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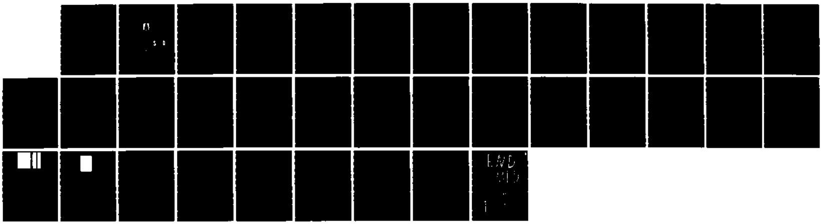

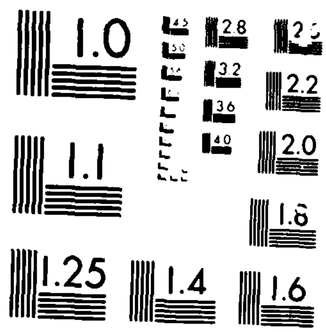

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12 (2): 93-101, 1985

Yichuan Xuebao (Acta Genetica Sinica)

Genetic Analysis of Polymeric Genes for Maltose Fermentation in Saccharomyces (1)

Ni Baofu   Chen Shiyi

(Department of Biology, Hangzhou University)

1. Genetic analyses of polymeric genes for maltose fermentation in three species of Saccharomyces were undertaken using S. globosus and S. chevalieri as recessive strains. The experimental results were as follows: 1) S. cerevisiae strain 2.982 contains two polymeric genes for fast maltose fermentation, MAL I and MAL II, each on different chromosomes; 2) S. carlbergensis strain 2.500 contains only one gene, MAL 6; 3) Among the progeny of a hybrid of S. uvarum strain 2.346 with S. chevalieri, S. uvarum has at least one gene, MAL I; 4) S. globosus 2.1161 contains genes MAL I, MAL II, and MAL 6 genes. In this paper three methods of comparative crossing between haploid cells, haploid cells and spores, and between spores were improved with increased hybridization frequencies.

This paper was received on March 28, 1984.

(1) [illegible] is given to Mr. Sheng Zujia, Shanghai Fudan University and Mr. Cai Jinke, Biological Institute of the Chinese Academy of Science for their reviews and encouragement. These projects are supported by the Science Fund of the Chinese Academy of Sciences.

Saccharomyces is an important industrial microorganism. Currently seven polymeric genes for maltose have been determined, since the inception of genetic research on maltose fermentation in the 50's. The identified genes for maltose fermentation are MAL I, MAL II, MAL 3, and MAL 4, respectively located on chromosomes VII, III, II, and XI. Recently it was reported that maltose fermentation is controlled by another gene and genetic system (2,8).

In this paper we report the isolation and characterization of the progeny strains of a hybrid maltose fermentation gene from S. cerevisiae 2.982 (from the Soviet Union), S. carlbergensis 2.500 (from America), S. uvarum 2.346, and S. chevalieri 2.213; the purpose was to provide theoretical criteria for the selection of better yeast (Saccharomyces) and also to give a basis for exploring the expression of Saccharomyces genes and their control.

MATERIALS AND METHODS

(I) Strains

Single spore strain 2.982-5D(a) and 2.982-7a(α) of S. cerevisiae 2.982 (from the Soviet Union), heterogeneous combination, maltose fermenter.

Single spore strain 2.500-1B of S. carlbergensis (from America), heterogeneous combination, maltose fermenter.

Isolated monocyte strain 2.346-11 of S. uvarum 2.346. Better spore forming ability; maltose fermenter.

Single spore strain 2.213-9C of S. chevalieri 2.213, homologuous combination; maltose non-fermenter, sucrose fermenter.

Single spore strain 2.1161-2C of S. globosus 2.1161 (from Soviet Union); homologous combination; does not ferment sucrose or maltose. /94

All of the above mentioned strains were provided by the Strain Culture Department of the Microbiological Institute, Chinese Academy of Science.

(II) Culture Media

YEPD solid culture medium: Yeast paste 1%, protein peptone 2%, glucose 2%, agar 2%, filtered through filter paper. YEPD liquid culture medium contains the above components except for agar.

Spore-producing culture medium: Sodium acetate 0.4%, cottonseed sugar 0.04%, agar 2%.

Culture medium for measuring maltose fermentation: Yeast paste 0.3%, protein peptone 0.5%, maltose 2%. Place in Durham's fermentation tube.

(III) Genetic Analysis Methods

Hybridization and dissection of the ascospore was performed under a microscope.

1. Dissection of ascospore: Johnston and coworker's method was used (3).

2. Hybridization method: Three different methods were used.

A. Cell to cell crossing: Place haploid cell fluids, each from a different cross, side by side on a glass plate. Several small drops of YEPD medium are placed by capillary in the center. Then, under the microscope, draw a 30-50 micron droplet of YEPD from the YEPD drop using a fine glass needle. Cross the two kinds of haploid cells in the droplet.

B. Crossing a cell with a spore: Because the spore and the nutriation cell are in different phases of their life cycle, it is found during hybridization that the spore generally does not germinate at the same time as the haploid cell. Moreover, the spore and cell tend to disperse in the small drop of YEPD; there is no easy way to maintain strict contact with each other, so that the frequency of zygote formation is low. To increase the hybridization frequency, initially culture the spore in the small droplet of YEPD liquid at 30 degrees C for about 1.5 hours. Then place the germinating spore and the haploid cell in the droplet of YEPD liquid for crossing. Continue culturing in 30 degrees C.

C. Crossing a spore with a spore: Cross two homologous spore strains in a microscopic droplet of YEPD liquid at 30 C for 1.5 hour; when an end of the spore obviously begins to germinate under the microcope, close the germinating ends of the two spores with a fine glass needle and continue culturing.

The above three methods of crossing need continuous microscopic observation to determine when a zygote forms. Select a germinating hybrid from the zygote, transfer it to a small drop of YEPD liquid for continuing culture, and allow the isolated hybrid to proliferate on the second day. Generally, the hybrid is immediately used for genetic analysis.

EXPERIMENTAL RESULTS

(I) Genetic analysis of polymeric genes for maltose fermentation in S. cerevisiae 2.982.

1. The determination of the number of maltose fermentation genes in S. cerevisiae 2.982: Using cell crossing of the single spore 2.982-5D and 2.982-2C, the diploid C1 was formed. Then the spore crossing method was used to cross C1 with recessive S. globosus strain 2.1161-2C. Six hybrids were obtained, C6-C11. All hybrids were mixed in the genes for maltose fermentation and could ferment maltose. C6 and C8 were randomly selected for analysis. In the 21 asci in which each contained four ascospores, the maltose fermenters isolated are shown in table 1.

