Molecular Markers for Rapidly Identifying Candidate Genes in *Chlamydomonas reinhardtii*: ERY1 and ERY2 Encode Chloroplast Ribosomal Proteins

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ABSTRACT

To take advantage of available expressed sequence tags and genomic sequence, we have developed 64 PCR-based molecular markers in *Chlamydomonas reinhardtii* that map to the 17 linkage groups. These markers will allow the rapid association of a candidate gene sequence with previously identified mutations. As proof of principle, we have identified the genes encoded by the ERY1 and ERY2 loci. Mendelian mutations that confer resistance to erythromycin define three unlinked nuclear loci in *C. reinhardtii*. Candidate genes ribosomal protein L4 (RPL4) and L22 (RPL22) are tightly linked to the ERY1 locus and ERY2 locus, respectively. Genomic DNA for RPL4 from wild type and five mutant *ery* alleles was amplified and sequenced and three different point mutations were found. Two different glycine residues (G102 and G112) are replaced by aspartic acid and both are in the unstructured region of RPL4 that lines the peptide exit tunnel of the chloroplast ribosome. The other two alleles change a splice site acceptor site. Genomic DNA for RPL22 from wild type and three mutant *ery* alleles was amplified and sequenced and revealed three different point mutations. Two alleles have premature stop codons and one allele changes a splice site acceptor site.

A large collection of chemically induced mutations exists in *Chlamydomonas reinhardtii* (Dutcher 2000; Harris 2001). Several approaches have been used to identify the gene products of various loci. A locus of interest can be cloned by complementation (Burton and Rochaix 1994; Zhang et al. 1994; Funke et al. 1997), by identifying new alleles with an insertional tag (Tam and Lefebvre 1993), by identifying new alleles with a transposable element (Schnell and Lefebvre 1993), or by positional cloning from a nearby physical marker (Dutcher et al. 2002). The availability of expressed sequence tags and genomic sequences that have mapped onto a physical/genetic map make it possible to identify candidate genes for various loci. Ranum et al. (1988) and Vysotskaia et al. (2001) have developed restriction fragment polymorphism markers for use with Southern blots and single nucleotide polymorphisms, respectively. The availability of mapped molecular markers that can be scored quickly and easily should make it possible to rapidly identify mutations that correspond to a candidate sequence.

Erythromycin is an antibiotic that blocks the peptide exit tunnel of bacterial and other prokaryotic-like ribosomes (Gale et al. 1981; Gabashvili et al. 2001). Resistance to erythromycin is conferred by mutations in ribosomal proteins L22 and L4 in bacteria. In addition, resistance is conferred by mutations in the 23S ribosomal RNA in domain V as well as by mutations in loci that encode 6-N,N'-adenosyl dimethyltransferase or dimethyladenosine transferase. The absence of methylation of 2508A in 23S rRNA confers resistance (Lai and Weisblum 1971). These sequences are possible candidate genes. The unicellular green alga, *C. reinhardtii*, is sensitive to erythromycin. Using radiolabeled erythromycin, Mets and Bogorad (1971) showed the site of action of erythromycin to be the chloroplast of Chlamydomonas.

Mutations in Chlamydomonas that confer resistance to erythromycin have been isolated in both nuclear and chloroplast loci. Genes for the 23S rRNA are located in the chloroplast and two mutations in the 23S rRNA confer resistance (Harris et al. 1989; Maul et al. 2002). Mutations in three nuclear loci (ERY1, ERY2, and ERY3) also confer resistance to erythromycin. These loci map to linkage groups X, XIV, and I, respectively (Hanson and Bogorad 1977, 1978; Eves and Chiang 1982). Alterations in the mobility of chloroplast ribosomal proteins from *ery* and *ery2* mutant strains have been observed on one- and two-dimensional gels (Mets and Bogorad 1972; Davidson et al. 1974).

Mutations that confer resistance to erythromycin provide proof of principle that we can identify and then map candidate sequences with respect to previously

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identified mutant loci. We have identified the gene products for two of the three nuclear ERY loci. The ERY1 locus encodes ribosomal protein L4 (RPL4) and the ERY2 locus encodes ribosomal protein L22 (RPL22).

