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Polymyxin B Nonapeptide Inhibits Mating in *Saccharomyces cerevisiae*

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Polymyxin B nonapeptide enhanced susceptibility of yeast cells to various hydrophobic antibiotics and to mating pheromones. At much lower concentrations, the nonapeptide severely inhibited mating. The inhibition was caused by interference with sexual agglutination.

Polymyxin B nonapeptide (PBN) is a cyclic peptide obtained by enzymatic removal of the fatty acid moiety from polymyxin B (3). PBN sensitizes enteric bacteria to various hydrophobic antibiotics by binding to and disorganizing the outer membrane (11–14). I have demonstrated that PBN is not toxic to yeast cells but, as with bacteria, it renders the cells permeable to various hydrophobic antibiotics, including those to which the yeast is normally resistant (1). In the present paper I show that PBN potentiates the effect of mating pheromones on haploid cells and, at the same time, drastically reduces or abolishes mating in *Saccharomyces cerevisiae*.

All strains used in this study are listed in Table 1. The potentiation by PBN of yeast mating pheromone α (MFα) action on *MAT***a** cells is shown in Fig. 1. PBN sensitized the cells so that inhibitory zones were easily seen at MFα concentrations that were barely effective in the control. However, PBN could not confer MFα sensitivity on an *ste2* mutant strain lacking MFα receptors (6), even when 8.5 μg of MFα was placed on a disk (not shown). This suggests that the drug does not create new entryways into the cells, but rather may use only the preexisting ones. Similar results were obtained with mating pheromone **a** (MF**a**) and *MAT*α cells (not shown).

When PBN was added to yeast mating mixtures, the yield of diploids was drastically reduced (Fig. 2). This occurred at PBN concentrations that were 10 to 50 times lower than those necessary to elicit a visible permeability response (1). At 0.005 A_{260} U/ml, the number of diploids from the YNN27 × A8534D mating pair was about 10% of the control mating and declined precipitously at higher levels of the drug. When the *MAT*α mating partner was a polymyxin B-resistant strain (GBH14 or GBH21), the diploid formation was about 1 order of magnitude less sensitive to PBN. Polymyxin B resistance in *MAT***a** strains (GBH4 and GBH24) did not improve diploid yield over that of the nonresistant control (Table 2).

Inclusion of PBN in a mixture of agglutinating cells resulted in a drastic reduction of the agglutination index (Fig. 3). Agglutination was measured by the procedure of Hartwell (6) with modifications. A low-salt minimal medium (LSM) was used because PBN effects are counteracted by standard concentrations of Ca^{2+} and Mg^{2+} (1). The LSM contained (per liter) 25 mg of $MgSO_4$, 25 mg of $CaCl_2$, 0.5 g of KH_2PO_4, 0.13 mg of $FeCl_3$, 1 ml of stock trace elements solution (see below), 1 ml of stock vitamin solution (see below), 1 mg of inositol, 0.5 g of arginine as the nitrogen source, 60 g of glucose, and other nutrients as needed to satisfy auxotrophic requirements (7). The stock vitamin solution contained (per liter) 2.5 mg of biotin, 50 mg of calcium pantothenate, 2.5 mg of folic acid, 25 mg of *p*-aminobenzoic acid, 50 mg of niacinamide, 50 mg of pyridoxine, 25 mg of riboflavin, and 50 mg of thiamine. The

TABLE 1. *Saccharomyces cerevisiae* strains used

Strain	Relevant genotype	Comment	Source
X2180-1A	*MAT***a**	MFa producer	V. L. MacKay
YNN27	*MAT*α *ura3-52 trp1-289*		R. W. Davis
A8534D	*MAT***a** *his3-40 ade2-1*		G. R. Fink
GBH4	*MAT***a** *his4-519 leu2-3, 112 pbs1-12*	Resistant to polymyxin B	This laboratory
GBH14	*MAT*α *trp1-289 pbs1-12*	Resistant to polymyxin B	This laboratory
GBH21	*MAT*α *ura3-52 trp1-289 pbs1-273*	Resistant to polymyxin B	This laboratory, from YNN27
GBH24	*MAT***a** *his3-40 ade2-1 pbs1-87*	Resistant to polymyxin B	This laboratory, from A8534D
RC629	*MAT***a** *sst1-2*	Supersensitive to MFα	R. K. Chan
3745-37-1	*MAT***a** *ste2-10::LEU2*$^+$	Lacks MFα receptors	L. H. Hartwell

