name:BAUG53905.b1 mate:1244013519
>gnl|ti|1243952391 name:BAUG19945.b1 mate:1243952487
>gnl|ti|1243997749 name:BAUG39095.g1 mate:1243994101

Supplementary Data – Table S3

List of additional genes identified in the neighbourhood of putative *MAT* genes in *Sp. roseus*

1	RRM	RNA binding protein (RRM super family)
2	Mit-Carr	Putative mitochondrial carrier protein
3	ABC	Putative ABC1 protein, mitochondrial precursor/ Ubuiquinone biosynthesis protein coq 8
4	KAP95	Putative Karyopherin β 1 (importin)
5	DDOST	Dolichyl diphosphodigosaccharide protein glycosytransferase 48 KDa subunit
6	RPS19/15	Ribosomal protein S19/S15
7	Rib L18	Ribosomal L18ae protein
8	RNAPOL	Putative DNA directed RNA polymerase III, 30/40 KDa subunit
9	LONP S16	Peptidase S16, (Lon protease)

Supplementary Data – Table S4

Sequences used for the construction of the phylogenetic tree

Ustilago maydis	Umay_Cla4	AAT39367
	Umay_Smu1p	Q4P5N0
Pleurotus djamor	Pdja_Cla4	AAS46755
Cryptococcus neoformans	Cneo_Ste20α	XP_570118
	Cneo_Ste20a	AAN75615
Cr. neoformans var. *grubii*	Crngru_Ste20α	AAN75173
Cr. gattii	Crgat_Ste20a	AAV28761
	Crgat_Ste20α	AAV28796
Pneumocytis carinii	Pncar_Ste20	AAG38545
Yarrowia lipolytica	Ylip_Pkin	XP_502152
Aspergillus fumigatus	Afum_Chm1	XP_754042
Candida albicans	Calb_Cla4	O14427
Magnaporthe grisea	Mgri_Chm1	XP_369805
Saccharomyces cerevisiae	Scer_Pkin	CAA57879
	Scer_Ste20p	NP_011856
Penicillium marneffei	Pmar_PakA	Q2VWQ3
Schizosaccharomyces pombe	Scpom_Pak1p	AAC49125
Rhodosporidium toruloides	RtSte20_A1	EU386160
	RtSte20_A2	EU386161
Sporobolomyces roseus	Sr_Ste20	[a] Scaffold 9, contig 11
Puccinia graminis	Pgra_ Smu1	[b] PGTG_14316.2, Supercontig 51: 338996-341873

[a] *Sporobolomyces roseus* genome sequencing project database, Joint Genome Institute (JGI): http://genome.jgi-psf.org/Sporo1/Sporo1.home.html

[b] *Puccinia graminis* f. sp. *tritici* genome Database, Broad Institute of MIT and Harvard: http://www.broad.mit.edu/annotation/genome/puccinia_graminis/Home.html

Supplementary Data – Fig. S1

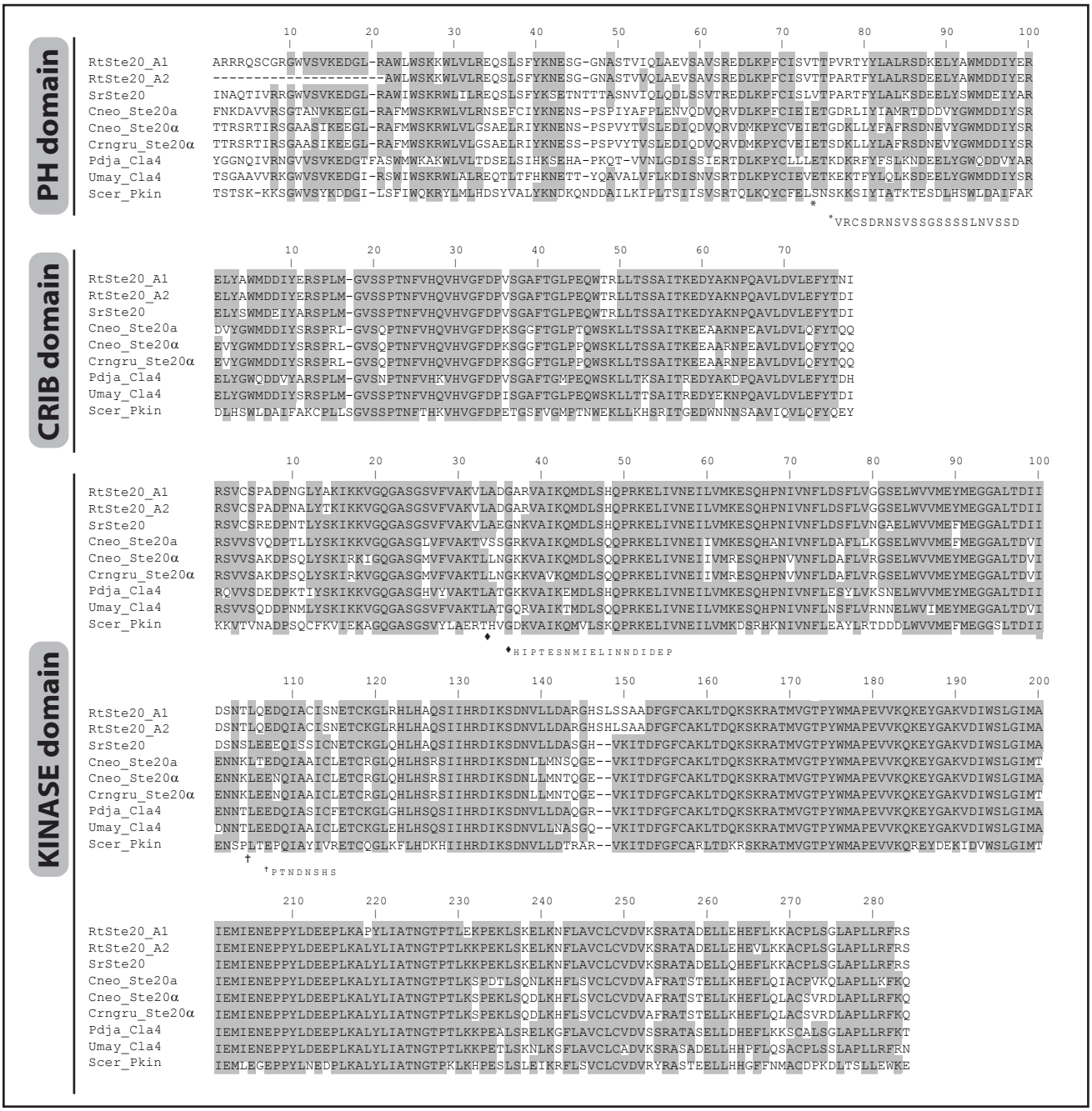

Fig. S1 – Alignment of the three conserved domains of several fungal PAK kinases: RtSte20_A1 and RtSte20_A2 (*Rhodosporidium toruloides, MAT A1* and *MAT A2)*; SrSte20 (*Sporobolomyces roseus)*, Cneo_Ste20a and Cneo_Ste20α *(Cryptococcus neoformans)*, Cngru_Ste20α *(C. neoformans var. grubii)*, Pdja_Cla4 (*Pleurotus djamor)*, Umay_Cla4 (*Ustilago maydis*) and Scer_Pkin (*Saccharomyces cerevisiae)*. $^{*,\blacklozenge,\dagger}$Denotes insertions that occur only in the Scer_Pkin sequence. Identical amino acids are shaded. The alignment was obtained using the T-Coffee web server.