Restoring Polyhydroxybutyrate (PHB) Depolymerase Expression in a Bald Mutant of *Streptomyces* sp. SFB5A

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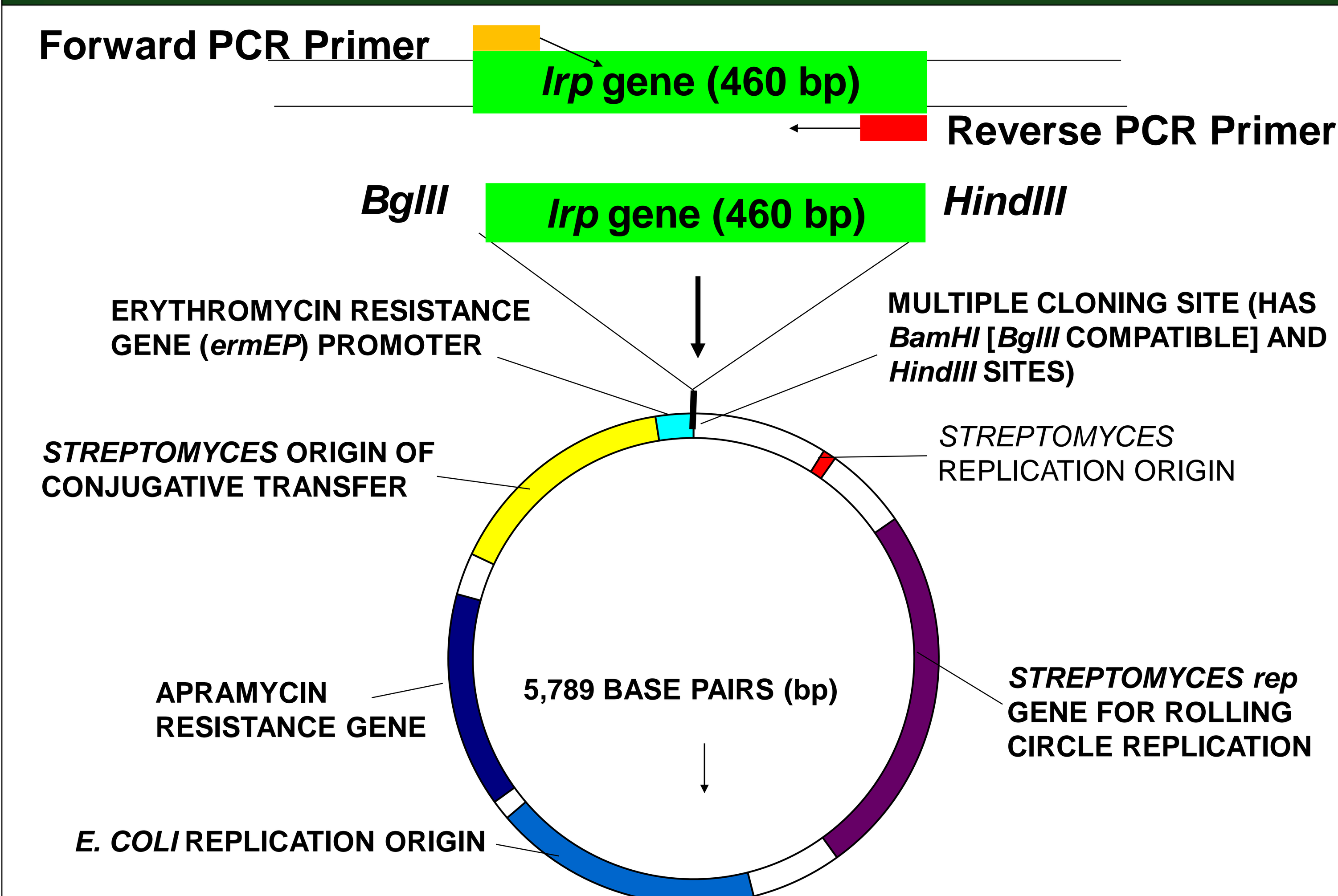
INTRODUCTION

Polyhydroxyalkanoates (PHAs) are biodegradable, plastic-like polymers. They are degraded to their monomers by PHA depolymerases secreted by environmental bacteria to allow for use of PHAs for growth. The PHA depolymerase of *Streptomyces* sp. SFB5A is encoded by the gene, *phaZ*. A "bald" mutant of this species, *bld4*, exhibits shiny rather than the wild type, powdery colonies and does not produce PHA depolymerase. This apparent loss of *phaZ* expression could be caused by a mutation in *phaZ* itself and/or a gene encoding one of its transcriptional regulators. A gene, *lrp*, just upstream from *phaZ* in the genome of *Streptomyces* sp. SFB5A, may code for a transcriptional regulator of *phaZ*. Our goal was to introduce this gene into *bld4* to see if it restores production of PHA depolymerase and/or morphology to that of the wild type (WT).