From table 1 it is apparent that the experimental data confirms the model of segregation of two genes on different chromosomes. This suggests that S. cerivisiae 2.982 contains 2 genes for maltose fermentation on different chromosomes.

Previous S. chevalieri homozygous recessive strains (1,7) were used in isolation and characterization of S. cerevisiae maltose fermentation genes. To show that the same conclusions can be reached using S. globosus substituted for S chevalieri, we hybridized the spore of S. chevalieri 2.213-9C with the diploid C1 of S. cerevisiae 2.982. From the spore hybrid, 4 hybrids were obtained, C69-C72. C69 was selected randomly for analysis. Of the 11 asci, 2 asci were 4+:0-; 8 asciwere 3+:1-; 1 asci was 2+:2-. Of the three asci the main product was 3+:1; this result was the same as when using S. globosus as a homozygous recessive, which /95 proves that we can also use S. globosus as a homozygous recessive for genetic analysis of maltose fermentation genes.

Table 1 Determination of the number of genes for maltose fermentation in *S. cerevisiae*

子囊类型 (发酵:不发酵) Ascus types (fermenter:nonfermenter)	双因子独立分离 Independent segregation of two genes			
	观察值 Observed value		理论值 Theoretical value	
	子囊数 No. of asci	百分率(%) Percent	子囊数 No. of asci	百分率(%) Percent
4+ : 0-	4	19	3.5	16.7
3+ : 1-	15	71.4	14	66.6
2+ : 2-	2	9.6	3.5	16.7

2. Isolation of a strain with a single maltose fermentation gene: If hybrid C8 contains two maltose fermentation genes on different chromosomes, then each of the four single spores in the same ascus can ferment maltose following meiosis. Each spore contains a gene for fermenting maltose. Therefore the number 2 ascus of the 4 single spore strain of maltose fermenters in the same ascus was taken for further analysis. A single spore of strain of 8-3C, a homozygous maltose non-fermenter, was serially crossed with non-fermenting strains; the results are shown in table 2.

Table 2 The segregation of single gene for maltose fermentation

杂交组合 Combination	杂交方式 Cross method	获得的杂种 Obtained hybrids	被分析的杂种 Analysed hybrids	杂种表型 Phenotype of hybrids	子囊类型 Ascus types			杂种所含的 *MAL* 基因数 No. of *MAL* genes in hybrids
					4+:0-	3+:1-	2+:2-	
8-2A × 8-3C	(2)	C97-106	C97	+	0	0	11	1
8-2B × 8-3C	(2)	C107-108	C107	+	0	0	6	1
8-2C × 8-3C	(3)	C85-96	C85	+	0	0	8	1
8-2D × 8-3C	(3)	C73-84	C73	+	0	0	16	1

3. Determination of two different maltose fermentation genes: The above result showed that S. cerevisiae 2.982 contains two maltose fermentation genes on different chromosomes. To further analyze these two genes, allelism determination was done by crossing 4 single spore strains with the above 2nd ascus. The results are shown in table 3.

Table 3 Determination of two polymeric genes for maltose fermentation in the hybrid C6

杂交组合 Combination	杂交方式 Cross method	获得的杂种 Obtained hybrids	被分析的杂种 Analysed hybrids	杂种表型 Phenotype of hybrids	子囊类型 Ascus types			等位性 Allelism
					$4^+:0^-$	$3^+:1^-$	$2^+:2^-$	
8-2A × 8-2C	(2)	C116-123	C117	+	11	0	0	等位基因 allele
8-2A × 8-2D	(2)	C134-135	C134	+	1	5	2	非等位基因 non-allelic genes
8-2B × 8-2C	(2)	C124-133	C124	+	2	6	2	非等位基因 non-allelic genes
8-2B × 8-2D	(2)	C136-143	C136	+	8	0	0	等位基因 allele

The above experimental results completely prove that S. /96
cerevisiae 2.982 contains two polymeric genes for maltose fermentation on different chromosomes. We temporarily called the MAL gene in 8-2A (or 8-2C) MAL I; the MAL gene in 8-2B (or 8-2D) we called MAL II (see figure 1).

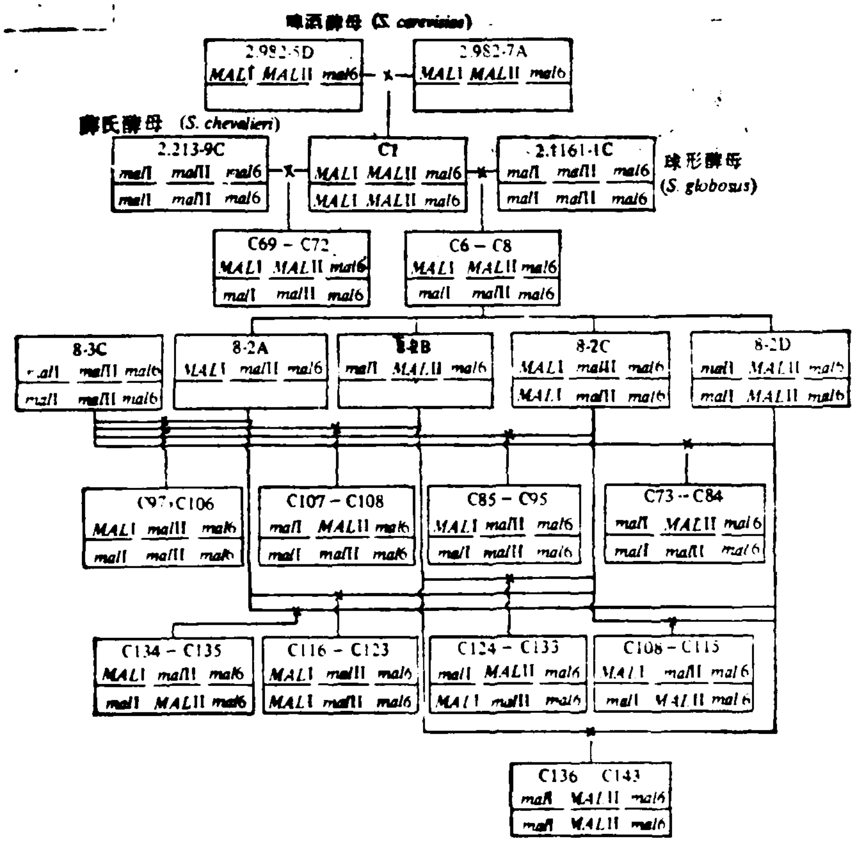

图1 啤酒酵母麦芽糖发酵基因鉴定谱系图

Fig. 1 Pedigree of identifying *MAL* genes in *S. cerevisiae*

(II) Isolation and Characterization of Maltose Fermentation Genes of S. carlbergensis 2.500 /97

1. Proof that S. carlsbergensis only has one maltose fermentation gene: Cai Jinke and coworkers identified the MAL 6 gene in S. carlsbergensis and proved it to be different from S. cerevisiae 2.1168 (from France), which contains MAL A, MAL B, and MAL C genes (1). To prove MAL 6 of S. carlsbergensis is also different from MAL I and MAL II of S. cerivisiae 2.982 (from the Soviet Union), we first isolated the MAL 6 gene.

