Supplementary data:

Impact of manganese on biofilm formation and cell morphology of *Candida parapsilosis* clinical isolates with different biofilm forming abilities Sulman Shafeeq, Srisuda Pannanusorn, Youssef Elsharabasy, Bernardo Ramírez-Zavala, Joachim Morschhäuser and Ute Römling

Strains/ Plasmids	Description	Source
<u>C. parapsilosis</u>		
ATCC 22019		Reference strain
SMI 416	Isolate from bloodstream infection	1
SMI 501	Isolate from bloodstream infection	1
SMI 630	Isolate from bloodstream infection	1
SMI 661	Isolate from bloodstream infection	1
SMI 681	Isolate from bloodstream infection	1
SMI 694	Isolate from bloodstream infection	1
SMI 706	Isolate from bloodstream infection	1
SMI 798	Isolate from bloodstream infection	1
SMI 828	Isolate from bloodstream infection	1
SMI 416 $bcr1\Delta$	BCR1 deletion in SMI 416	This study
ATCC 22019 $bcr1\Delta$	BCR1 deletion in ATCC 22019	This study
ATCC 22019 <i>bcr1</i> ∆ + pBCR1	<i>BCR1</i> complementation in ATCC 22019 <i>bcr1</i> Δ strain	This study
Plasmids		
pBCR1CpM2	pSFS4 containing left and right flanking region of <i>BCR1</i> gene	1
pBCR1CpMK1	Left flanking region of <i>BCR1</i> in pBCR1CpM2 is replaced with <i>BCR1</i> gene including upstream region	1

Table S1: List of strains used in this study.

References

1. Pannanusorn, S. *et al.* Characterization of biofilm formation and the role of BCR1 in clinical isolates of Candida parapsilosis. *Eukaryot. Cell* **13**, 438–451 (2014).

Figure S1: Impact of different concentrations of the divalent cations cobalt (Co²⁺), copper (Cu²⁺), iron (Fe³⁺), nickel (Ni²⁺) and zinc (Zn²⁺) as nitrate salts on the biofilm forming ability of no (SMI 416), low (SMI 706) and high (SMI 828) biofilm formers of *C. parapsilosis* after 48 h at 37 °C (A, B and C) and 30 °C (D, E and F). Biofilms developed on a polystyrene surface in 96 well plates were stained with crystal violet and the OD₆₀₀ was measured after dissolution in 30% acetic acid. Graphs represent the mean values calculated from three independent experiments with three biological replicates each. Error bars indicate the standard deviation.





Figure S2: Impact of different concentrations of Mn^{2+} on biofilm forming ability of no (SMI 416 and ATCC 22019), low (SMI 706) and high (SMI 828) biofilm formers of *C. parapsilosis* at 37 °C after 48 h. Biofilms were developed on a polystyrene surface in 96 well plates were stained with crystal violet and the OD_{600} was measured after dissolution in 30% acetic acid. Graphs represent the mean values calculated from three independent experiments with biological replicates each and error bars indicate the standard deviation. Statistical significance of the differences in biofilm formation in the presence of Mn^{2+} compared to 0 mM Mn^{2+} was determined by one-way ANOVA (ns-not significant, *P < 0.05, **P < 0.01 and ***P < 0.001).



Figure S3: Impact of Mn^{2+} on the biofilm forming ability of *C. parapsilosis* ATCC 22019, SMI 501, SMI 630, SMI 661, SMI 681, SMI 694 and SMI 798 at 30 °C after 48 h. Biofilms were developed on a polystyrene surface in 96 well plates were stained with crystal violet and the OD_{600} was measured after dissolution in 30% acetic acid. Data points represent the mean values calculated from three independent experiments with three biological replicates each and error bars indicate the standard deviation.