FIGURE 1: Diagram of Ligation with *lrp* Insert and pIJ86



The *lrp* gene will be amplified using the polymerase chain reaction (PCR) and specific primers containing *BglII* and *HindIII* restriction sites. It will be ligated into the *BamHI and HindIII* sites of the plasmid vector, pIJ86 (the *BglII* site of the *lrp* insert is compatible with the *BamHI* site in the vector).

pIJ86 can replicate in both *E. coli* and *Streptomyces* and contains an apramycin resistance gene that serves as a selectable marker in both species. The pIJ86-*lrp* construct will first be introduced into a non-methylating, conjugative strain of *E. coli*, (ET12567/pUZ8002), and then transferred to *Streptomyces* by intergeneric conjugation. Ideally, the *lrp* DNA can be transcribed under control of the constitutive *ermEP* promoter of pIJ86 and translated to yield Lrp protein.

FIGURE 3: Electrophoresis of PCR-Amplified *lrp* Gene

The *lrp* gene was amplified by PCR from *Streptomyces* sp. SFB5A genomic DNA with the designed forward and reverse primers from Fig. 1. The PCR schedule was 30 cycles of denaturation (94°C, 30 sec.), annealing (57°C, 30 sec.), and extension (72°C, 30 sec.). PCR products were electrophoresed on a 2.0% agarose gel. A PCR product of 460 bp was obtained (lane 1); its size was estimated by comparison to a DNA Step Ladder (50 bp) ™ low range size ladder (lane 2). The product was extracted and ligated to the pIJ86 vector, forming a *pIJ86-lrp* construct, which was then used to transform *E. coli* DH5α competent cells.

lrp gene
(460 bp)