We hybridized 2.500-1B with S. globosus 2.1161-2C using the method of cell and spore crossing. We obtained hybrids C46-C54. C50 was randomly selected for analysis. The 11 ascospore is 2+:2-, which proves the work of Cai Jinke, et al. We also named the MAL gene in 50-4B or 50-4C, which can ferment maltose, as MAL 6.

2. *Allelism determination* of MAL 6, MAL I, and MAL II: To prove that MAL 6 of S. carlsbergensis is different from MAL I and MAL II of S. cerivisiae 2.982, we undertook allelism determinations, the results of which are in table 4.

Table 4 The allelism test between *MAL*6 and *MAL*I or *MAL*II

杂交组合 Combination	杂交方式 Cross method	获得的杂种 Obtained hybrids	被分析的杂种 Analysed hybrids	杂种表型 Phenotype of hybrids	子囊类型 Ascus types			等位性 Allelism
					4+:0-	3+:1-	2+:2-	
[illegible] × 50-4B (*MAL*II) (*MAL*6)	(3)	C213-220	C218	+	1	5	1	非等位基因 non-allelic genes
8-2C × 50-4C (*MAL*II) (*MAL*6)	(3)	C228-236	C228	+	3	7	25	非等位基因 non-allelic genes

The experimental data (table 4) clearly indicate that MAL 6 in S. carlsbergensis 2.500 is distinct from MAL I and MAL II in S. cerevisiae 2.982.

(III) Genetic Analysis of Maltose Fermentation Gene in S. uvarum 2.346

1. The lethal factor of S. uvarum 2.346 and the maltose fermentation gene: To obtain the single spore strain of S. uvarum 2.346, 36 asci were dissected. Three non-germinated spore states were found; even among those which germinated, some did not form colonies; some germinated and formed new strains. The single spore strain of S. globosus or S. chevalieri were used as homozygous recessive strains to hybridize with the monocyte isolated strain 2.346-11. Upon dissection of the hybrids, 4 single spore strains from the same ascus could not be obtained. Thus it was difficult to determine the number of maltose fermentation genes. The lethal factor in S. uvarum 2.346 needs further exploration.

The viability of the hybrid progeny using S. chevalieri 2.213-9C as a homozygous recessive strain is relatively high. Using the spore crossing method we selected 28-8A, which can ferment maltose, and 28-11, which cannot ferment maltose (homozygous combination), from hybrid C28, a single spore strain from the 2 homozygous strains; from 28-11, which cannot ferment maltose (homozygous combination), we obtained 5 hybrids (C144-C148). C146 was randomly selected for analysis. It had 9 asci which were 2+:2-, which proves 28-8A only contained one maltose fermentation gene.

2. The determination of allelism of the MAL gene of 28-8A and MAL I, MAL II, and MAL 6: To determine the differences and similarities of the MAL gene of 28-8A and MAL I, MAL II and MAL 6, we selected homozygous combination of single spore 146-2D, which

forms a capsule, and contains MAL of 28-8A. This was hybridized with a strain (without capsule formation) containing MAL I, MAL II, and MAL 6. Allelism determination was done on the maltose fermentation genes. The results are shown in table 5.

Table 5 Determination of the gene for maltose fermentation in *S. uvarum*

杂交组合 Combination	杂交方式 Cross method	获得的杂种 Obtained hybrids	被分析的杂种 Analyzed hybrids	杂种表型 Phenotype of hybrids	子囊类型 Ascus types 4+:0-	3+:1-	2+:2-	等位性 Allelism
146-2D(MAL1)× 50-4B(MAL6)	(3)	C318	C318	+	1	4	1	非等位基因 non-allelic genes
		C319	C319	+	2	4	0	
		总数 Total			3	8	1	
146-2D(MAL1)× 8-2C(MAL1)	(3)	C306—311	C310	+	14	0	0	等位基因 allele
			C311	+	16	0	0	
		总数 Total			30	0	0	
146-2D(MAL1)× 8-2D(MAL11)	(3)	C312—315	C314	+	2	5	2	非等位基因 non-allelic genes

From table 5 it can be seen that 146-2D contains a maltose fermentation gene which is not allelic with MAL II of 8-2D and MAL 6 of 50-4B; but it is the same as MAL I of 8-2C. The hybrid pedigree is shown in figure 2. /98

