ON THE GENE CONTROLLING THE RATE OF AMYLASE PRODUCTION IN *BACILLUS SUBTILIS*

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The auther (Yuki, 1967) previously reported of the intra-strain transformation of amylase gene in *Bacillus subtilis*. In that study, it was noticed that different strains of the bacteria had different rates of amylase production, and that when an *amy*$^-$ mutant of a strain was transformed to *amy*$^+$ by DNA of another strain producing amylase at a higher rate than the former, there occurred two types of amylase producers, one produced amylase at the rate similar to that of the donor strain and the other at the rate similar to that of the original strain of the *amy*$^-$ recipient. From this observation, the existence of a genetic factor which controls the rate of amylase production was predicted. Some genetic studies were undertaken to confirm the presence of such a factor, and the results are described in this paper.

MATERIALS AND METHODS

Three genealogically different strains of *Bacillus subtilis*, 1-168 (*trp*$_2$), 27 and 1088, were used in this study. DNAs of 27 and 1088 strains can transform 1-168 strain to prototroph. Amylase produced by 1088 strain, named amylase-1088, differs electrophoretically from amylase of 1-168 strain, amylase-4 (Yuki, 1967). Genetic symbols, *amy*4 and *amy*^{1088}, were used to denote the genes which specified amylase-4 and amylase-1088, respectively. Electrophoretic mobility of amylase produced by 27 strain

was not known, since the activity in the culture medium was low. Amylase negative derivatives of 1-168 strain, 1-103 (*trp*$_2$ *aro*$_{116}$ *amy*$_{12}$), 1-108 (*aro*$_{116}$ *amy*$_3$), 1-115 (*his*$_2$ *aro*$_{116}$ *amy*$_{14}$), 1-116 (*his*$_2$ *aro*$_{116}$ *amy*$_{15}$) and 1-118 (*aro*$_{116}$ *amy*$_4$) strains were used in the genetic analysis as recipients. When these strains were transformed by wild type DNA, *aro*$_{116}$ and *amy* were transformed jointly (Yuki and Ueda, 1968).

Media, method of preparation of transforming DNA, procedure of transformation, assay method of amylase activity, and method of agar gel electrophoresis were described previously (Yuki, 1967). Amylase producing transformants were scored among *aro*$^+$ transformants selected on the appropriate selective plates containing 0.2 % starch.

RESULTS

As can be clearly be seen in Fig. 1, the rate of amylase production by 1-168 and 1088 strains considerably differs from each other. In the case of 27 strain, amylase activity was hardly detectable in the culture medium by the assay method employed in this study. But, 27 strain was certainly an amylase producer at a very low rate, since a small and faint

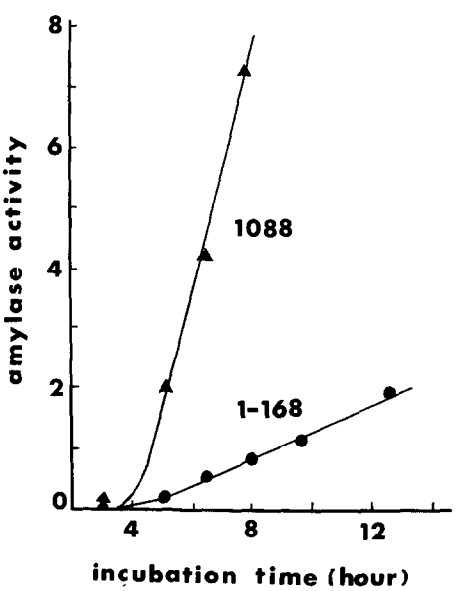

Fig. 1. Production of amylase by 1-168 and 1088 strains into the culture medium. 1-168 and 1088 were cultivated with shaking in nutrient broth at 37°C and amylase activity in the culture medium was assayed at intervals.

halo of hydrolysed starch could be observed around its colony grown for two days on nutrient agar containing starch. The difference in the rate of amylase production of these strains can readily be distinguished on the agar plate containing 0.2 % starch by the size of halo around their colonies, which was mostly used to distinguish the rate of amylase production in the following genetic analysis.

When transformation of 1-103 strain to *amy*$^+$ by DNA of 1088 was performed, approximately 10 % of the *amy*$^+$ transformants was surrounded with smaller halo similar to that of 1-168, and the remaining 90 % was with larger halo similar to that of 1088 (Table 1). Among amy transformants

Table 1. Size of halo of *amy*$^+$ transformants of 1-103 elicited by DNA of 1088 and 27 strains.

Donor	*amy*$^+$/*arg*$_{116}^+$	No. of *amy*$^+$ examined	Type of halo	
			*amy*H-168	*amy*H-1088
1088	40.5 %	30	4	26
	44.5 %	118	12	106
			*amy*H-168	*amy*H-27
27	39.7 %	136	4	132
	34.4 %	93	2	91

with large halo, amylase-4 producers were found to occur, and amylase-1088 producers with small halo were also found. These results clearly indicate that each strain of 1-168 and 1088 has, respectively, a gene which control their inherent rate of amylase production and the gene is closely linked to *amy*$_{12}$. The gene might have no relation to the determination of molecular structure of amylase.

The data shown in Table 1 indicate that the low rate of amylase production of 27 strain might also be controlled by a gene, although the frequency of *amy*$^+$ transformants with halo of similar size to that of 1-168 was lower than that obtained when 1088 strain was used as donor.