Figure S4: Impact of Mn^{2+} on the attachment of *C. parapsilosis* strains SMI 416 and ATCC 22019, and their derivates. Attachment of wild type (SMI 416 and ATCC 22019), their *BCR1* mutants (SMI 416 *bcr1* Δ and ATCC 22019 *bcr1* Δ), and *BCR1* complementation (ATCC 22019 *bcr1* Δ +p*BCR1*) at 30 °C after 3 h of incubation (A and B). Relative attachment was calculated with attachment of the respective wild type at 0 mM Mn²⁺ set as 100%. Cells were allowed to attach to a polystyrene surface in 96 well plates and were subsequently stained with crystal violet. The OD₆₀₀ was measured after dissolution of the crystal violet in 30% acetic acid. Graphs represent the mean values calculated from three independent experiments with three biological replicates each and error bars indicate the standard deviation. Statistical significance of the differences in attachment in the presence of 2 mM Mn²⁺ compared to 0 mM Mn²⁺ was calculated by one-way ANOVA (ns-not significant).



B



Figure S5: Cell viability in biofilms of *C. parapsilosis* SMI 416 wild type formed after 48 h at 30 °C in the presence of 2 mM Mn²⁺. (A) live cells; (B) dead cells; and (C) live/dead cells. Cells were stained with the LIVE/DEADTM BacLightTM Viability Kit according to the manufacturer's instructions.



Figure S6: Impact of different concentrations of Mn^{2+} on the biofilm forming ability of wild type (SMI 416 and ATCC 22019), their *bcr1* Δ mutants (SMI 416 *bcr1* Δ and ATCC 22019 *bcr1* Δ) and *BCR1* complementation (ATCC 22019 *bcr1* Δ +p*BCR1*) at 37 °C (A and B). Biofilms developed on a polystyrene surface in 96 well plates were stained with crystal violet and the OD₆₀₀ was measured after dissolution in 30% acetic acid. Graphs represent the mean values calculated from three independent experiments with three biological replicates each and error bars indicate the standard deviation. Statistical significance of the difference in biofilm formation (wild type SMI 416 and SMI 416 *bcr1* Δ) in the presence of Mn²⁺ compared to 0 mM Mn²⁺ was calculated by one-way ANOVA (ns-not significant, **P < 0.01, and ***P < 0.001).



Figure S7: Pseudohyphae (A) and hyphae (B, C and D) stained with Calcofluor white (CFW).



Figure S8: Multiple sequence alignment of Bcr1 of *C. viswanathii*, *C. tropicalis*, *C. dubliniensis*, *C. albicans*, *L. elongisporus*, *C. parapsilosis and C. orthopsilosis*.