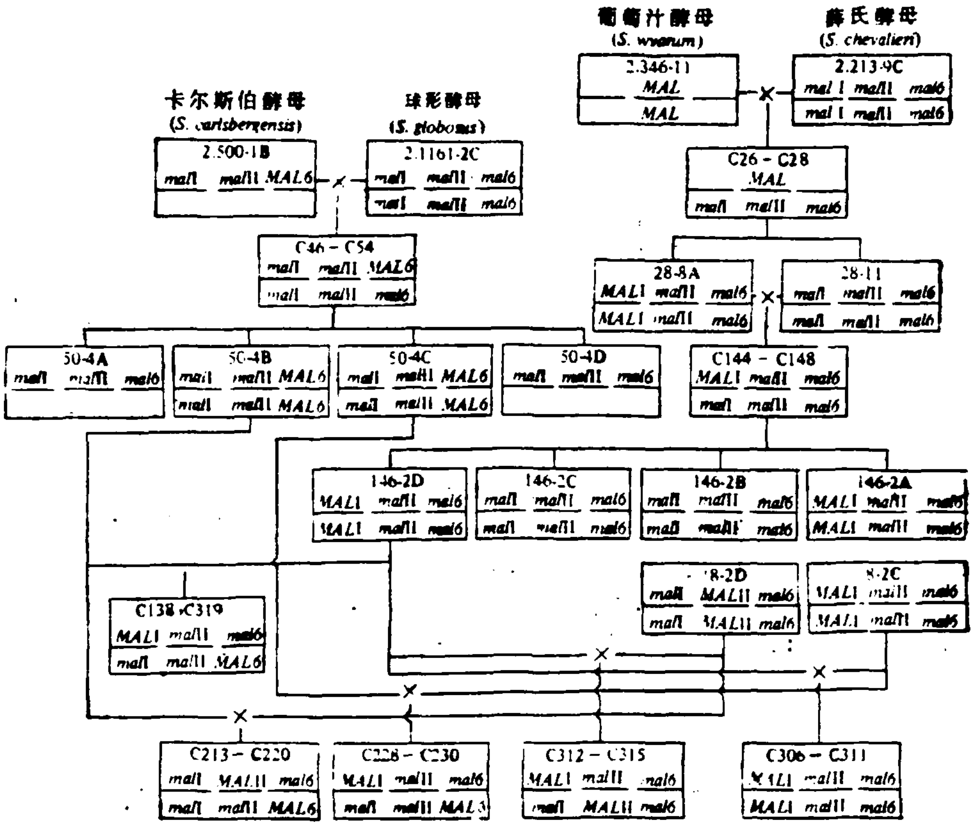

图2 卡氏酵母和葡萄汁酵母麦芽糖发酵基因鉴定系谱图

Fig. 2 Pedigree of indentifying *MAL* genes in *S. carlsbergensis* and *S. uvarum*

DISCUSSION

/99

From the experimental results it can be seen that maltose fermentation in Saccharomyses is controlled by many polymeric genes. The number of whole asci and the percentage of the two genes segregating in the isolated groups confirms the theoretical values (see table 6).

Table 6 The segregation of two genes for maltose fermentation

杂交组合 Combination	被分析的杂种 Analysed hybrids	子囊类型 Ascus types		
		4+:0-	3+:1-	2+:2-
C1 × 2.1161-2C	C6,C6	4	15	2
8-2A × 8-2D	C134	1	5	2
8-2B × 8-2C	C124	2	6	2
8-2D × 50-4B	C218	1	5	1
8-2C × 50-4C	C228	3	7	2
8-2D × 146-2D	C314	2	5	2
146-2D × 50-4B	C318,C319	3	8	1
观察值 Observed value	子囊数 no. of asci	16	51	12
	百分率 percent	20.2	64.6	15.2
理论值 Theoretical value	子囊数 no. of asci	12.9	52.2	12.9
	百分率 percent	16.7	66.6	16.7

Winge, et al., developed a sensitive assay tube for fermentation, and used it to identify two fast maltose fermentation genes, MAL 1 and MAL 2, and one slow maltose fermentation gene MAL 3 (7) from "yeast foam." Cai Jinke identified 3 fast fermentation genes, MAL A, MAL B, and MAL C, from S. cerevisiae 2.1168 , but did not find MAL 3 (1), using Winge's experimental conditions. The genetic analysis of the maltose fermentation gene of Saccharomyces, S. chevalieri was formerly used as the recessive strain. Our experiments proved that S. globosus can be substituted for S. chevalieri as a recessive strain in the genetic analysis of the maltose fermentation genes. At first the rapid maltose fermentation gene from S. cerevisiae 2.982 was isolated and characterized. It will require further study to see if the strain contains MAL 3, accounting for the low fermentation. The reason for the low fermentation by MAL 3 will require more research. It is known that MAL I is closely linked to SUC I and MAL 3 to SUC 3, each located on different chromosomes. If we can take S. globosus, which does not ferment maltose or sucrose, as a double recessive strain and obtain the MAL 3 gene, then we can perform linkage analysis of isolated MAL and SUC genes, therby determining MAL I, SUC I, and SUC 3. Since S. chevalieri cannot ferment maltose but does ferment sucrose, it contains 3 sucrose genes. Thus, if the MAL gene is selected for genetic analysis, the above linkage analysis cannot be used. The genetic analysis of the sucrose fermentation gene of S. chevalieri will be reported in another paper. The MAL 2 and MAL 4 genes can be used to identify one another by genetic linkage analysis.

The traditional classification of yeast basically depended upon the size of the cell and its shape, the sugars fermented and assimilated, and physiological characteristics. In recent years many researchers have studied new methods of yeast classification. Among them is the classification method based upon molecular genetics of the nuclear genes of Saccharomyces. Because of the stability of the DNA molecular classification, it is more meaningful than the microbiological approach taken by other research methods (6). In 1952 Lodder divided 2 kinds of S. uvarum and S. carlbergensis according to size, length, and width of cell. In 1972 he combined these 2 above kinds into one. We find the MAL 6 contained in S. uvarum is different from S. carlsbergensis in the progeny strain of hybrids of S. uvarum and S. chevalieri, but it is the same with maltose fermentation gene MAL I of S. cerevisiae. Regarding the question of whether the uvarum yeast contains MAL genes besides MAL I is not known; the main impediment is that few spores survive. Up to the present, there is no report of the genetic analysis of the S. uvarum MAL gene. We believe further research is needed. /100

The Saccharomyces belong to the ascomycetes and have a typical combination to form better hybrid species for genetic analysis. Therefore research on various methods of hybridization has also received a certain amount of attention, for example, Lindegren's mass hybridization. S.Y. Chen for the first time used the method of hybridizing different combinations of haploid single cells with cells. Regarding spore hybridization, Roberts has advocated a temperature of 15 degrees C for 16-18 hours after crossing to increase hybridization frequency (4). Iguti suggested the maximal culture temperature for hybridization frequency is 20 degrees C; this is based upon culture temperature sides. Each obtained different hybridization frequencies. Roberts obtained

6.7% (4). Winge obtained 16.3% (7). Cai Jinke and coworkers got about 10% (1). In an experiment in which the hybrids reported in the literature are taken into 9 combinations crossing pairs of 322 spores resulted in 34 hybrids, a hybridization frequency of 10.5%, conforming with literature reports. Moreover, using the method recommended in this paper, in 21 combinations of 823 pairs of spores crossings, resulting in 286 hybrids, a hybridization frequency of 34.8%; this is a greatly increased hybridization frequency. It was reported that the chances of a successful cell and spore crossing are small. Therefore, using the method recommended by this paper, in 9 combinations were crossed 457 spores, resulting in 65 hybrids, a frequency of 14.2%. It is known that the combination process has three periods: cell adhesion, cell linkage and nuclear fusion. DNA duplication in different kinds of combination of haploid cells inhibits cell cleavage. Cell adhesion depends largely upon a and alpha factors, their dispersion, their concentration in the culture medium and the degree of cell contact. The cause of the increased hybridization frequency in the method introduced in this paper is probably due to the culturing in a microscopic droplet of medium, which increases the concentration of a and alpha factors in the medium; a drop of medium from a capillary is not suitable (average diameter more than 1 mm). Secondly, the surface tension of the liquid increases the degree of contact between cells. The spore hybrid decreases the ability because the germinating parts of the spore, even though their physiological state is assimilated, still have an inability to link. Regarding the crossing of cells with spores, it is the permitting of the spore to germinate that mimics the haploid nutritional cell, thereby increasing the hybridization frequency. Improvements of this method has certain practical value in the genetic research on hybrid culture of Saccharomyces and research on genetics.