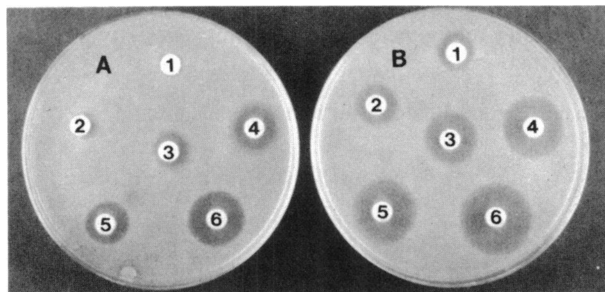

FIG. 1. Response of yeast cells (RC629 *MATa sst1-2*) to mating hormone α (MFα) in absence (A) and presence (B) of PBN. The drug was prepared and plate assays were carried out as described previously (1). Cells (2×10^7/ml) and PBN (4.6 A_{260} U, about 25 mg) were mixed in top agar (0.7% in YEPD [7]), and the mixture was poured onto a YEPD agar plate (total volume 23 ml). After the agar overlay had solidified, sterile filter disks (6 mm in diameter) were placed on the surface, and MFα (Sigma Chemical Co.) was applied (1, 0.017 μg; 2, 0.034 μg; 3, 0.085 μg; 4, 0.17 μg; 5, 0.34 μg; 6, 0.85 μg). Plates were incubated at 30°C for 24 h.

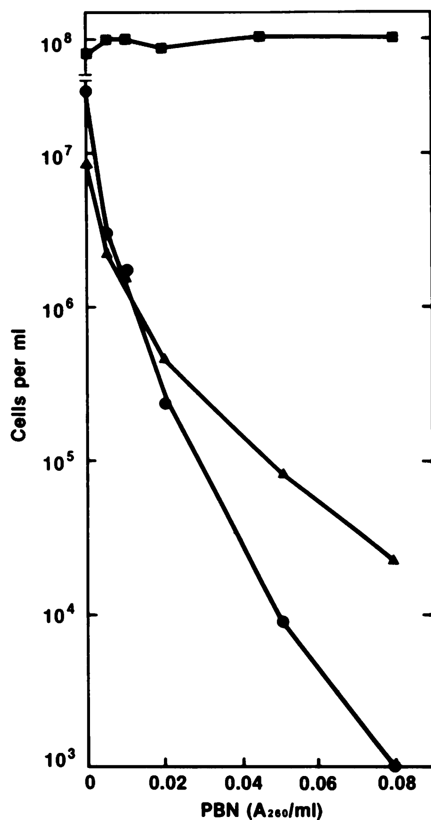

FIG. 2. Inhibition of mating by PBN. Cells were grown in liquid YEPD to early log phase (2×10^7 to 3×10^7 cells per ml), washed once with YEPD, and suspended in fresh YEPD at density of 10^7 cells per ml. Cultures of the opposite mating type (0.5 ml each) were combined in tubes (20 by 150 mm) and incubated in a New Brunswick G25 shaker at 30°C and 100 rpm for 15 h. PBN was included as indicated. After incubation, suspensions were vortexed vigorously for 30 s, and dilutions were plated on SD agar (7) (●, ▲) and YEPD agar (■) to calculate the number of diploids and total viable cells, respectively. Mating pairs: A8534D × YNN27 (●, ■) and A8534D × GBH14 (▲).

TABLE 2. Effect of PBN on mating between polymyxin B-sensitive and-resistant strains[a]

Expt	Mating pair	Diploids formed (cells/ml)		
	MATa × *MATα*	− PBN	+ PBN[b]	(%)
1	A8534D × YNN27	1.2×10^7	1.3×10^4	(0.11)
	A8534D × GBH14 (*pbs1-12*)	6.3×10^6	8.1×10^4	(1.29)
	GBH4 × YNN27 (*pbs1-12*)	2.4×10^7	2.2×10^4	(0.09)
	GBH4 × GBH14	1.2×10^7	1.5×10^5	(1.25)
	A8534D × YNN27 (40 hrs)	2.2×10^7	1.5×10^4	(0.07)
2	A8534D × YNN27	1.1×10^7	1.0×10^3	(0.009)
	A8534D × GBH21 (*pbs1-273*)	1.4×10^7	1.0×10^4	(0.072)
	GBH24 × YNN27 (*pbs1-87*)	1.1×10^7	1.0×10^3	(0.009)
	GBH24 × GBH21	2.3×10^7	1.7×10^4	(0.074)