BCR1_CANVI BCR1_CANTR BCR1_CANDU BCR1_CANAL BCR1_LODEL BCR1_LODEL BCR1_CANPA BCR1_CANOR Clustal Consensus	10 20 30 40 50 60 70 80 90 MSSNTQIYHKEQPHSQPGQPLLHHHTHGHPHASSSSSSSSHHNSLPPLPPIPPPHLPPLTTT-STNSTTSSGSSIPPPAIPS MSSNTQYYQKESPHSQPGQSLLHHG-HGHGVNNNSLPPLPPIPPPHLPPISNNNSHNSSTNSGASIAPPPVIS MSGTSQVLQNDSHQSQHSMAYNQRQSMIYPQAQVPHQQQHSLPPLPPIPPHLPPISNNNSHNSSNSNELINNGSVQPPLPPF MSGTSQVLQNDSHQSQHASMAYNQRQPMMYPPPPQQPLHSQNSLPPLPPIPPAPSLPPISNNISNSNSNELINNGSVQPPLPPF MHRRNPVLMSTMSNQQQYANGOFQMYHQDQRQQEQRQQQALPPISNLPSIHSQQQGQQGQQGQQYSTSQQETHSMPPTSS MNSIQSPSSPQQQ-QHSHSQQLPPTQPHLPGINSLPPISAHHYSSHRPFGSSSQSPPQSQHYPQSQRSQINATLPF MNSIQSSSSPRQSQMQQQQQHLPQPHLPGITSLPPISAHHHSSHRPFNSS-QSPQQYPQSQRSQLNSTLPF * :	100 SS-TNQQQ SSSTTNQQQ PPPITSNQQ SSSSSTSSAT PPPIISSQQ PPPIISSQH
BCR1_CANVI BCR1_CANTR BCR1_CANDU BCR1_CANAL BCR1_LODEL BCR1_CANPA BCR1_CANOR Clustal Consensus	110 120 130 140 150 160 170 180 19	0 200 ABSIAS AESVAS AESSAS AESSASS TESAASS TESAASS ** *
BCR1_CANVI BCR1_CANTR BCR1_CANDU BCR1_CANAL BCR1_LODEL BCR1_CANPA BCR1_CANOR Clustal Consensus	210 220 230 240 250 260 270 280 29 	0 300 KLLIERSKTKK KLLIERSKSKK KLLIERSKSKK KLLIERSKQKK KLLIERSKLKK KLLIERSKLKK
BCR1_CANVI BCR1_CANTR BCR1_CANDU BCR1_CANAL BCR1_LODEL BCR1_CANPA BCR1_CANOR Clustal Consensus	310 320 330 340 350 360 370 380 350 KLKQQEKKQQQQQQLKAQA <	0 400 2PAPIGIAPPP IPHAH-PPQQP PQPP PQPP PQPP
BCR1_CANVI BCR1_CANTR BCR1_CANDU BCR1_CANAL BCR1_LODEL BCR1_CANPA BCR1_CANPA BCR1_CANOR Clustal Consensus	410 420 430 440 450 460 470 480 49 PPSQLPPPPGPAGASISQPLAHASSSYFDQYRQTS -QSHLTSLPPPPKISAHP -SKPLPPLPHQIE - SSQLPPPPP-PPGPTVSQPLAHATSSYFDQYRQT -QNHLTSLPPPPKISAHP -SKPLPPLPHQIE - MP -QQQQQ -HQQGIPAGYLDPYRQYP -NTFDPMALPPPPKINAHP -QKPLPPLPHQIG - PP -PAQQQVPGQQCPQQQGMPAGYLDPYRQYP NTFDPMALPPPPKINAHP -QKPLPPLPHQMGGQ - FANHNPHSPN -TYPLAPSYIYPPQPSQQPHQNPHPHLQPTNGSY -HKPLPPLPHQMGGQQ - HQQQQPLQ -GQPFSSHVPIPSQVPMCGYSVP -LQQSQSQ -HKPLPPLPHQVDQSQ-SLQ QQQQQQQQPSLPQAQTFTSQVPMPGGYMNGQYSVP -LQQSHSQSHLQQSQFAQHSPDGQQSMSQSTYHKPLPPLPHQVDQSQ-SLQ	0 500 QETSE QETSE QVNAS .QTTLPQINKD QQTTLPPIQRN
BCR1_CANVI BCR1_CANTR BCR1_CANDU BCR1_CANAL BCR1_LODEL BCR1_CANPA BCR1_CANOR Clustal Consensus	510 520 530 540 550 560 570 580 59 SDVQS	0 600
BCR1_CANVI BCR1_CANTR BCR1_CANDU BCR1_CANAL BCR1_LODEL BCR1_CANPA BCR1_CANOR Clustal Consensus	61062063064065066067068069MPDATYPPPKSATSAASMTPNLASPISPLFQQSFNQGNSRNGNMSSNYPSGSNPRAS-TITTSTVSTKSPMSQHFSIISTSS-NWTNLSDAVYPPPPKSATSAASMTPNLASPISPLFQSSFNQGTSKSGNGNNAGVSGSNPRAS-TITTSTVSIKSPMSQHFSIISSS-NWTNTSDAVYPPPPKSATSAASLTPNLASPLSPLFHQSFSQTTLKSTPDPRGSSTLTNSSVSIKSPMSQHFSILSGNS-NVSNAQNGQYPPPPKSATSAASLTPNLASPLSPLFHQSFSQTTLKS	0 700 INTTNSLPSVN INTTNSLPSVN INTTNSLPSVS INTTNSLPSVS INTTNSLPSVS INTSINLPTMQ VNGMKSPLSQ INGTMKSPLSQ * *
BCR1_CANVI BCR1_CANTR BCR1_CANDU BCR1_CANAL BCR1_LODEL BCR1_CANPA BCR1_CANOR Clustal Consensus	710 720 730 740 750 760 770 780 79 NLPHPNWGGSSGSSGYNKSFHERQSSQLSDSILVYS	0 800 NKEVKSN NKDVKSN RKDIKST RKDIKSN EARROGQSSN NNN HNGNGTP