REFERENCES

(1) Cai Jinke: 1978. Yichuan Xuebao, 5(2): 89-98.

[2] Evans, I. S. et al.: 1976. *Genet. Res.* 27(1): 89—93.
[3] Johnston, J. R. et al.: 1959. *J. Bacteriol.* 78: 292.
[4] Mortimer, R. K. et al.: 1969. *In* "The Yeasts", ed. A H Rose et al., Academic Press New York. Vol. 1, 303—460.
[5] Mortimer, R K. et al.: 1980. *Microbiol. Rev.* 44 (6): 519—570.
[6] Phaff, H. J. et al.: 1978. *In* "The Life of Yeasts", London, England.
[7] Winge, O. et al.: 1950. *Compt. Rend. Lab. Carlsberge Ser Physiol.* 25(2): 35—83.
(8) Naumov, G.I.: 1976. Genetika, (11): 86-100.

Genetic Analyses of Polymeric Genes for Maltose Fermentation in *Saccharomyces*

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ABSTRACT

Through a series of extensive crosses of three species of *Saccharomyces* with recessive strains *S. globosus* or *S. chevalieri*, genetic analyses of polymeric genes for fast maltose fermentation were undertaken by means of micromanipulation technique. The results were as follows: (1) A strain of *S. cerevisiae* (No. 2.982) contains two polymeric genes for fast maltose fermentation; *MAL*I and *MAL*II in different chromosomes. (2) A strain of *S. carlsbergensis* (No. 2.500) contains only one gene for maltose fermentation; *MAL*6. (3) We found that among the progeny of a hybrid of *S. uvarum* (No. 2.346) with *S. chevalieri*, *S. Uvarum* possesses at least one gene for maltose fermentation, which is identical with *MAL*I from *S. cerevisiae*, but not identical with *MAL*6 from *S. carlsbergensis*. (4) *S. globosus* may be considered as a recessive parent as *S. chevalieri* to identify genes for maltose fermentation, since it contains following genes: *mal*I, *mal*II and *mal*6.

In this paper, cross methods of comparing between haploid cells, a haploid cell with a spore and between spores were improved in some aspects, and discussed in detail. Various strains with monogene, and with different combinations of these genes as above, were obtained in our experiments.

12 (3): 106-112, 1985

Yichuan Xuebao (Acta Genetica Sinica)

Identification and Localisation of Transposition Genes in Transposon Tn 233 (CH)

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Tn233 (CH) is a transposon isolated from a resistant Shigella strain in our clinic; its physical map reveals a structure basically similar to transposon Tn21. Plasmid pBR322::Tn233 (CH) DNA with transposon Tn233 (CH) was partially digested with restriction endonucleases Èco RI and Bam Hi. The DNA fragments produced were religated with T4-DNA ligase. The resulting DNA molecules were used to transform Escherichia coli C600. A number of deleted transposition-defective mutants and deleteted mutants which were still able to translocate were isolated. The results of complementation test show that the transposition function of deleted transposition defective mutants of Tn233 (CH) can be complemented by deleted mutants still capable of transposition. Then the deletions were mapped by restriction endonuclease analysis, and the location of transposition genes of Tn233 (CH) was verified.

Received December 5, 1983.

Bacterial transposons are one kind of DNA which can move within a plasmid. They contain genes concerned with the process of transposition as well as genes unrelated to transposition, e.g., genes conferring antibiotic resistance or for heat stable enterotoxins, etc. (8). During the past years in research on transposon molecular and genetic structure, research on transposon Tn3 has included its complete nucleotide analysis and revealed its genetic structure. In addition to determining the location of the ampicillin resistance gene, it was found that its two ends have 38 bp inverted repetitive sequences. Genes concerned with the transposition function of Tn3 (tnpA), i.e., a lysozyme gene (tnpR) and a DNA segment related to the IRS (or res) transposon were identified (7). Additionally, research was also begun on transposon genes in Tn2603 (9) and Tn21 (5). This work has important significance for understanding the transposition mechanism and the evolutionary relationships of some transposons.

Tn233 (CH) is a transposon isolated in plasmid DR233 from a drug resistant strain of Shigella in our laboratory. It contains a resistance gene to streptomycin, sulfanilamide, and mercury ion (1). We have previously presented its physical (6,2) and genetic (4) maps. It is basically the same as transposon Tn21. This paper reports the research results in identifying and localizing the transposition gene in transposon Tn233 (CH).

Materials and Methods

The strain, its culture conditions, plasmid DNA preparation, assay by agarose gel electrophoresis, bacterial transformation, and drug resistance were reported previously (3).

The structure of deleted mutant plasmid pBR322::Tn233 (CH) DNA: The complete digestion of plasmid DNA with restriction endonucleases was carried out in a buffer with Tris 100 mM pH7.5, $MgCl_2$ 10 mM, NaCl 50 mM, and DTT 2 mM. The reaction mixture was incubated at 37 degrees C for 30 minutes; the reaction was stopped by heating to 65 degrees C for 10 minutes. For partial digestion the same reaction system was used, but the restriction endonucleases were added at different times after dilution; the degree of enzymatic hydrolysis was determined by agarose gel electrophoresis. After stopping the reaction, the mixture containing the enzymatic hydrolysis buffer was diluted two times and cooled to 8 degrees C. DTT and ATP were then added to a final concentration of 10 mM and 1 mM, respectively. One unit of /107
T4-DNA ligase is then added and the reaction mixture is incubated for 24 hours at 8 degrees C, then used to transform bacteria. Eco RI is a product of Dong Feng Reagent Factory. Bam HI is prepared using the method of Wilson, et al. (10). T4 DNA ligase was donated by Dr. Wang Dajian.

