

## Introduction

*Bacillus subtilis* derived Oxalate Decarboxylase (OxDC) is a Mn-dependent enzyme that catalyzes the degradation of mono-protonated oxalate to carbon dioxide and formate. In the absence of this enzyme, oxalic acid forms insoluble metal complexes, leading to negative impacts in the human body, agriculture, and the paper and pulp industries [1]. Within this study we describe the expression and purification of *B. sub* OxDC utilizing a *Bacillus subtilis* host. *Bacillus subtilis* is very well known for its secretory pathway of proteins out of the cell, which has been utilized industrially for the production of proteins. The pHT43 stable vector was designed to perform high level of expression of proteins in the cytoplasm and then shuttle them out of the cell. The chosen cell strains 1A751 and WB800N are extracellular protease deficient lines to prevent degradation of the secreted proteins of interest. After successful purification of OxDC the enzymatic mechanism will be studied utilizing Electron Paramagnetic Resonance, having the benefit of the manganese centers as molecular probes for following the catalysis.

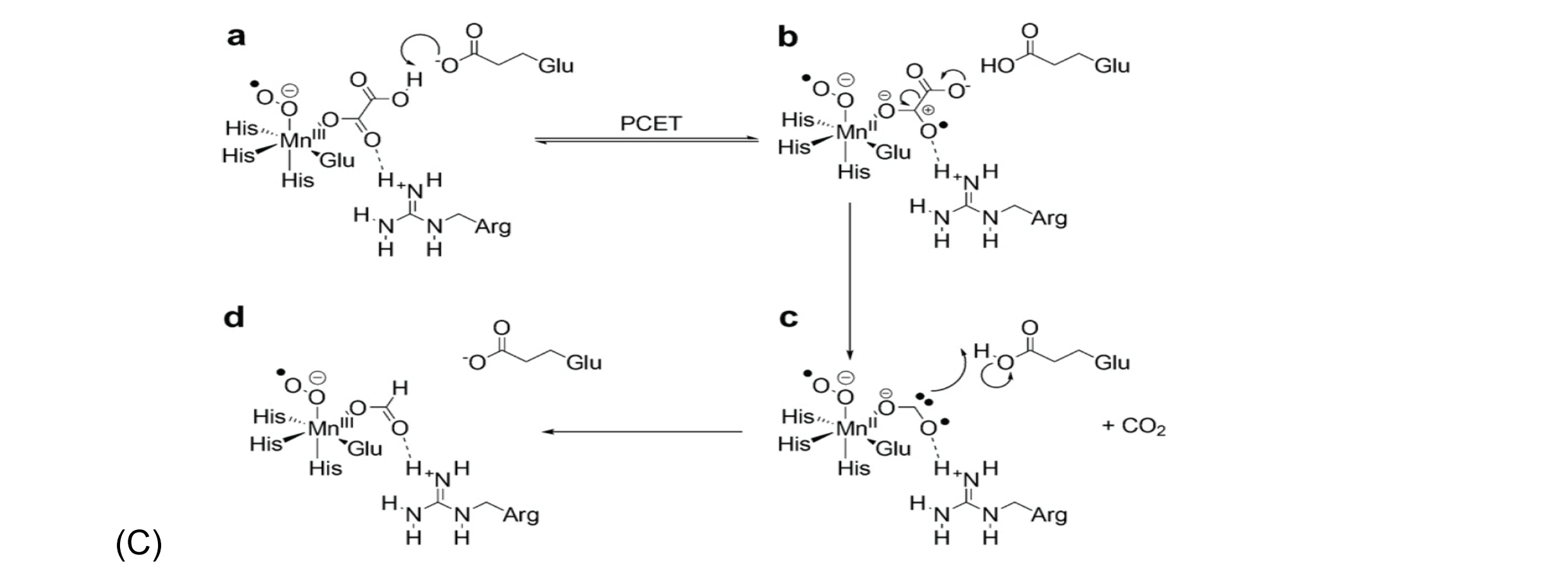
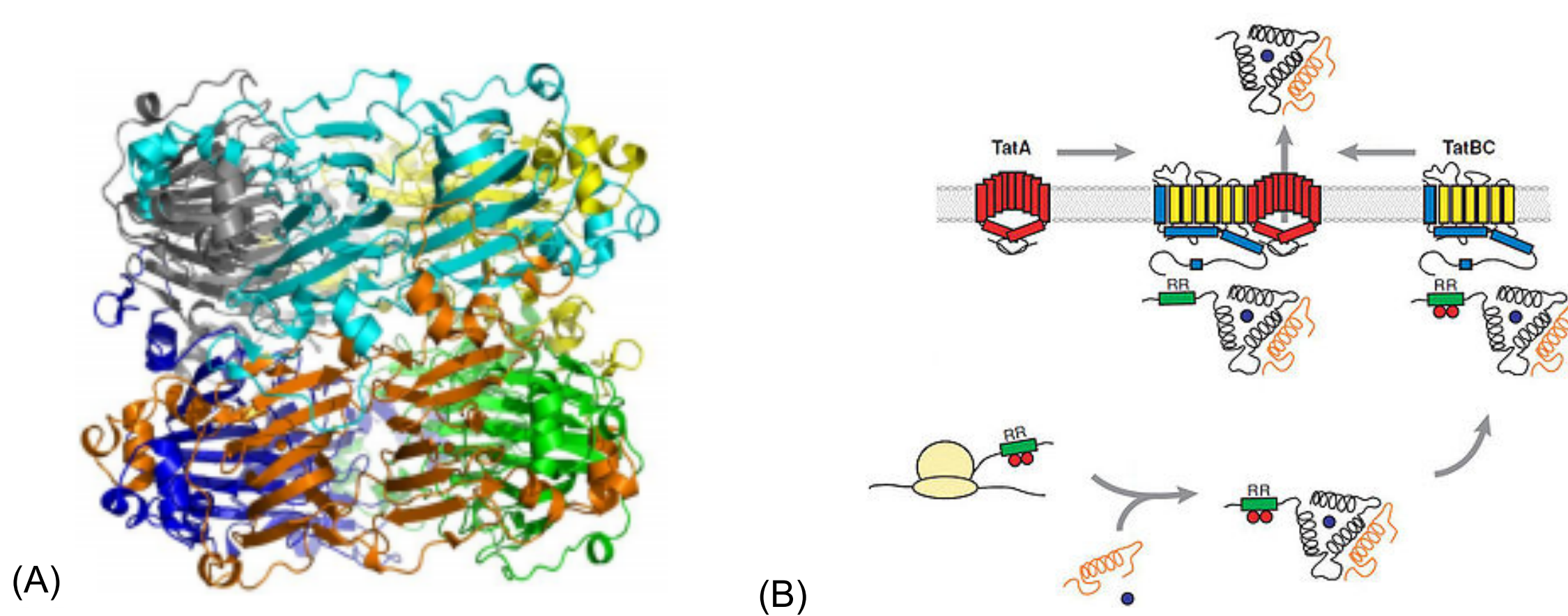


