SHORT PAPER

Genetic mapping of blasticidin S-resistant gene, *bls 2*, in *Saccharomyces cerevisiae*

BY Junpei ISHIGURO and Masami HAYASHI

*Department of Biology, Faculty of Science, Konan University,
Okamoto 8-9-1, Kobe 658*

(Received July 14, 1986)

ABSTRACT

Genetic mapping of blasticidin S-resistant gene, *bls 2*, was made by tetrad analysis in the yeast *S. cerevisiae*. The *bls 2* locus was placed between *met 1* and *MAL 4* loci located on the right arm of chromosome XI. The map distances calculated with a equation by Perkins were 12.1 cM from *MAL 4* and 21.3 cM from *met 1*.

Blasticidin S, an aminoacyl nucleoside antibiotic, is a potent inhibitor of protein synthesis in both prokaryotic and eukaryotic cells; this antibiotic blocks peptidyl transfer or peptide bond formation in the elongation process of protein synthesis (Yamaguchi *et al.* 1965; Yamaguchi and Tanaka 1966; Barbacid *et al.* 1975). In the yeast, *S. cerevisiae*, two recessive genes, designated as *bls 1* and *bls 2*, have been found responsible for resistant phenotypes toward the antibiotic (Ishiguro and Miyazaki 1985). Chromosomal mapping of the resistant mutations clarifies the gene locations of the proteins associated with the resistant phenotypes. The resistant gene, *bls 1* was found linked to an *ilv 3* locus on the right arm of chromosome X (Ishiguro and Miyazaki 1985). In the present study, genetic mapping of the resistant gene, *bls 2*, was carried out. The strains used are listed in Table 1. The genetic procedures for crossing, diploid isolation, sporulation, asci dissection, and testing of markers were those described by Sherman *et al.* (1982). During the linkage analysis, the *bls 2* gene was found linked to a *met 1* locus on the right arm of chromosome XI. To localize the gene on the chromosome precisely, phenotypic segregations among *bls 2*, *met 1*, and *MAL 4* were examined by tetrad analysis using a cross between J4007-4B and HM007 (Table 1). The result indicated the gene, *bls 2*, to be placed between the *met 1* and *MAL 4* loci (Fig. 1). The map distances calculated with a equation derived by Perkins (1949) were 12.1 cM from *MAL 4* toward a centromere and 21.3 cM from *met 1* toward a telomere of the chromosome (Fig. 1). A map distance between *met 1*

Table 1. *List of Strains*

Strain	Genotype	Source
J4007- 4B	<i>a bls 2 lys 4</i>	this study
IS447-19B	<i>α can 1 cdc 4 his 2 hom 3 leu 2-1 lys 9 met 1 pet 8 rad 4 trp 2 ura 3</i>	B-I. Ono
1403-7A	<i>a suc MAL 4 MGL 3 gal 3 gal 4 trp 1 ura 3</i>	YGSC*
HM007**	<i>α MAL 4 met 1 his 2 ura 3 can 1 rad 4 gal 1</i>	this study

* Yeast Genetic Stock Center, Berkeley

** A segregant obtained from a cross between IS 447-19B and 1403-7A.

Gene Pair	Ascus Type		Map Distance	
	PD	NPD	T	c M
bls2-MAL4	162	1	44	12.1
bls2-met1	124	1	82	21.3

Fig. 1. Mapping of the gene, *bls 2*, on the right arm of chromosome XI. PD, NPD and *T* are the parental ditype, nonparental ditype and tetratype asci, respectively. Map distance (X_p) was calculated with a equation derived by Perkins: $X_p = 50 \times [(T + 6NPD)/(PD + NPD + T)]$. Media for scoring of *bls 2*, *MAL 4* and *met 1* were YPD (1% yeast extract, 2% peptone, 2% glucose) containing 100 μ g/ml of blasticidin S, YPM (1% yeast extract, 2% peptone, 2% maltose) containing bromocresol purple as the indicator dye (Khan and Eaton 1971) and a synthetic complete medium (Sherman *et al.* 1982) lacking methionine, respectively. All media contained 2% agar for solidification.

and *MAL 4* was thus 33.4 cM, and the value is consistent with that reported previously as approximately 34 cM (Mortimer and Schild 1985), supporting localization of *bls 2* on the chromosome deduced from the present study. The previous study showed both resistant genes to be likely responsible for the changes in the permeability of the antibiotic to the cells (Ishiguro and Miyazaki 1985). Although the gene products are not yet identified, *bls 1* and *bls 2* loci may encode the structural genes for the membrane proteins or for the modifying enzymes which affect the permeability of the cell membrane.

We thank Dr. B-I. Ono and YGSC for kindly providing the mapping strains.

REFERENCES

- BARBACID, M., FRESNO, M. and VAZQUEZ, D. (1975) Inhibitors of polypeptide elongation on yeast polysomes. *J. Antibiotics* **28**, 453-462.

- ISHIGURO, J. and MIYAZAKI, M. (1985) Characterization of blasticidin S-resistant mutants of *Saccharomyces cerevisiae*. *Current Genet.* **9**, 179-181.
- KHAN, N. A. and EATON, N. R. (1971) Genetic control of maltase formation in yeast. I. Strains producing high and low basal levels of enzyme. *Molec. Gen. Genet.* **112**, 317-322.
- MORTIMER, R. K. and SCHILD, D. (1985) Genetic map of *Saccharomyces cerevisiae*, edition 9. *Microbiol. Rev.* **49**, 181-212.
- PERKINS, D. D. (1949) Biochemical mutants in the smut fungus *Ustilago maydis*. *Genetics* **34**, 607-626.
- SHERMAN, F., FINK, G. R. and HICKS, J. B. (1982) Methods in yeast genetics. Laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- YAMAGUCHI, H., YAMAMOTO, C. and TANAKA, N. (1965) Inhibition of protein synthesis by Blasticidin S. I. Studies with cell-free systems from bacterial and mammalian cells. *J. Biochem.* **57**, 667-677.
- YAMAGUCHI, H. and TANAKA, N. (1966) Inhibition of protein synthesis by Blasticidin S. II. Studies on the site of action in *E. coli* polypeptide synthesizing systems. *J. Biochem.* **60**, 632-642.