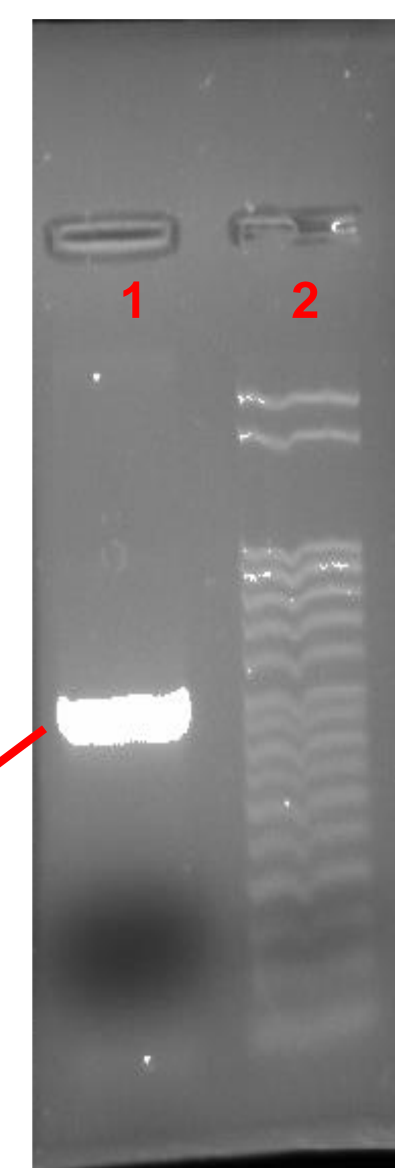
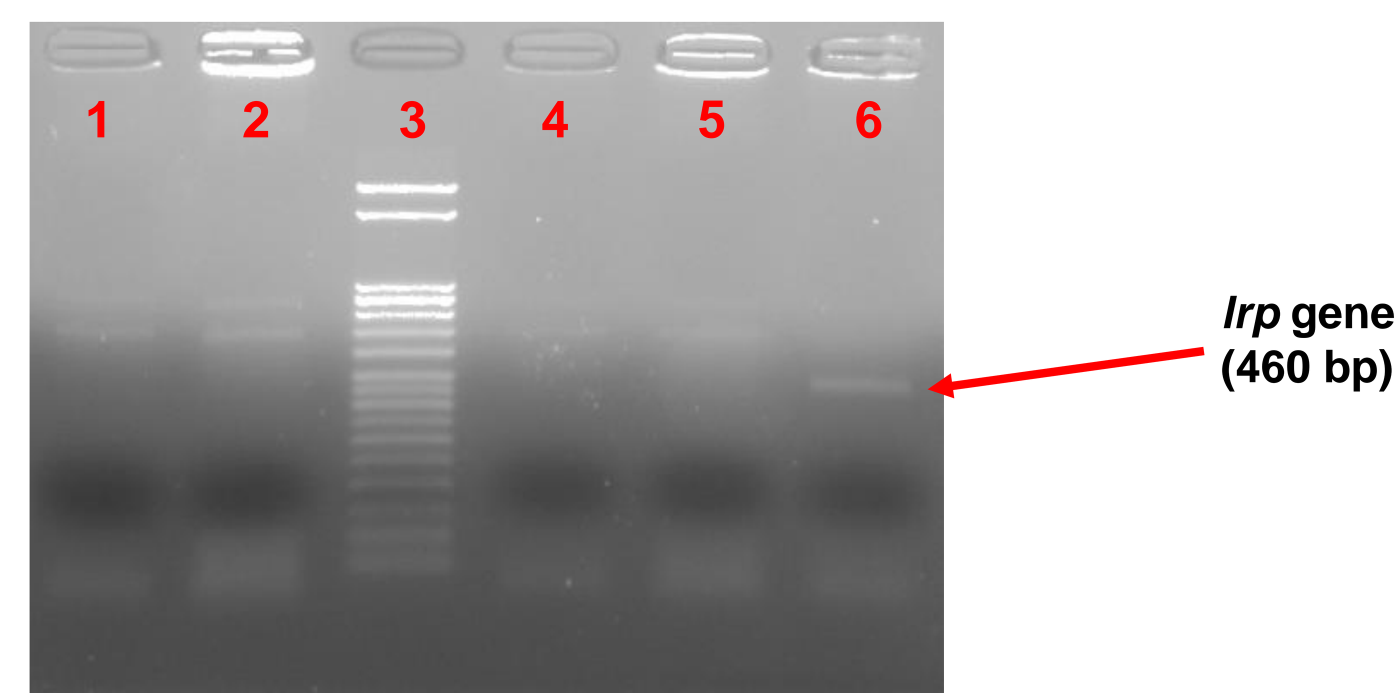
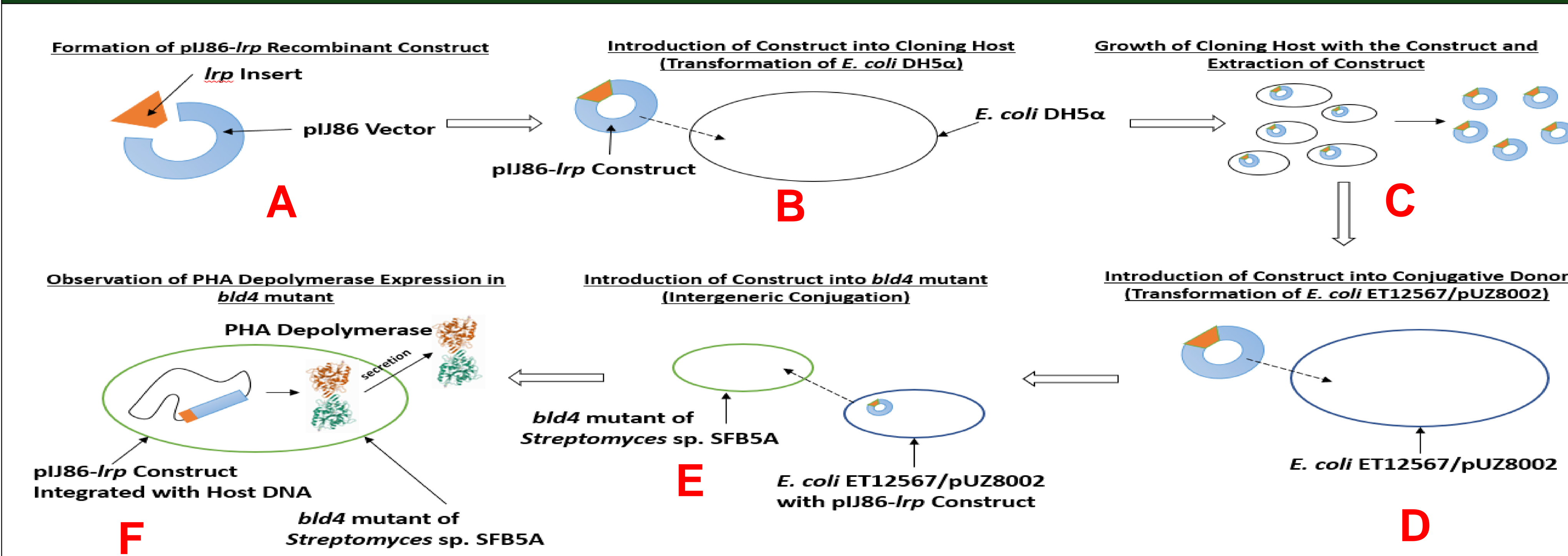