Figure 1. (A) Oxalate decarboxylase structure (B) TAT-dependent pathway as the proposed mechanism for secretion of fully folded enzyme (C) Proposed mechanism of decarboxylation

## Acknowledgements

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## References:

- [1] Miller, C., et al., *Toxicology & Applied Pharmacology*. **2000**,162,132-141
- [2] Libert, B., et al., *Agric. Food Chem.* **2011**, 35, 1234-1242

## Results

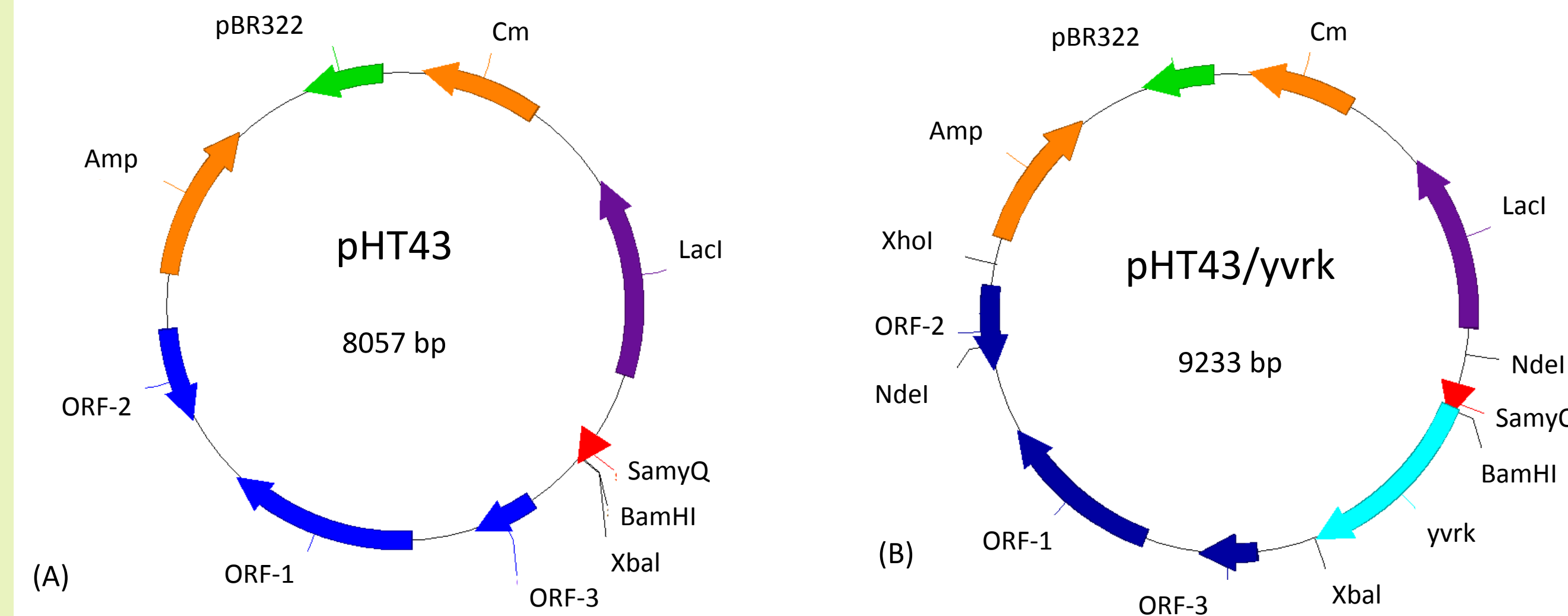


Figure 2. (A) pHT43 – stable vector system that is IPTG inducible with secretion pathways (B) pHT43/yvrk – vector ligation product

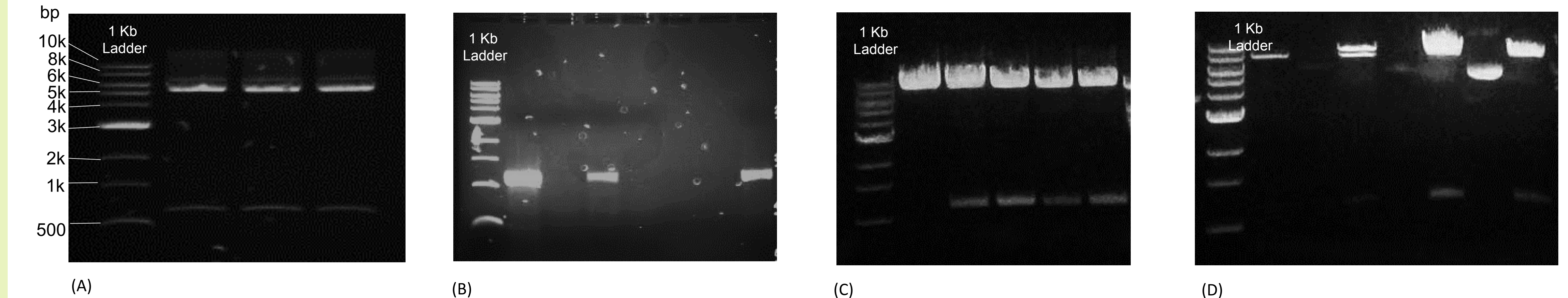


Figure 4. All gels shown (A-D) have a 1 kb Biotech ladder (A) Purified digested pET32a/yvrk (B) Purified yvrk insert (C) Initial double digestion of the ligated pHT43/yvrk product with XbaI and BamHI restriction enzymes. (D) Side by side comparison of digested and undigested (supercoiled) products of the pHT43/yvrk plasmid.