[a] See legend to Fig. 2 for experimental details.
[b] Experiment 1, 0.05 A_{260} U/ml; experiment 2, 0.08 A_{260} U/ml.

solution was filter sterilized and stored at 4°C. The stock trace elements solution contained (per liter) 25 mg of $ZnCl_2$, 15 mg of $CuSO_4 \cdot 5H_2O$, 5 mg of $Cr_2(SO_4)_3$, 10 mg of $H_2MoO_4 \cdot 4H_2O$ (85%), 25 mg of H_3BO_3, and 25 mg of $NiCl_2 \cdot 6H_2O$. The solution was filter sterilized and stored at 4°C.

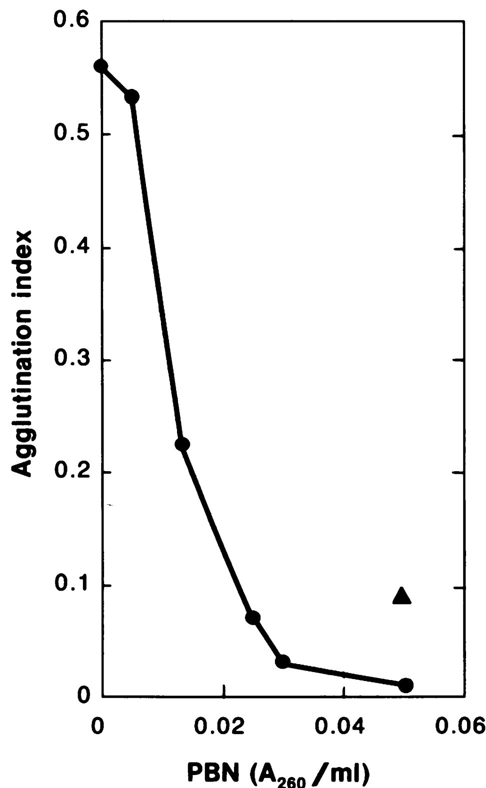

FIG. 3. Inhibition of agglutination by PBN. The ability of cells to agglutinate in the absence or presence of PBN was measured as described in the text. PBN was either present throughout induction period (●) or added after induction had been stopped with cycloheximide (▲). The agglutination index was determined on duplicate samples, and averages were plotted.

The strains tested (YNN27 and A8534D) were grown in LSM to a density of 2×10^7 to 4×10^7 cells per ml. The YNN27 culture was diluted with LSM to yield 4×10^6 cells per ml after all additions had been made. MF**a** (supernatant liquid from a stationary culture of X2180-1A in LSM) was added (0.10 volume) to induce agglutinin production. The cells were incubated at 25°C at 100 rpm for 1.25 h, and another 0.10 volume of MF**a** was added. After 1 h, cycloheximide was added to 100 μg/ml, and the culture was placed on ice until needed (used within 1 to 2 h).

The culture of A8534D was diluted with LSM to 4×10^6 cells per ml. MFα was included at 10 μg/ml to induce agglutinin synthesis. PBN was added as required. After 2.25 h at 25°C, the induction was stopped with cycloheximide. The induced A8534D culture (1 ml) was mixed with 1 ml of induced YNN27 culture, and the protocol of Hartwell (6) was followed. The agglutination index (A.I.) was calculated as [0.5 (A + B)-C]/0.5 (A + B), where A is the A_{660} of the A8534D culture, B is the A_{660} of the YNN27 culture, and C is the A_{660} of the mixture of the two cultures. When C = 0, the cultures agglutinate completely; when C = 0.5(A + B), there is no agglutination. The inhibitory effect of PBN was evident whether the drug was present throughout the period of agglutinin induction, or was added after the induction was stopped with cycloheximide (Fig. 3). Therefore, it appears that PBN inhibits diploid formation because it interferes with agglutination of cells competent for mating.