MATERIALS AND METHODS

Strains and culture media: Crosses were made between CC1952 and several laboratory strains derived from strain 137c using protocols described previously (Harris 1989). CC-1952 is a strain isolated from the wild that has extensive molecular polymorphisms relative to the lab strain, 137c (Gross et al. 1988). Strains are listed in Table 1. Media were as described (Luss and Dutcher 1991).

In agreement with current nomenclature rules for C. reinhardtii, the alleles at the ERY1 loci are changed from ery1a, ery1b, ery1c, and ery1d to ery1-1, ery1-2, ery1-3, and ery1-4. Ery11, ery12, and ery14 (Harris et al. 1974) are named ery1-6, ery1-7, and ery1-8. The same changes have been made for ERY2 alleles and are listed in Table 1.

PCR protocol: Primers for the mapping markers were chosen using Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi; Rozen and Skaltsky 2000). DNA was isolated as described (Johnson and Dutcher 1991). The mapping was performed in 25 µl reactions using Taq polymerase with 20 mM Tris, 50 mM KCl, pH 8.3 buffer with 20 pmol of each primer, 5% DMSO, 1 mM dNTP, and MgCl₂ concentrations as indicated in Table 2. The cycling parameters were 95°C for 5 min, 95°C for 1 min, at the temperature in Table 2 for 1 min, 72°C for 1 min per kilobase of product length, repeated 30 times, and 72°C for 10 min. Restriction digests were performed directly on the PCR products as indicated in Table 2. Products were displayed on 2% agarose gels with ethidium bromide.

 Colony PCR: DNA from colonies from tetrads of ery1 × CC1952 and ery2 × CC1952 was made using the REDextract-N-AMP blood PCR kit (Sigma, St. Louis). The kit was used according to the manufacturer’s directions with the exception that 20 µl reactions were used. Restriction digests were performed in the PCR mix directly.

Screening the bacterial artificial chromosome library: PCR products from genomic DNA were used to screen filters obtained from Genome Systems (now available from Clemson University Genomics Institute) using random primed labeling methods as described elsewhere (Dutcher et al. 2002). Identification of additional bacterial artificial chromosome (BAC) clones was made by searching the JGI database of BAC end sequence (http://bahama.jgi-psf.org/produ/bin/chlamy/home.chlamy) using BLASTn or tblASTn (Altschul et al. 1990).

Sequencing: Isolated genomic DNA from wild type and ery1 mutant strains was amplified using primers GCAGTTCGTCAT TGTATTAGGT and CGTCTCATGAATTGATGTTGTT GCC AGGCCATCTAAGCTAA. KlenTaq long and accurate polymerase (Barnes 1994) was used with the following conditions to amplify DNA for sequencing: 35 cycles of 1 min at 94°C, 2 min at 52°C, and 10 min at 68°C. A final 30-min extension period at 68°C was included. The product was purified from a 1% agarose gel using the gel purification kit from QIAGEN (Valencia, CA). For ERY2, four pairs of primers were used with KlenTaq long and accurate polymerase to amplify DNA with the following conditions: 35 cycles of 1 min at 94°C, 2 min at 56°C, and 10 min at 68°C. No gel purification was needed. The DNA was sequenced using BigDyeV3 in conjunction with the protein and nucleic acid chemistry laboratory (Washington University School of Medicine). For ERY1 DNA, seven forward and seven reverse primers were used for sequencing. For ERY2 DNA, three forward and three reverse primers were used for sequencing. The Sequencher Program (Gene Codes, Ann Arbor, MI) was used for assembling the sequence reads into contigs.

Modelling the protein structure: The protein sequences of RPL4 and RPL22 were submitted to SwissModel (http://www.expasy.org/swissmodel/) to be fit to the crystal structures of the homologous proteins from Harloarcula marismortui (IFKK and Deinococcus radiodurans (1LNR; Guex et al. 1999). The predicted chloroplast signal peptide was removed for the modeling, as there was no similarity with the bacterial sequences used for crystallization. The predicted structure was examined using Deep View: Swiss-PDF viewer.