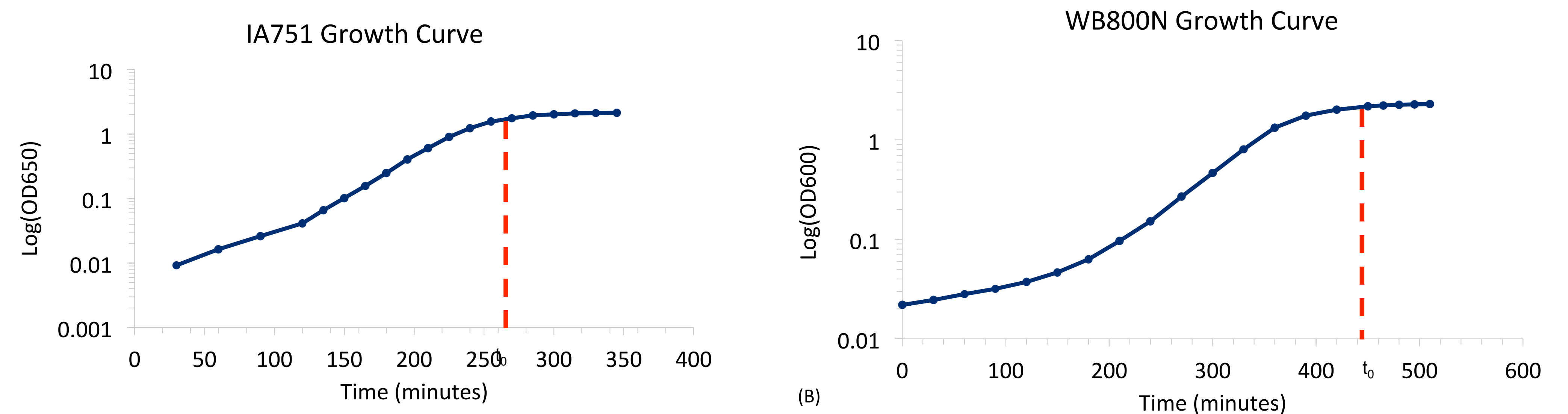


Figure 5. (A) IA751 growth curve in order to find the beginning of stationary phase to start  $t_0$  for optimal transformation of the plasmid (B) WB800N growth curve in order to find the beginning of stationary phase to start  $t_0$  for optimal transformation.

## Discussion

Previously, Oxalate Decarboxylase was expressed and purified using an *E. coli* based system. Within this work, the goal was to move to a *Bacillus subtilis* based expression system to utilize the benefits of its high expression levels and secretion pathways. The gene encoding for *Bacillus subtilis* Oxalate Decarboxylase was initially isolated by double digestion of the pET32a vector containing it (Figure 4a). The gene was then PCR amplified (Figure 4b) utilizing primers shown in Figure 3. The amplified gene was then ligated in the pHT43 vector, which was confirmed by double digestion of the ligated product (Figure 4c). BLAST alignment of the sequence versus what was expected showed that there was a 100% match between the new plasmid and established plasmid and gene sequences. The chosen cell lines are extracellular protease deficient cell strains that will prevent the degradation of the secreted OxDC. Preparation of competent *Bacillus subtilis* cells require that cells be induced in the stationary phase, as they develop their natural competence. Cells were then grown in a  $\text{CaCl}_2$  based medium for 90 minutes after entrance into stationary phase marked by  $t_0$  in Figure 5. Transformation of ligation product was then carried out by addition of DNA to freshly prepared competent cells and then grown overnight on LB agar plates treated with  $5\mu\text{g/ml}$  chloramphenicol to screen for successful transformation. Future variables that will be tested to optimize protein yield are: varying the amount of IPTG inducer, changing post-induction incubation temperature, and changing the time of induction.