Molecular Cloning and Characterization of the *lux* Genes from the Secondary form of *Xenorhabdus luminescens*, K122

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Xenorhabdus luminescens is a luminous bacterium which is symbiotic with the entomopathogenic nematode *Heterorhabditis*. The bacteria play an important role in pathogenesis of insect larvae. Once the nematode enters the larvae, *Xenorhabdus* spp are released from the gut of the nematode into the hemocoel of the insect. Here both the bacteria and the nematodes proliferate and kill the insect within 48 hours [1, 2]. It has been reported that bacteria-free nematodes or nematodes with symbiotic bacteria other than *Xenorhabdus* are often greatly curtailed in their growth and reproduction inside insect hosts [3, 4].

Phase variation is a common feature of *Xenorhabdus* spp. The primary form of *Xenorhabdus* spp occurs naturally in the infective stage nematode. Cultures of the primary form of *Xenorhabdus* spp give rise to secondary forms after prolonged static incubation in broth media. The primary form of *Xenorhabdus* spp provides better growth conditions for the nematodes than does the secondary form [3]. Unlike the primary form of *Xenorhabdus* spp, the secondary form is deficient in pigment and protease and lipase activities, and is about 100 fold less luminous than the primary form in vivo [4]. The primary form symbiont is of major importance in the nematode-bacteria association. Although the two forms of *Xenorhabdus* display no significant difference in their pathogenicity to the Galleria larvae[3], the form change can result in a dramatic yield decrease during mass production of the nematodes in vitro[5]. Reversion from the secondary form of *Xenorhabdus* back to the primary form has been demonstrated for some *Xenorhabdus* spp but not for *X. luminescens* [6]. The genetic mechanism of conversion from primary form to secondary form is not understood. All of the observations are consistent with the view that the secondary variants arise not from random mutations, but from genetic events[7].

The bioluminescence which is 100 times stronger in the primary form than in the secondary form of *X. luminescens* requires the expression of the *lux* genes. We have cloned these genes as a first step towards reaching an understanding of the genetic mechanism of phase switching. In order to clone the *lux* genes, a genomic DNA bank has been constructed from *X. luminescens*, K122. Chromosomal DNA isolated from the secondary form was partialy digested with 0.02 units of enzyme Sau3A at 37 °C for 30 minutes. The partially digested DNA fragments were then cloned into the BamHI site of plasmid pUC19, and transformed into the competent cells of *E. coli* Tg-1. Two clones, pL1217 and pL1219, were selected out from the DNA bank under dark conditions. Clones pL1217 and pL1219 have 10.5 kb and 17 kb inserts repectively, and must contain the 5 *lux* genes which encode the two subunits of luciferase and the enzymes of the fatty acid reductase complex in order to generate luminescence in *E. coli*.. To further study the 10.5 kb insert from pL1217, physical mapping of the genes has been done with 11 different enzymes (Fig. 1). The results of mapping showed that the restriction enzyme sites in genes from K122 were slightly different from the sites in genes of other *X. luminescens* strains [8, 9]. For example, the BamHI site was found in the genes from the K122 strain, but not in the genes from the Hm strain[8],

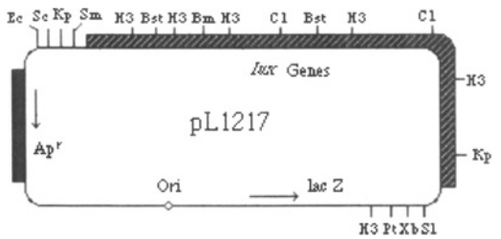

Figure 1. Restriction enzyme map of plasmid pL1217. Abbreviations are: Ec, EcoRI; Sc, SacI; Kp, KpnI; Sm, SmaI; H3, HindIII; Bst, BstEII; Bm, BamHI; Cl, ClaI; Sl, SalI; Xb, XbaI and Pt, PstI. Ap^r is the ampicillin resistence gene. Ori is the origin of replication. the arrow indicates the direction of gene transcription. The hatched box shows the cloned insert containing the *lux* genes.

and two ClaI sites in the genes from K122 were spaced 2.2 kb apart whereas the Hm genes had a 1 kb inserval between these two sites.

Expression of the *lux* genes in *E. coli* was tested after the transformation of pL1217 into *E. coli* strain Tg-1. A single colony of *E. coli* Tg-1/pL1217 was streaked onto LB/containing ampicillin agar and grew at 37 °C overnight. The primary and secondary forms of K122 were grown at same time on LB agar plates at 28 °C. The *lux* genes were constitutively expressed under the control of their own promoter in *E. coli* Tg-1/pL1217. The*lux* genes produced stronger light in *E.coli* than the light produced from these genes in the primary form of K122 although the *lux* genes in pL1217 were originally cloned from the secondary form of K122, which does not luminesce.

We have used pL1217 as a probe to do Southern blot and Northern blot analysis of DNA and RNA respectively from *X. luminescens*, K122 (see ref. 10 for methods). Genomic DNA from the primary and secondary forms of K122 was cut with HindIII and the fragments were separated on an agarose gel. After transferring the DNA onto a Nylon membrane, the Southern blot was hybridized to ^{32}P-labelled probe pL1217 (Fig. 2A). The result show that both primary and secondary forms have the same hybridization pattern. This means that the difference in bioluminescence is not caused by major structural changes within the *lux* genes. It was expected that the *lux* genes were transcribed in the primary form K122 but not in the secondary form. In order to test this hypothesis, RNA was prepared from the primary and secondary form cultures, and a Northern blot of the RNA was probed with ^{32}P-labelled pL1217 (Fig. 2B). It appears that the *lux* gene RNA is present in both primary and secondary forms of K122. Indeed there seems to be more *lux* gene RNA in cells of the secondary form than in the primary cells (fig. 2B). This result suggests that, during the switch from primary to secondary form, the expression of the *lux* genes may be regulated at either the translational or the post-translational level. We hypothesize that the RNA is modified in such a manner as to become both more stable and less translatable in secondary form cells.

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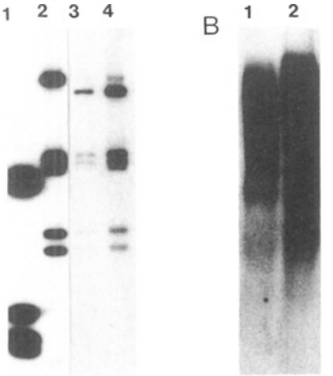

Figure 2. Southern blot (A) and Northern blot (B) analysis of the *lux* genes of *X. luminescens*, K122. (A) Lanes: 1, molecular weight markers; 2, HindIII digested pL1217;3, primary form of K122; 4, secondary form of K122. (B) Lanes: 1, primary form of K122; 2, secondary form of K122.