RESULTS

Physical markers for mapping: To facilitate the rapid mapping of sequence obtained from expressed sequence tags (ESTs) and genomic sequence, we have developed molecular PCR-based mapping markers that distinguish between alleles in two Chlamydomonas strains (137c and CC1952; Gross et al. 1988; Vysotskai et al. 2001). Many of the loci were selected on the basis of their previously known map position (http://www.biology/duke.edu/chlamy_genome/) and loci within 10–15 map units of their respective centromeres were preferentially chosen. PCR products for 64 markers were amplified using the primers and conditions that are listed in supplementary material available on the web (Appendix 1 at http://www.genetics.org/supplemental/). These markers ranged in length from 200 to >1000 bp. Eleven of the 64 markers generate PCR products in the two strains that are distinguishable on 2% agarose gels. The remaining 55 markers require digestion by a restriction enzyme to produce a distinguishable marker as indicated in Appendix 2, available online at http://www.genetics.org/supplemental/.

Meiotic progeny panel: Genomic DNA was isolated (Johnson and Dutcher 1991) from 172 meiotic progeny from crosses of 137c-derived strains and the polymorphic strain, CC1952 (Table 1). These DNAs were placed into 96-well microtiter plates for monitoring the segregations of the markers in Appendix 1, available online at http://www.genetics.org/supplemental/. Figure 1 shows 2.0% agarose gels for three different markers. The segregation for LC1 on linkage group II and LC5 on linkage group XIX was performed with 94 meiotic progeny and two parental strains; segregation for acetyl glutamate kinase on linkage group I was performed with 82 meiotic progeny and two parental strains. The last two lanes in the bottom right of Figure 1, A–C, contain the parental DNA. We find that 40–100% of the DNA samples from meiotic progeny produce products that can be interpreted with an average success rate of 80%. The segregation of the 62 markers was determined for up to 172 meiotic progeny; some markers were analyzed from only one of the plates of progeny. The linkage analysis is shown graphically in Figure 2.
Two ERY Loci Encode RPL22 and RPL4

TABLE 1
Strains used in this work

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<th>Strain</th>
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<td>Chlamydomonas Genetics Center (ery2b)</td>
</tr>
<tr>
<td>OC-893</td>
<td>ery2-4; mt+</td>
<td>Chlamydomonas Genetics Center (ery2d)</td>
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(The full set of data is shown in Appendix 2 at http://www.genetics.org/supplemental/).

Mapping ESTs to candidate genetic loci: Many mutations have been identified in *C. reinhardtii*, but few of their gene products are known (DUTCHER 2000; HARRIS 2001). We set out to determine if we could identify the gene products for several previously characterized loci using a candidate gene approach. Loci that confer resistance to erythromycin often encode ribosomal proteins in eubacteria (SPAHN and PRESCOTT 1996). Three nuclear loci that confer resistance to erythromycin in *C. reinhardtii* (*ERY1–ERY3*) (DAVIDSON et al. 1978) have previously been identified.

ESTs for ribosomal proteins: Resistance to erythromycin in bacteria is conferred by mutations in ribosomal proteins L4 and L22, in domain V of the 23S rDNA gene, and in methyladenosine transferase. We searched the EST database for homologs of L4 and L22 ribosomal proteins (RPL4 and RPL22) and found matches (Table 2). Bogorad and colleagues established that a single ribosomal protein was altered in some *ery1* alleles. This protein was called L6 in their numbering scheme on the basis of its mobility in two-dimensional gels. Although no equivalence to the *Escherichia coli* L6 protein was implied by their numbering (HANSON and BOGORAD 1978), we found an EST for the Chlamydomonas L6 homolog and established a linked molecular marker for it as well. We identified an EST that shows similarity to a dimethyl adenosine transferase (DMAT) as well as its genomic DNA sequence (Li et al. 2003).

DNA from 40, 43, and 40 meiotic progeny from crosses of *ery1*, *ery2*, or *ery3* by CC1952, respectively, was obtained and used in colony PCR with primers for RPL4, RPL22, and the DMAT homolog. We were unable to generate a polymorphism for RPL6 so we used a nearby gene, BI99. We observed complete linkage between RPL4 and *ERY1* and between RPL22 and *ERY2*. BI99 and DMAT showed no linkage to any of the *ERY* loci (Appendix 2 at http://www.genetics.org/supplemental/). BI99 showed linkage to markers on linkage group IX, and DMAT showed linkage to markers on linkage group III (Appendix 2 at http://www.genetics.org/supplemental/).