Transposition assay: E. coli C600 donor strain (with the plasmid R144drd3 or its derivatives and pBR322::Tn233 (CH) or its derivatives) and E. coli 802 (Nal^r) recipient strain are respectively put in nutrient broth and grown overnight. Using a 2% innoculation volume, inoculate a fresh bottle of nutrient broth and culture for 4 hours with agitation at 37 degrees C. Incubate without agitation for an additional 2 hours. After an appropriate dilution of the culture medium, smear the medium onto nutrient agar culture plates containing different antibiotics. After the colonies grow out, count the number of transformants and record the type of antibiotic resistance. From the proportion of transformants with R144drd3::Tn233 (CH) (measurement includes some deleted derivatives of Tn233) and only R144drd3, one can calculate the transposition capability of Tn233 (CH) or its deleted mutants.

Results and Discussion

(I) The structure of deleted mutant Tn233 (CH) and the measurement of its transposition ability

Using restriction endonuclease Eco RI to partially digest plasmid pBR322::Tn233 (CH) DNA with transposon Tn233 (CH), followed by T4 DNA ligation to form different length circular DNA fragments, pBR322::Tn233 (CH) derivatives were formed. These contained different length DNA deletions. These derivatives were used to transform E. coli C600, an antibiotic sensitive cell, plated in nutrient agar containing ampicillin (50 microgram/ml). After the colonies grew out, they were assayed for the resistance markers of the transformants (Ap, Tc, Sm, Su, Hg). From these, 12

Table 1 Transposition ability of Tn233 (CH) and its deleted mutants

供体菌株 Donor strain *E. coli* C600		受体菌株（抗性标记） Recipient strain (Resistant markers)	转移接合子（细胞数/毫升） Transconjugants (cells/ml) 选择用营养琼脂平板中含有 Nutrient agar plates containing			a/b	转座能力 Transposition ability
携带的质粒 Plasmids carried	抗性标记 Resistant markers		Nal, Km, Sm, (a)	Nal, Km, Hg (a)	Nal, Km (b)		
1. pBR322::Tn233(CH) 2. R144drd3	Ap, Tc, Sm, Su, Hg Km	*E. coli* 802 (Nal)	8.5×10^{5}	–	3.4×10^{8}	2.5×10^{-3}	+
1. pBR322::Tn233 △E12 (pTE12) 2. R144drd3	Ap, Tc, Sm, Su Km	*E. coli* 802 (Nal)	0	–	5.7×10^{8}	$<10^{-9}$	–
1. pBR322::Tn233 △E14 (pTE14) 2. R144drd3	Ap, Tc, Sm, Su Km	*E. coli* 802 (Nal)	0	–	5.9×10^{8}	$<10^{-9}$	–
1. pBR322::Tn233 △E15 (pTE15) 2. R144drd3	Ap, Tc, Sm, Su Km	*E. coli* 802 (Nal)	1.3×10^{5}	–	5.1×10^{8}	2.6×10^{-4}	+
1. pBR322::Tn233 △E66 (pTE66) 2. R144drd3	Ap, Tc, Sm, Su Km	*E. coli* 802 (Nal)	1.0×10^{6}	–	5.1×10^{8}	2.0×10^{-3}	+
1. pBR322::Tn233 △E67 (pTE67) 2. R144drd3	Ap, Tc, Sm, Su Km	*E. coli* 802 (Nal)	6.3×10^{5}	–	3.4×10^{8}	2.3×10^{-3}	+
1. pBR322::Tn233 (CH) 2. R144drd3	Ap, Tc, Sm, Su, Hg Km	*E. coli* 802 (Nal)	–	1.1×10^{5}	1.2×10^{9}	0.9×10^{-4}	+
1. pBR322::Tn233△E41 (pTE41) 2. R144drd3	Ap, Tc, Hg Km	*E. coli* 802 (Nal)	–	0	7.6×10^{8}	$<10^{-9}$	–
1. pBR322::Tn233 △E51 (pTE51) 2. R144drd3	Ap, Tc, Hg Km	*E. coli* 802 (Nal)	–	0	8.8×10^{8}	$<10^{-9}$	–
1. pBR322::Tn233 △B5 (pTB5) 2. R144drd3	Ap, Tc, Hg Km	*E. coli* 802 (Nal)	–	1.0×10^{5}	7.9×10^{8}	$1.3\times10^{-}$[illegible]	+

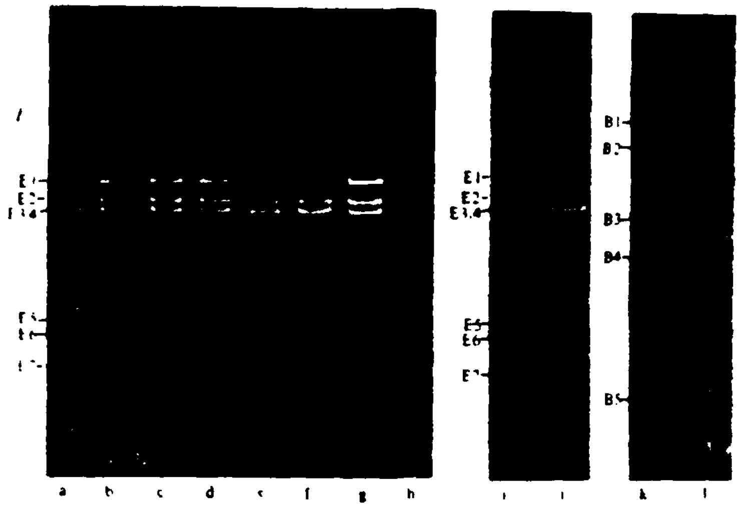

图1 pBR322::Tn233 (*CH*) 及其缺失变种的质粒 DNA 经限制性内切酶 *Eco*RI 或 *Bam*HI 消化后的琼脂糖凝胶电泳

Fig. 1 Agarose gel electrophoresis of pBR322:: Tn233 (*CH*) and its deleted mutant plasmid DNAs digested with restriction endonucleases *Eco*RI or *Bam*HI

strains of transformants (Ap^r, Tc^r, Sm^r, Su^r) which lost mercury ion resistance were selected. Similarly, 13 (Ap^r, Tc^r, Hg^r) transformants were found which had lost streptomycin and sulfanilamide resistance. When assaying the transposition ability, initially the method of linking plasmid R144drd3 (Km^r) to transform the above reconstructed pBR322::Tn233 (CH) was used. Then, according to the linking method described above, it was crossed with E. coli strain 802 (Nal^r), to assess whether Tn233 (CH) deleted mutants still transpose plasmid R144drd3 and transform the R144drd3 recipient E. coli 802 cells. Table 1 lists the assay results of the transposition ability of Tn233 (CH) deleted mutants in 7 transformant strains. In the transformants sensitive to mercury ion, the deleted mutant (Tnp-) of Tn233 (CH) lost transposition ability in plasmid pTE12 and pTE14. Deleted mutants (Tnp+) still conserved transposition ability in pTE15, pTE66, and pTE67. In the transformants sensitive to streptomycin and sulfaniliamide, Tn233 (CH) deleted mutants also lost their transposition ability in plasmids pTE41 and pTE51. Additionally,