FIGURE 4: Screening of *E. coli* transformants for pIJ86-*lrp*



Colony PCR on *E. coli* DH5α colonies transformed with pIJ86-*lrp* constructs was performed using the same PCR primers and protocol used for the amplification of the *lrp* gene (Figs. 1 and 3); but colony material was used as the template DNA. PCR products were visualized by gel electrophoresis on a 2.0% agarose gel. Lanes 1, 2, 4, 5, and 6 contained PCR products from selected transformants, while lane 3 held the DNA step ladder (50 bp). The transformant tested in lane 6 showed the expected 460 bp band, although it was faint. Restriction digestion (Fig. 5) was used to confirm results of the colony PCR.

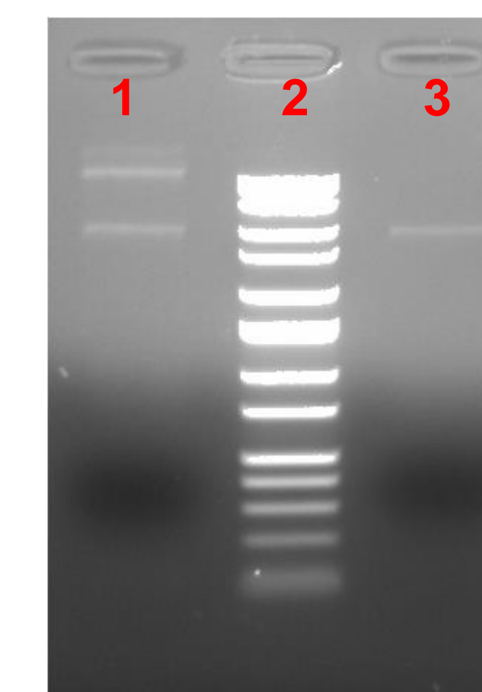
FIGURE 2: Flowchart of Project



After PCR amplification of *lrp* and ligation to the pIJ86 vector (A), the resulting construct will be used to transform *E. coli* DH5α cells (B). Positive clones will be identified by colony PCR, and pIJ86-*lrp* recombinant plasmid DNA extracted from them (C). The construct will be introduced first into *E. coli* ET12567/pUZ8002 (D), and then into *bld4* via intergeneric conjugation (E). Exconjugants will be screened for PHA depolymerase synthesis by appearance of powdery, wild type colonies and clearing zones on agar plates containing PHB granules (F); at right is a comparison of WT and *bld4* colony morphology on agar containing PHB.

WT *bld4*

FIGURE 5: Restriction Digest of Plasmid DNA from pIJ86-*lrp* Constructs



Plasmid DNA from the suspected clone (lane 6, Fig. 4) was extracted, digested with *HindIII,* and electrophoresed on a 1.0% agarose gel to verify presence of the pIJ86-*lrp* construct. This should yield a fragment of 6255 bp. Lanes 1 and 3 contained plasmid DNA from two broth cultures of the suspected clone, while lane 2 contained a wide range DNA size ladder. A band of about 4000 bp was observed, which was less than the expected size; additional bands were also obtained in lane 1. We are currently screening other transformants to see if their colony PCR and restriction digest patterns on agarose gels match with the expected size patterns. This time, we will use pIJ86 primers that flank the site where *lrp* would be inserted; this would result in a PCR product of 654 bp.

SUMMARY

- We successfully amplified and isolated the *lrp* gene from the genomic DNA of the bacterium *Streptomyces* sp. SFB5A via PCR.
- The PCR product was successfully ligated to the pIJ86 cloning vector and then used to transform *E. coli* DH5α cells.
- A positive clone was tentatively identified by colony PCR, but a subsequent restriction digest shows a restriction fragment of the wrong size.

FUTURE WORK

- Additional *E. coli* DH5α transformants will be screened for the pIJ86-*lrp* construct using PCR with pIJ86-specific primers
- *E. coli* ET12567/pUZ8002 will be transformed with the pIJ86-*lrp* construct.
- The pIJ86-*lrp* construct will be introduced into *bld4* mutant of *Streptomyces* sp. SFB5A by intergeneric conjugation with *E. coli* ET12567/pUZ8002.
- Exconjugants will be screened for PHA depolymerase by growing *Streptomyces* sp. SFB5A on agar plates containing PHB granules and checking for the appearance of clearing zones.

ACKNOWLEDGEMENTS

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