Figure 1.—Segregation of LC1, LC5, and AGK in meiotic progeny. DNA from 94 meiotic progeny was used for PCR with primers to (A) LC1, a dynein light chain; (B) LC5, a dynein light chain, and (C) AGK, acetyl glutamate kinase. In A–C, the 137c and CC1952 parents are shown at the bottom right.
No loci that confer drug resistance have been mapped to either of these regions to date.

**ERY1 encodes RPL4:** Previously, seven alleles that confer resistance to erythromycin were shown to be linked to one another (Hanson and Bogorad 1977) and this locus was designated **ERY1** (Harris 1989). We sequenced 2536 bp of genomic DNA from wild type and from five of the mutant strains. The predicted Chlamydomonas protein is 243 amino acids long. It has 58% identity to the homolog in *Nicotiana tabacum* and 57% identity to the homolog in *Nostoc sp. PCC7120*, a cyanobacterium. The amino terminus, which is likely to be the chloroplast transit signal, shows little similarity among these three proteins (Figure 3). Two alleles, *ery1-2* and *ery1-6*, have a single nucleotide change of G to A that results in a change in the splice site from GT to AT at the end of exon 2. This change results in the loss of the restriction enzyme recognition site for *Bst* and cosegregates with erythromycin resistance in 43 meiotic progeny for the *ery1-2* allele and in 12 meiotic progeny for *ery1-6*. The *ery1-5* allele has a single nucleotide change from G to A that results in a glycine-to-aspartic acid change at amino acid 102. This mutation results in the acquisition of a *Bcc*I recognition site and cosegregates with erythromycin resistance in 1077 bp of genomic DNA from wild type and from three of these mutant strains. The predicted Chlamydomonas protein is 171 amino acids long. It is 57% identical to the homolog from *Medicago sativa* and 55% identical to the homolog from *Porphyra purpurea*, a red alga. The *ery2-2* and *ery2-5* alleles have a G-to-T change that results in amino acid 112. This mutation results in the acquisition of a *Bff* recognition site and cosegregates with erythromycin resistance in 9 *ery1-7* meiotic progeny. Thus, we observed no recombination between the *ery1* DNA polymorphisms and the erythromycin resistance phenotype. In addition, five different alleles have three different missense mutations.

**ERY2 encodes RPL22:** Previously, seven linked alleles were identified as conferring resistance to erythromycin (Mets and Bogorad 1972; Davidson et al. 1978) and this locus was designated **ERY2** (Harris 1989). We sequenced 1077 bp of genomic DNA from wild type and from three of these mutant strains. The predicted Chlamydomonas protein is 243 amino acids long. It has 58% identity to the homolog in *Nicotiana tabacum* and 57% identity to the homolog in *Nostoc sp. PCC7120*, a cyanobacterium. The amino terminus, which is likely to be the chloroplast transit signal, shows little similarity among these three proteins (Figure 3). Two alleles, *ery1-2* and *ery1-6*, have a single nucleotide change of G to A that results in the introduction of an amber codon at amino acid 108 (Figure 4). The *ery2-2* and *ery2-5* alleles have a G-to-T change that results in the introduction of an amber codon at amino acid 108 (Figure 4). The *ery2-4* allele has a G-to-A change that results in a change in the splice site from GT to AT at the end of exon 2. This change results in the loss of the restriction enzyme recognition site for *Bst* and cosegregates with erythromycin resistance in 43 meiotic progeny for the *ery1-2* allele and in 12 meiotic progeny for *ery1-6*. The *ery1-5* allele has a single nucleotide change from G to A that results in a glycine-to-aspartic acid change at amino acid 102. This mutation results in the acquisition of a *Bcc*I recognition site and cosegregates with erythromycin resistance in 1077 bp of genomic DNA from wild type and from three of these mutant strains. The predicted Chlamydomonas protein is 171 amino acids long. It is 57% identical to the homolog from *Medicago sativa* and 55% identical to the homolog from *Porphyra purpurea*, a red alga. The *ery2-2* and *ery2-5* alleles have a G-to-T change that results in the introduction of an amber codon at amino acid 108 (Figure 4). The *ery2-4* allele has a G-to-A change that results in an alteration in the splice site from the consensus GT to AT. If the message in *ery2-4* was not spliced, the protein would be truncated prematurely at a stop codon in the intron. These changes result in the acquisition of the restriction recognition sites for *Bfa* and the loss of an *Hph*I recognition site for the *eri2-2* and *ery2-4* alleles, respectively.