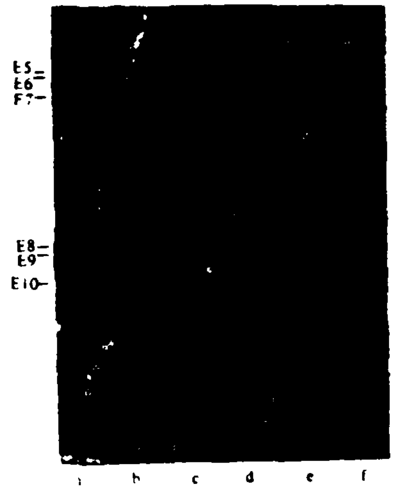

图2 pBR322:: Tn233 (CH) 及其缺失变种质粒 DNA 经限制性内切酶 EcoRI 消化后的聚丙烯酰胺凝胶电泳

Fig. 2 Polyacrylamide gel electrophoresis of pBR322::Tn233 (CH) and its deleted mutant plasmid DNAs digested with restriction endonuclease *EcoRI*

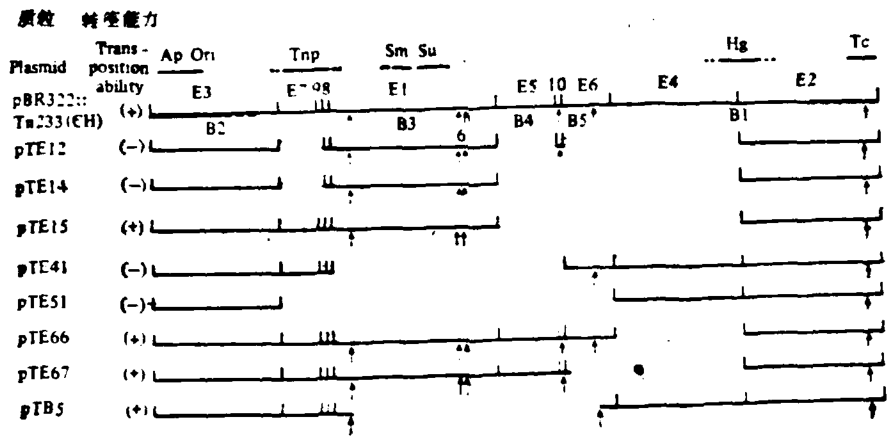

图3 pBR322:: Tn233 (CH) 及其缺失变种的物理图
图中 | 表示 *EcoRI* 切点，↑ 表示 *BamHI* 切点位置。

Fig. 3 Physical maps of pBR322::Tn233 (CH) and its deleted mutants
The map shows *EcoRI* (¦) and *BamHI* (↑) sites.

Table 2 Complementation of deleted transposition-defective mutants by deleted transposition wild-type Tn233 (CH) in trans

供体菌株 Donor strain *E. coli* C600		受体菌株（抗性标记） Recipient strain (Resistant markers)	转移接合子（细胞数/毫升） Transconjugants (cells/ml)			a/b	转座能力 Transposition ability
携带的质粒 Plasmids carried	抗性标记 Resistant markers		选择用营养琼脂平板中含有 Nutrient agar plates containing: Nal, Km, Sm (a)	Nal, Km, Hg (a)	Nal, Km (b)		
1. pBR322:: Tn233△E12 (pTE12) 2. R144drd3:: Tn233△F5 (pRB5)	Ap, Tc, Sm, Su Km, Hg	*E. coli* 802 (Nal.)	$2.1\times10^{\text{[illegible]}}$		$1.3\times10^{\text{[illegible]}}$	$1.6\times10^{\text{[illegible]}}$	+
1. pBR322:: Tn233△E14 (pTE14) 2. R144drd3:: Tn233△B5 (pRB5)	Ap, Tc, Sm, Su Km, Hg	*E. coli* 802 (Nal.)	$2.5\times10^{\text{[illegible]}}$	–	$1.1\times10^{\text{[illegible]}}$	$2.5\times10^{\text{[illegible]}}$	+
1. pBR322:: Tn233△E41 (pTE41) 2. R144drd3:: Tn233△F15 (pRF15)	Ap, Tc, Hg Km, Sm, Su	*E. coli* 802 (Nal.)	–	$8.3\times10^{\text{[illegible]}}$	$3.1\times10^{\text{[illegible]}}$	$2.6\times10^{\text{[illegible]}}$	+
1. pBR322:: Tn233△E51 (pTE51) 2. R144drd3:: Tn233△F15	Ap, Tc, Hg Km, Sm, Su	*E. coli* 802 (Nal.)	–	4.5×10^{3}	[illegible]$.8\times10^{\text{[illegible]}}$	$4.6\times10^{\text{[illegible]}}$	+

using the same method and substituting Bam HI for Eco RI, strains of transformants sensitive to streptomycin and sulfanilamide were obtained. The tranpositional ability of the Tn233 (CH) deleted mutants which retained transposition activity in plasmid pTB5 are shown in table 1.

(II) Restriction map of Tn233 (CH) deleted mutants

Plasmid DNA was extracted from 8 strains of transformants (see table 1) whose transpositional activity had been measured. These were completely digested by restriction endonucleases Eco RI and Bam HI followed by agarose and polyacrylamide gel electrophoresis.

