

NOTES

Fitness Effects of Replichore Imbalance in *Salmonella enterica*[∇]

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Received 7 June 2010/Accepted 12 September 2010

A fitness cost due to imbalanced replichores has been proposed to provoke chromosome rearrangements in *Salmonella enterica* serovars. To determine the impact of replichore imbalance on fitness, the relative fitness of isogenic *Salmonella* strains containing transposon-held duplications of various sizes and at various chromosomal locations was determined. Although duplication of certain genes influenced fitness, a replichore imbalance of up to 16° did not affect fitness.

The bacterial chromosome is a dynamic molecule that can undergo various types of rearrangements, including inversions, translocations, and duplications. These rearrangements can alter gene order and change replichore length. Replichores are defined as the halves of the chromosome between the origin of replication and the terminus region in the vicinity of the *dif* site (4, 6, 10, 15). In most bacteria, both replichores are approximately the same length, with each comprising 180° around the circular chromosome. In this state, the replichores and DNA replication are balanced. Imbalance is introduced when one replichore becomes longer than the other. For example, if the replichores comprise 200° and 160° around the circular chromosome, the replichores are 20° imbalanced. Various studies over the years have investigated the effect of imbalanced DNA replication on fitness in *Escherichia coli* (8, 9, 16, 17). In a recent analysis, *E. coli* strains that contained asymmetrical interreplichore inversions were found to have growth defects when the imbalance was at least 50° (8). While these studies have demonstrated that imbalanced replichores can affect fitness, the approaches used to introduce the imbalance either do not occur or are extremely rare in nature.

Duplications play important evolutionary roles because the increase in gene copy number can facilitate adaptation to certain growth conditions (20), as well as being a source for the evolution of new genes (3). Typically duplications occur at frequencies between 10⁻³ and 10⁻⁵ in unselected cultures (2) but are lost at rates of up to 1,000-fold more often if they do not provide a selective advantage (19). Duplications also result in replichore imbalance. However, the effect of duplications on fitness in relation to replichore balance has not been investigated.

A major hurdle in studying the effects of duplications on fitness is that if the duplication is detrimental, haploid revertants will outgrow the parental strain containing the duplica-

tion. To circumvent this problem, we used a set of 11 isogenic *Salmonella enterica* strains with transposon-held duplications (5) (Fig. 1 and Table 1). Culturing these strains in the presence of chloramphenicol selects for the duplication because if the duplication collapses, the transposon is lost and the cells become chloramphenicol sensitive. As the size of the duplications in these strains varies, so does the amount of introduced replichore imbalance, ranging from 5° to 23°. In addition, fitness effects due to the location of the duplication versus the size of the duplication were also discerned, as the collection includes duplications of similar sizes located in different regions of the chromosome.

The relative fitness of these strains was investigated by measuring growth rates and by assaying growth in a mixed culture with an isogenic competitor strain. Single colony isolates streaked from frozen stocks were used to inoculate broth cultures. Growth was measured in E medium supplemented with 0.2% glucose (minimal medium), Luria-Bertani medium (LB), or LB supplemented with 1× E-salts and 0.2% glucose (LBEDO) (18). Competition assays were done in LB. Media were supplemented with chloramphenicol (20 mg/ml) to maintain the duplications, and the solid media used in competition

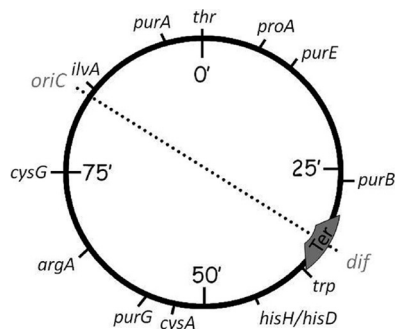


FIG. 1. Genetic map of *S. enterica* serovar Typhimurium LT2 showing genes used as endpoints in constructing transposon-held duplications of the regions between genes. Balanced replichores are indicated by the symmetry of the *oriC*-Ter axis. Duplications increase the length of one replichore relative to the other, imbalancing axis symmetry.

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[∇] Published ahead of print on 24 September 2010.