DNA from 20 meiotic progeny from crosses of *ery2-2 × CC1952* and 20 meiotic progeny from crosses of *ery2-4 × CC1952* were analyzed for segregation of the mutant alleles with respect to the CC1952 allele. We observed no recombination between the *ery2* DNA polymorphism and the erythromycin resistance phenotype. The resistance phenotype is tightly linked to the physical marker.
In addition, three different alleles have two different point mutations.

Resistance to other macrodilide antibiotics: Tylosin and spiramycin are used extensively for treatment of animals with bacterial infections. These antibiotics, like erythromycin, bind in the narrow part of the peptide exit tunnel to occlude peptide exit (Hansen et al. 2002). The side chain of spiramycin contacts RPL4 and the side chain of tylosin contacts RPL22. We asked if the mutations in ery1 and ery2 confer resistance to these related antibiotics. Wild-type strains are sensitive to 75 μg/ml of both compounds. The seven ery1 alleles confer resistance to both tylosin and spiramycin from 75 to 300 μg/ml while the three ery2 alleles confer resistance only to tylosin in the same concentration range.

DISCUSSION

We have developed 64 PCR-based molecular markers that can be easily scored. Thirty of these are based on the data from the Chlamydomonas Genome Project that provided a framework. New markers include genes for the enzymes of the tryptophan biosynthetic pathway. We have mapped the genes for anthranilate synthase-β (ASB), phosphoribosyl transferase (PRT), anthranilate phosphoribosyl isomerase (PAI), indole 3-glycerol phosphate synthase (IGPS), and tryptophan synthetase-α (TSA; Figure 2; Table 2). Originally, MAA loci were identified by resistance to 5-methylanthranilic acid (Dutcher et al. 1992; Palombella and Dutcher 1998). Many mutations that confer resistance to 5-MAA in Arabidopsis are in genes that encode enzymes of the tryptophan biosynthetic pathway (Last and Fink 1988). TSA maps to linkage group XII/XIII near the MAA1 locus, IGPS maps to linkage group XIV near the MAA4 locus, PAI maps to linkage group III, PRT maps to linkage group IV, and ASB maps to linkage group XV. Thus, the linkage between these loci and these genes provides further evidence for the efficacy of placing genomic and expressed sequence tags onto the genetic map to facilitate the identification of the genes that correspond to previously identified mutations.

We show that ERY1 encodes ribosomal protein L4 by linkage and sequencing multiple alleles. Davidson et al. (1978) suggested the protein encoded by the ery1-2 allele had both a different isoelectric point and a different molecular weight from the wild-type protein. The change in the splice site acceptor site would be consistent with this observation. Under several scenarios, translation will stop prematurely in the third exon.

The G102D and G112D changes are near the alteration (ASB) that confers resistance to erythromycin in E. coli (Chitt-
Figure 3.—Alignment of ribosomal protein L4 from Chlamydomonas with the sequences from tobacco (GenBank T01789), Nostoc sp. PCC 7120 (NP_488254), and E. coli (NP_417778) using Clustal X. The identities among the four sequences are indicated by an asterisk above the alignments. The identities among three of the four sequences are indicated by a period above the alignments. The similarities among the four sequences are indicated by a colon above the alignments. Amino acids G, P, S, and T are colored orange; amino acids H, K, and R are colored salmon; amino acids F, W, and Y are colored blue; amino acids D and E are colored purple; the amino acid P is colored yellow; and amino acids I, L, M, and V are colored green. The predicted chloroplast signal sequence is indicated by a black line below the alignments. The five sequenced ery1 alleles have three changes. Three alleles (ery1-3, ery1-5, and ery1-7) have two different point mutations that change a glycine to an aspartic acid (shown in blue below the alignment). The ery1-2 and ery1-6 alleles have a splice site acceptor change as discussed in the text; the end of the affected exon is indicated by a blue +.

In resistant E. coli, the K63 is changed to a glutamic acid. Clearly, this is a region that is important for conferring resistance. In this K63E mutant in E. coli, erythromycin fails to bind to the ribosome (CHITTUM and CHAMPNEY 1994, 1995). The change from glycine to aspartic acid in ery1-3 is consistent with the change in isoelectric point observed by DAVIDSON et al. (1974).