Comparing with the restriction enzyme map of pBR322::Tn233 (CH) DNA from figures 1 and 2, one can find that pTE12 lost 5 restriction fragments, i.e., E7, E9, E5, E6, and E4; pTE14 lost 6 restriction fragments, E7, E9, E5, E10, E6, and E4; pTE15 lost 4, E5, E10, E6 and E4; pTE66 lost the E4 segment; pTE67 lost 2 segments, E6 and E4. From the genetic map of Tn233 (CH) it is known that the part of the sequence of the mercury ion resistance is located in the E4 segment. The above mentioned 5 strains of Tn233 (CH) deleted mutants lost E4 segments, so that the /109 host cells are sensitive to mercury ion, which confirms the results of resistance testing. pTE51 lost 3 restriction fragments E1, E5 and E10; pTE41 lost 7 restriction fragments E7, E9, E8, E1, E5, E10, and E6; pTB5 lost 4 restriction fragments, B3, B6, B4, and B5. From the Tn233 (CH) genetic map we know the resistance genes for streptomycin and sulfanilamide are located in the E1 or B3 fragments (E1 and B3 overlap). The 3 strains of Tn233 (CH) deleted mutants lacking E1 or B3 fragments are sensitive to streptomycin and sulfanilamide, confirming the results of the resistance assay. The 8 strains of Tn233 (CH) deleted mutants which lost E7, E9, E8 and E1 lost the tranposition ability (figure 3). But in plasmid pTB5, even in Tn233 (CH) deleted mutants which lost B3, B6, B4, and B5 the transposition ability was retained. B3, B6, and B4 have large overlaps with the right of E1, indicating that only a small part of the left side of E1 has the transposition function. We can thus know E7, E8, E9, and a small part of the left side of E1 DNA sequences are concerned with transposition functions of Tn233 (CH). The E3 fragment is located to the left of the E1 fragment (figure 3). The end of Tn233 (CH) is located in the E3 fragment. The E3 fragment has a duplication of the start region (Ori) of vector plasmid pBR322. If fragment E3 is deleted, then the whole plasmid pBR322: :Tn233 (CH) cannot exist, so that E3 deleted mutants cannot be obtained. Therefore it is difficult to identify sequences in the E3 fragment of Tn233 (CH) concerned with transposition.

(III) Complementation of transposition function of Tn233 (CH) mutants Tnp+ and Tnp-

From restriction map analysis of Tn233 (CH) deleted mutants, it is clear that E7-E9-E8 and part of the sequences on the left of E1 are concerned with the transposition function of Tn233 (CH). We can also know that both ends of Tn3 have inverted repeated sequences, transposase gene tnpA, lysozyme gene tnpR and the IRS (also called res) sequences which are important to transposition of Tn3 (7). Therefore for distinquishing DNA sequences concerned with transposition function located in E7-E9-E8 and part of E1 fragments of Tn233 (CH), it must be found whether they contain a marked enzyme gene or not a marked protein, but only if they contain indispensible IRS sequences for the transposition process. This was done by the complementation test of Tn233 (CH) Tnp+ mutant to the transposition of the Tnp- mutant. Experimentally, the transposed pTB5 only carries the resistance to mercury ion; the deleted transposon (called Tn233ΔB5), sensitive to streptomycin and sulfanilamide, with plasmid R144drd3 formed plasmid R144drd3::Tn233ΔB5. After conjugation, this plasmid was

transferred to a strain containing the pTE12 plasmid. From table 1 it is known that the deleted transposon in pTE12 only carries the resistance to streptomycin and sulfanilamide; it is sensitive to mercury ion, but it has no transposition function (called Tn233ΔE12, pTE12 plasmid also called pBR322::Tn233ΔE12). Then we obtained strains with these two plasmids, R144drd3::Tn233ΔB5 and pBR322::Tn233ΔE12. These stains are donors to be crossed with E. coli strain 802 (Nal^r). Transconjugants were selected in different antibiotic plates. Because the resistance markers were different in these 2 deleted transposons, one can see if Tnp- mutant transposon of Tn233ΔE12 can transpose. Using this method we combined 4 strains respectively with Tnp+ and Tnp- mutant transposon plasmids (table 2). The result of the transposition experiments indicated that the Tnp+ mutants have a complementary function to Tnp- in trans; this indicated the deleted function of Tnp- mutants is a marked enzyme gene. Because we did not measure the whole intermediate formed by transposition like Tn3 donor and recipient plasmids, we could not determine if the Tnp- deleted mutant, except for the tnpA gene, is similar to that in Tn3, i.e., if it includes the tnpR gene. In the experiment we found when R144drd3 plasmid carries two Tn233 (CH) mutant transposons, the transposon is unstable, i.e., in most of the plasmids R144drd3 culture in antibiotic free medium, only one kind of resistance /112
marker of a mutant transposon was left. This phenomenon will be studied in the next step.

We indicated in the previous report that restriction maps and genetic maps of Tn233 (CH) and Tn4, Tn21, and Tn2603 have many similar parts (4). Between them, Tn233 (CH) is basically similar to Tn21. Tanaka (9) et al., used the B2 and H2 fragments of cloning plasmid pMK1::Tn2603. By doing complementation tests of the transposition function of B2 and H2 segments in cloned plasmid pMK1:Tn2603 to Tn2602 Tnp- deleted mutant, he determined the position of the tnpA gene in Tn2603. De La Cruz (5) et al. compared the complementation effect of Tn21 deleted mutants to another transposon Tn1721 (TC) Tnp- deleted mutant; he found the location of tnpA gene in Tn21. We used the group of Tn233 (CH) deleted mutants of different resistance genes (streptomycin, sulfanilamide or mercury ion resistance genes). We did complementation analysis between Tnp+ and Tnp- mutants to determine the transposition ability of Tn233 (CH) derivatives, thus obtaining the experimental results which directly reflect Tn233 (CH) itself.

REFERENCES

(1) Hong Mengmin, et al.: 1979. Yichuan Xuebao, 6(3):239.

(2) Hong Mengmin, et al.: 1983. Kexue Tongbao, 5:304.

(3) Chen Jianmin, et al.: 1981. Yichuan Xuebao, 8(3):189.

(4) Wang Zongyang, et al.: 1984. Ibid., 11(2):81.

[5] De La Cruz et al.: 1982. *J. Bact.*, 151: 222.

[6] Hong, M. M. et al.: 1982. *Abst.* 13th *Intern. Congr. Microbiology*, August, p. 88.

[7] Heffron, F. et al.: 1979. *Cell*, 18: 1153.

[8] Kleckner, N.: 1981. *Ann. Rev. Genet.*, 15: 341.

[9] Tanaka, M. et al.: 1982. *J. Bact.*, 151: 723.

[10] Wilson, G. A. et al.: 1975. *J. Mol. Biol.*, 97: 123.

Identification and Localization of Transposition Genes in Transposon Tn 233 (CH)

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ABSTRACT

Plasmid pBR322::Tn233(CH) DNA was partially digested with restriction endonucleases *Eco*RI or *Bam*HI. The DNA fragments generated by partial digestion were religated by T4-DNA ligase. The resulting DNA molecules were used to transform *Escherichia coli* C600. A number of deleted transposition-defective mutants and deleted mutants which were still able to translocate (transposition wild-type) were isolated. The results of complementation test show that the transposition function of deleted transposition-defective mutants of Tn233(CH) can be complemented by deleted transposition wild-type in trans. Then the deletions were mapped by restriction endonucleases analysis. Thus, the location of transposition genes of Tn233(CH) was verified.

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