TABLE 1. Properties of the *S. enterica* strains used in this study

Strain	Alias	Genotype	Duplication location (min)	Duplication size (kbp)	Replichore imbalance (°)	Reference
MST1	LT2	Wild type			1.9	11
MST3813	SV4200	Dup [<i>trp248</i> MudP <i>hisD9953</i>]	38–44	332.1	11.5	5
MST3814	SV3193	Dup [<i>hisH9962</i> MudP <i>cysA1586</i>]	44–53	399.7	13.7	5
MST3815	SV4015	Dup [<i>cysA1586</i> MudP <i>purG2149</i>]	53–56	158.4	5.7	5
MST3816	SV4193	Dup [<i>purG2149</i> MudP <i>argA9001</i>]	56–64	430.7	14.7	5
MST3817	SV4194	Dup [<i>argA9000</i> MudP <i>cysG1573</i>]	64–75	484.6	16.3	5
MST3818	SV1601	Dup [<i>cysG1573</i> MudP <i>ilvA2642</i>]	75–85	486.4	16.4	5
MST3819	SV4195	Dup [<i>ilvA2648</i> MudP <i>purA1881</i>]	85–95	495.3	16.7	5
MST3820	SV4142	Dup [<i>purA1881</i> MudP <i>thr469</i>]	95–0	248.7	8.8	5
MST3821	SV1604	Dup [<i>thr469</i> MudP <i>proA692</i>]	0–8	367.1	12.6	5
MST3822	SV1603	Dup [<i>proA692</i> MudQ <i>purE2164</i>]	8–12	230.2	8.1	5
MST3823	SV1611	Dup [<i>purE2514</i> MudP <i>purB1879</i>]	12–27	723.2	23.3	5
MST1529	TT11183	<i>srl-203::Tn10d</i> (Cam)				1
TYT4480		<i>rrfH::pCE36</i>				This work
MST5198		<i>rrfH::pCE36 srl-203::Tn10d</i> (Cam)				This work

experiments also contained 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; 40 mg/ml) to differentiate between the duplication and competitor strains.

In minimal medium, most of the strains had a generation time of approximately 40 min, except for MST3813, MST3823, and MST3819, which grew somewhat slower (Fig. 2). In LB, the average generation times of all of the duplication-bearing strains were longer than that of the wild type. When grown in LBEDO, all of the strains had generation times of 11 to 15 min. Observed differences in growth rate were not due to lower viability as determined by plate counts. There was no correlation between duplication size and generation time in any of the three media.

Competition indices were determined from mixed cultures with the isogenic competitor strain MST5198 and either the wild-type strain or one of the duplication-bearing strains. The competitor strain contained an integrated plasmid encoding a promoterless *lacZ* gene (7) driven by the *rrfH* promoter and *Tn10d*(Cam) conferring chloramphenicol resistance integrated into the *srl* locus (Table 1). Strains were grown to saturation overnight, and 10⁻⁴ dilutions were used to inoculate mixed cultures. Samples taken at *t* = 0 (input) and subsequent time points (output) were diluted and spot plated in triplicate. The

competition index (C.I.) was calculated as follows: C.I. = (no. output Lac⁻ CFU/no. output Lac⁺ CFU)/(no. input Lac⁻ CFU/no. input Lac⁺ CFU).

Data are shown for 6 and 12 h, but readings were taken at time points of up to 1 week. There was no correlation between the duplication size and the C.I. (Fig. 3). Most of the duplication-bearing strains competed as well as or slightly better than the wild type against the competitor strain. Two exceptions were MST3819 and MST3823, which both competed significantly more poorly against the competitor strain than did the wild-type strain (Student's *t* test *P* < 0.05). While these two strains contain the largest duplications, the size of the duplication in MST3819 was close to the size of the duplications in MST3817 and MST3818. Strains MST3817 and MST3818 were either similar or slightly better competitors than the wild type, indicating that the fitness defect in MST3819 is not due to replicore imbalance.

To confirm that the duplications in MST3819 and MST3823 were responsible for the observed growth defects, isolates of each strain with collapsed duplications were obtained by growing cultures of each strain without chloramphenicol and then screening for chloramphenicol-sensitive derivatives. The loss of the duplication restored wild-type growth to both strains.