Isolation of ribosomes from ery2 mutant strains suggested that the Ery2-4 protein had an alteration that was observable by one-dimensional gel electrophoresis with urea (METs and BOGORAD 1972, 1974), but the other mutant proteins had no alteration in their mobility. We are surprised that other alleles did not show alterations in these older studies as ery2-2 and ery2-3 alleles have amber codons that should produce truncated proteins. It is likely that all three mutant proteins are truncated.
Two ERY Loci Encode RPL22 and RPL4

Figure 4.—Alignment of ribosomal protein L22 (RPL22) from Chlamydomonas with the sequences from *M. sativa* (GenBank T09389), *P. purpurea* (NP_053919), and *E. coli* (NP_417774) using Clustal X. The identities among the four sequences are indicated by an asterisk above the alignments. The identities among three of the four sequences are indicated by a colon above the alignments. The similarities among the four sequences are indicated by a period above the alignments. Amino acids G, P, S, and T are colored orange; amino acids H, K, and R are colored salmon; amino acids F, W, and Y are colored blue; amino acids D and E are colored purple; the amino acid P is colored yellow; and amino acids I, L, M, and V are colored green. The predicted chloroplast signal sequence is indicated by a black line below the alignments. The *ery2-2* and *ery2-4* alleles have a stop codon that terminates the predicted proteins at the blue asterisk. The *ery2-5* allele has an altered splice site acceptor change as discussed in the text; the end of the affected exon is indicated by a blue +.

dence that this region corresponds to the signal sequence.

Erythromycin physically blocks the peptide exit tunnel (Gabashvili et al. 2001). In *E. coli* mutant strains, L4 mutant ribosomes do not bind erythromycin and have a smaller tunnel size while L22 mutant ribosomes are able to bind erythromycin, but have a larger tunnel opening (Gabashvili et al. 2001). On the basis of the crystal structure of the *H. marismortui* protein (Hansen et al. 2002) and cryoelectron microscopy, RLP22 lines the peptide exit tunnel. The ery2 alleles, which are predicted to result in truncated proteins, may create a peptide exit tunnel opening that is larger so that the erythromycin molecule cannot block the tunnel.

Erythromycin is one member of the macrolide family of antibiotics. It has a 14-membered lactone ring and one sugar group. Spiromycin and tylosin have 16-membered lactone rings and two sugar groups attached. X-ray crystallography of ribosomes from *H. marismortui* in the presence of these antibiotics shows that these compounds form covalent bonds with the 23S rRNA. The forosamine sugar moiety of spiromycin contacts protein L4 and the mycinose sugar moiety of tylosin lies along the peptide exit tunnel and contacts protein L22 (Hansen et al. 2002). The *ery1* mutations in RPL4 confer resistance to both of the compounds, but the *ery2* mutations in RPL22 confer resistance only to tylosin. It is possible that the interaction of the mycinose sugar moiety of tylosin positions it so that the absence of the carboxy terminus of RPL22 is not sufficient to open the peptide exit tunnel. We modeled the sequence of RPL22 from Chlamydomonas onto the structure of RPL22 from *H. marismortui* using SwissModel (Guex et al. 1999). There are three major differences between the known structure and the modeled structure for Chlamydomonas (Figure 5). First, the divergent amino terminus of the L4 protein cannot be predicted from the crystal structure. Second, one of the helices in *H. marismortui* is missing from the modeled structure. Third, the angle of the extended loop is different (Figure 5A). To show the extent of the truncation predicted in the *ery2-2* or *ery2-4* alleles, the wild-type model (in green) is superimposed on the truncated model (in black). The loop, which is thought to line the peptide exit tunnel, is missing. At present, we have no biochemical data to support the idea that the truncated protein is present.

The region of RPL4 that contains the glycine-to-aspartic acid mutations at 102 and 112 was not modeled onto the *H. marismortui* L4 protein as this region was unordered and is not in the crystal structure. It is reasonable to suspect that this region forms a hydrophobic face and that the addition of aspartic acid to this face would disrupt it and possibly block erythromycin binding.
Strains with the ery2 mutation may serve as an excellent recipient for transformation as one could select both positively and negatively for the different alleles. The mutant strain is unable to grow at 15°C, which would allow for selection of the wild-typeERY2 DNA while resistance to erythromycin at 25°C is dominant to the wild-type allele (Hanson and Bogorad 1977).

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