An *amy*$^+$ transformant (named s-1) with a big halo similar to that of 1-168 was found to produce amylase with different electrophoretic mobility from amylase of 1-168. The amylase of s-1 was assumed to be the same as amylase of 27 strain.

Genetic symbols, *amyH*-168, *amyH*-27 and *amyH*-1088, were used to denote the genes which controlled the inherent rate of amylase production of 1-168, 27 and 1088 strains.

To map *amyH* gene, an *amy*$^-$ mutant, 1-99 (*amy*$_{17}$ *amyH*-1088), was isolated from an *amy*$^{1088+}$ *amyH*-1088 transformant of 1-67 (*trp*$_2$ *amy*$_2$) elicited by DNA of 1088, and its DNA was exposed to 1-103, 1-108, 1-115, 1-116 and 1-118 strains. Frequencies of *amy*$^+$ recombinants among *aro*$^+$ transformants selected were examined. The *amy*$^-$ mutations were mapped in *amy* locus in the order: *amy*$_4$ - *amy*$_{14}$ - *amy*$_3$ - *amy*$_{12}$ - *amy*$_{15}$ (Yuki and Ueda, 1968). Thus, from the frequencies of *amy*$^+$ recombinants (Table 2), it can be deduced that *amy*$_{17}$ is located between *amy*$_3$ and *amy*$_{12}$. As can be seen

Table 2. Genetic experiment to map *amyH* with respect to the *amy*$^-$ mutations.

Recipient	*amy*$^+$/*aro*$^+$	Type of halo *amyH*-1088	*amyH*-168
1-118 (*amy*$_4$)	30/1125 (0.027)	5	25
1-115 (*amy*$_{14}$)	19/886 (0.021)	3	16
1-108 (*amy*$_3$)	60/4030 (0.015)	14	46
1-103 (*amy*$_{12}$)	21/2800 (0.0075)	15	6
1-116 (*amy*$_{15}$)	8/805 (0.0099)	6	2

in Table 2, when 1-108, 1-115 and 1-118 were the recipients, approximately 80 % of the *amy*$^+$ recombinants was with smaller halo (*amyH*-168), but when 1-103 and 1-116 were the recipients, approximately 80 % was with bigger halo (*amyH*-1088). This results indicate that *amyH* is located on the opposite side of *amy* to *aro*$_{116}$.

An attempt was performed to isolate mutants in which the rate of

amylase production would be affected. For this purpose, an *amy*$^+$ *amyH-27* transformant (ss-1) of 1-103 elicited by DNA of 27 strain was mutagenized by nitrosoguanidine and plated on the nutrient agar containing starch. By this method, mutant having higher rate of amylase production, could readily be detected as colony surrounded by a distinct halo, since halo of ss-1 was faint and hardly visible on the plate. Thus, were obtained three mutants, named H1, H2 and H3, having bigger halo of hydrolysed starch. Electrophoretic analysis showed that amylase produced by these mutants was identical to that produced by s-1. It might be possibly explained that ss-1 was producing amylase-27 at a very low rate and that the mutations affected only the rate of amylase production, with no effect on the electrophoretic mobility of the amylase. Since most of *amy*$^+$ transformants of 1-103 elicited by DNAs extracted from the mutants showed distinct halo, it does not seem that the mutation had occurred at the distant site from *amy* locus.

DISCUSSION

The data described above indicate that the rate of amylase production in *B. subtilis* is controlled by a specific factor, named *amyH* in this paper, which is located at the close vicinity of *amy*, on the opposite side of *amy* to *aro*$_{116}$. The *amyH* was found not to affect the electrophoretic mobility of amylase. In the previous paper (Yuki, 1967), *amy* was assumed to be the structure gene of amylase. So, it seems that *amyH* controls only the rate of production of amylase which is specified by the structure gene, locating closely to it. The electrophoretic mobility was assumed to be specified by a specific site, named E, which was present within the structure gene of amylase (Yuki, 1967). Although *amyH* can be certainly distinguished from E site, there still remains the possibility that *amyH* occupy the extremity of the structure gene.

Pardee and Beckwith (1962) reported *lac*$^+$ revertants of *lac*$^-$ of *o*^{o}

(i^-) type which produced β-galactosidase constitutively at widely different levels, and concluded that operator region of lac operon might control the fixed rate of synthesis of enzymes involved in the operon with little effect on the molecular form of the enzymes. Jacob, Ullman and Monod (1964) have suggested, in lac operon, the existence of a specific site associated with the structure genes whose maximal rate of expression it controls, and have named it the promotor. The promotor was precisely localized between o and z by Scaife and Beckwith (1966). Richmond (1966) reported the penicillinase micro-mutants of Staphylococcus aureus which had much less enzyme level than that of wild type strain. He concluded that the mutants were not due to the mutation of the regulator gene of penicillinase, and assumed that the mutation might be in a region analogous to the operator gene of lac operon of Escherichia coli. Although, in amylase system of B. subtilis, such a regulatory system as characterized in lac operon of E. coli is not still established, the most probable explanation might be, at the present level of study, that amyH found in this study might correspond to the promotor and specify the speed of m-RNA transcription of the structure gene of amylase. However, it can not be neglected that the control of the rate of amylase production might act at the level of translation or else.

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