These results attest to the complex manner in which PBN interacts with *S. cerevisiae* cells. At relatively high concentrations, the drug sensitized cells to various antibiotics and to mating pheromones. At low concentrations, PBN severely decreased mating efficiency. PBN was not metabolized in the course of incubation, and the inhibition was as strong after 40 hrs as it was after 15 h (Table 2). The effect must be largely caused by interference with the agglutination reaction because PBN does not prevent the synthesis of mating pheromones or the formation of "schmoos" within the concentration range of 0.005 to 0.05 A_{260} U/ml (not shown). It is interesting that a polymyxin B resistance mutation (*pbs1*) makes the mating process less sensitive to the presence of PBN, but only if the MATα mating partner carries the mutation (Fig. 2 and Table 2). The reason for this directionality of PBN action is not known. It is possible that this is linked to the greater hydrophobicity of MF**a** than of MFα (8) and the resulting stronger effect of MF**a** on *MAT*α cells in the mating assay.

A great deal of attention has been devoted recently to the genetics and biochemistry of agglutination response in *S. cerevisiae* (4, 5, 9, 10, 15). The synthesis of sexual agglutinins is under *MAT* locus control (10). Recent work has identified a complex set of mutations affecting the expression of agglutinability (4, 5, 9). Some of the mutations (*cag1* and *sag2*) are linked to the *MAT* locus and others (*cag2* and *ag*α1) are not. With the observed PBN effect on agglutinability, it may become possible to dissect the system further by using PBN for in vitro and in vivo studies on the biochemistry and genetics of agglutinin reactivity and complex formation.

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LITERATURE CITED

1. **Boguslawski, G.** 1985. Effects of polymyxin B sulfate and polymyxin B nonapeptide on growth and permeability of the yeast *Saccharomyces cerevisiae*. Mol. Gen. Genet. **199:** 401–405.
2. **Chan, R. K., and C. A. Otte.** 1982. Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by **a**-factor and α-factor pheromones. Mol. Cell. Biol. **2:**11–20.
3. **Chihara, S., T. Tobita, M. Yahata, A. Ito, and Y. Koyama.** 1973. Enzymatic degradation of colistin. Isolation and identification of α-*N*-acyl αγ-diaminobutyric acid and colistin nonapeptide. Agric. Biol. Chem. **37:**2455–2463.
4. **Doi, S., and M. Yoshimura.** 1985. A mutation affecting sexual agglutinability in *MAT*α locus of *Saccharomyces cerevisiae*. Curr. Genet. **9:**191–196.
5. **Doi, S., and M. Yoshimura.** 1985. Alpha mating type-specific expression of mutations leading to constitutive agglutinability in *Saccharomyces cerevisiae*. J. Bacteriol. **161:**596–601.
6. **Hartwell, L. H.** 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. J. Cell. Biol. **85:**811–822.
7. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1983. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
8. **Strazdis, J. R., and V. L. MacKay.** 1982. Reproducible and rapid methods for the isolation and assay of **a**-factor, a yeast mating hormone. J. Bacteriol. **151:**1153–1161.
9. **Suzuki, K., and N. Yanagashima.** 1985. An α-mating type-specific mutation causing specific defect in sexual agglutinability in the yeast *Saccharomyces cerevisiae*. Curr. Genet. **9:**185–189.
10. **Thorner, J.** 1981. Pheromonal regulation of development in *Saccharomyces cerevisiae*, p. 143–180. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Vaara, M., and T. Vaara.** 1983. Sensitization of Gram-negative bacteria to antibiotics and complement by a nontoxic oligopeptide. Nature (London) **303:**526–528.
12. **Vaara, M., and T. Vaara.** 1983. Polycations sensitize enteric bacteria to antibiotics. Antimicrob. Agents Chemother. **24:**107–113.
13. **Vaara, M., and T. Vaara.** 1983. Polycations as outer membrane-disorganizing agents. Antimicrob. Agents Chemother. **24:**114–122.
14. **Vaara, M., and P. Viljanen.** 1985. Binding of polymyxin B nonapeptide to gram-negative bacteria. Antimicrob. Agents Chemother. **27:**548–554.
15. **Yamaguchi, M., K. Yoshida, and N. Yanagishima.** 1984. Isolation and biochemical and biological characterization of an a-mating type-specific glycoprotein responsible for sexual agglutination from the cytoplasm of a-cells, in the yeast *Saccharomyces cerevisiae*. Arch. Microbiol. **140:**113–119.