	810	820	830	840	850	860	870	880	890	900
BCR1 CANVI	WLRGVLNDEENR	· · · · · · · · ·		 		.		 SMMVDKI	 DOEED	GGDVS
BCR1 CANTR	WLRGVLNDEEE					AKGDGDND		SMMVDKEDI	DOEDEEENKN	QEDCS
BCR1_CANDU	WLRGVLNDDTN		DPQENF	LTSPSPA	PTLPPVSSI	TNG-TNDGNK		DVIMSESE:	3ESQSQ	SQTNL
BCR1_CANAL	WLKGVLNDDNNNNNN	NNN	E QQQQQ	QLQDPKLASPA	PTLPPVSSI	TGGGTSNGND		SMMVDNME	NKNDVS	QSNVI
BCR1_LODEL	WLKGVLNSDDGNNKK	TNNEDSMMIDG	DGNGNGDGN	NLGKENRDSME	LSLSREASI	NSVIGSGSNEKE	SPHRRSQTQ	LPPIEGTSME	KNDSVDVALD	RKPAS
BCR1_CANPA	SLPSIKNLPIPG					-NFHSSNGGG		PMNVNNAS	/GGGER	RDVRN
BCR1_CANOR	SLPSIKNLPIPG					-NSLSSNGTT		NMNTS	ISDGEM	RDVRD
Clustal Consensus	* .: *					• •		:		
	910	920	930	940	950	960	970	980	990	1000
		.				.				
BCR1_CANVI	VTNNTSSSNNNY	STSTEVIDDSM	RTSTPS	-IKSEHSNDTI	HTTTVVLK-	KE QPQQPI	VP			
BCR1_CANTR	IANNTTSNNNY	STSTEVIDESM	RTSTPS	-IRSDNSNDTI	HSTTVVLK-	KDHQAPSI	VSTS			
BCR1_CANDU	INSNGKSKQESGP	TLESTELT	NIGASS	-IKSTHSTETI	QTTMVIVN-	KSDTPTAT	TTITTTNESS	SNTNATT		
BCR1_CANAL	ID SNNNNG DKMV NKL	NFQSIELHDNN	NNNTPS	-IKSTHSTETI	QTTMVVVN-	KSDPPPLS	STPTTNTNTN	TNSTATTN		
BCR1_LODEL	VNKHANIGASSIL	SVQNETLKRPL	ILNHDNDE	GIDGGHSSQSV	HTLLSNRSG	LYSGKPLQLPTI	PPVNSGASG	SGSGGSGINN	JDRRD GN M TN	GALPS
BCR1_CANPA	VNGHSIG	SVGSLIHNEEE	STTIAS	TSNNNANVTA	STSMNTTT-	SETLGKS	SN			
BCRI_CANOR	TNGHSIG	SVGSLIHNDE-	STNVTN	-TTDVTTTSTV	NTTVGDSA-	SSSTGKS	3N			
Ciustai Consensus	•	• •	•	• •• •	•					
	1010	1020	1030							
		.								
BCR1_CANVI	HVSKKPTINN	LISPSPNEPVS	IKEE							
BCR1_CANTR	HVSKKPTINN	LISQSPNEPVS	IKEE							
BCR1_CANDU	SEYVSKKPTINN	LISPSPPQ								
BCR1_CANAL	ATGYVSKKPTINN	LISQ								
BCR1_LODEL	VPASPYVSKKPTINS	LIM								
BCR1_CANPA	WLKGVLNN	DEKRQ								
BCR1_CANOR	WLKGVLNN	EEKRQ								
Ciustal Consensus	* .:*.									