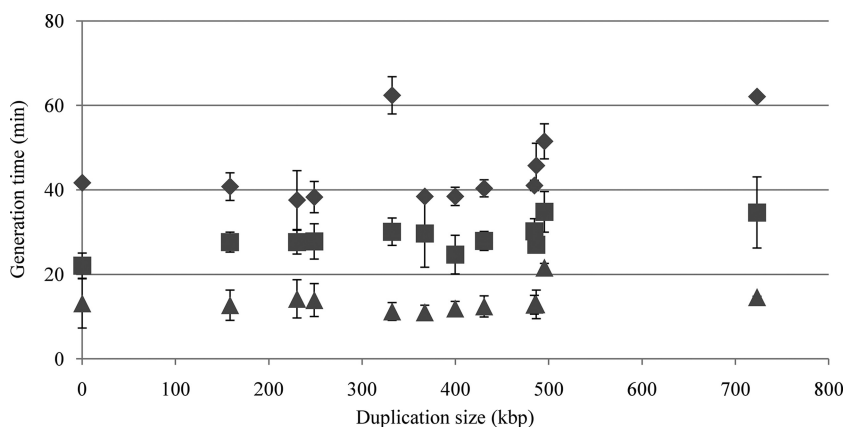


FIG. 2. Generation times of strains as a function of duplication size. Strains were grown in minimal medium (◆), LB (■), or LBEDO (▲) aerobically at 37°C. Error bars show standard deviations.

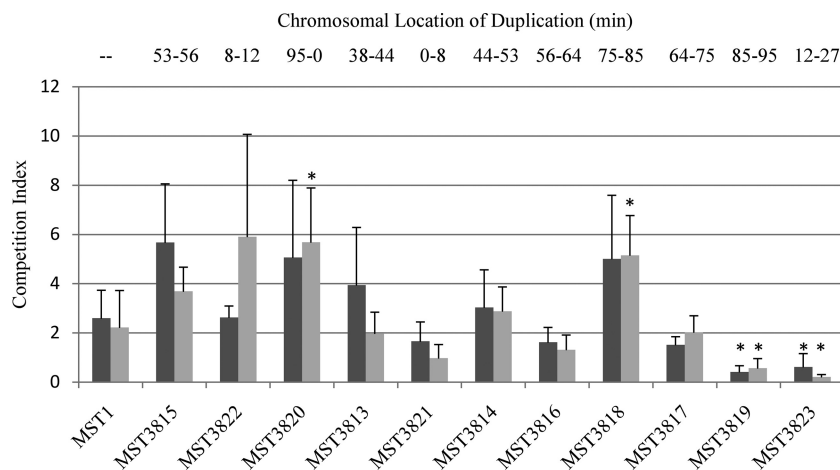


FIG. 3. Competition indices of the wild-type and duplication-bearing strains after 6 (■) and 12 h (▣) of growth in mixed cultures with MST5198 ($n = 6$). Strains are in order of increasing duplication size, with the locations of duplications indicated at the top. Indices statistically significantly different ($P < 0.05$) from that of the wild-type strain (MST1) are marked (*). Error bars show standard deviations.

The frequencies of duplication collapse in cultures of MST3818 and MST3819 were also compared. Since these two strains have duplications of similar sizes, the collapse frequencies of the duplicated regions, and concomitant loss of chloramphenicol resistance, were expected to be similar. While the reversion frequency of MST3818 cells was 3.5×10^{-3} cells/generation, MST3819 cells reverted with a frequency of 2.8×10^{-2} cells/generation, resulting in 97 to 98% of MST3819 cells being chloramphenicol sensitive and haploid after reaching stationary phase.

In conclusion, the results of this study show that a replicore imbalance of up to 16° introduced by transposon-held duplications in the chromosome does not have a measurable effect on the fitness of *S. enterica*. The duplicated regions in these strains are larger than the islands acquired via horizontal gene transfer, which have been purported to disrupt replicore balance sufficiently to promote chromosome rearrangements. A duplication that introduced a replicore imbalance of 23° did have a growth defect, but whether the defect is from replicore imbalance or the gene content of the duplication could not be distinguished. This study suggests that the disruption of replicore balance by acquisition of horizontally transferred genes is not the cause of chromosome rearrangements in host-specific *S. enterica* serovars as hypothesized (12–14).

We thank Josep Casadesús for kindly providing the duplication-bearing strains, Moselio Schaechter for valuable discussions and advice, and Michele McKeand for technical assistance.

This research was supported in part by grant U54CA132384 from the National Cancer Institute.

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