Characterization of putative Transcriptional Regulator Orf90 from Staphylococcus aureus Plasmid pSK41

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INTRODUCTION

The rapid emergence of antibiotic resistant bacteria threatens global health. Antibiotic resistance primarily originates through **conjugative plasmid transfer** (CPT).

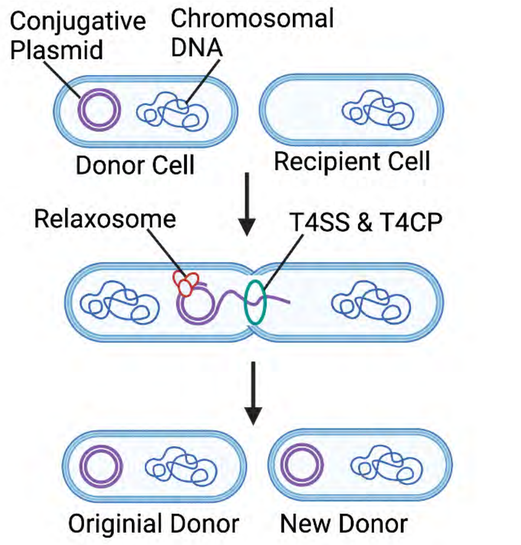


Fig 1. 3 components that facilitate CPT.

Many plasmid-encoded proteins are unstudied. Orf90 is an uncharacterized plasmid-encoded protein located on pSK41, a plasmid with multiple antibiotic resistances. (Fig 2.)

Fig 2. Portion of pSK41 displaying the Origin of Transfer (OriT), where CPT begins and where the relaxosome forms. Orf90 is in an operon group adjacent to OriT.

The Basic Local Alignment Search Tool (BLAST) was used to identify members of the Orf90 gene family. Recombination protein RmuC yielded the highest sequence similarity. Orf90 may regulate Orf77, an entry-exclusion protein. **Here, we present the initial characterization of Orf90 to illuminate its role in pSK41 conjugation.**

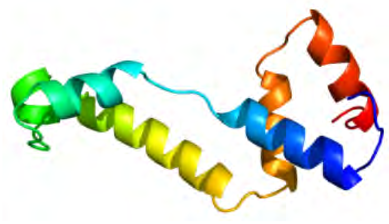


Fig 3. Phyre2 Structure Prediction from Orf90 Sequence

- N-terminus depicted in red, C-terminus depicted in blue
- 60% of protein modeled, 56.8% confidence level
- Predicted structure is completely alpha-helical

METHODOLOGY & RESULTS

HIS-ORF90 PURIFICATION

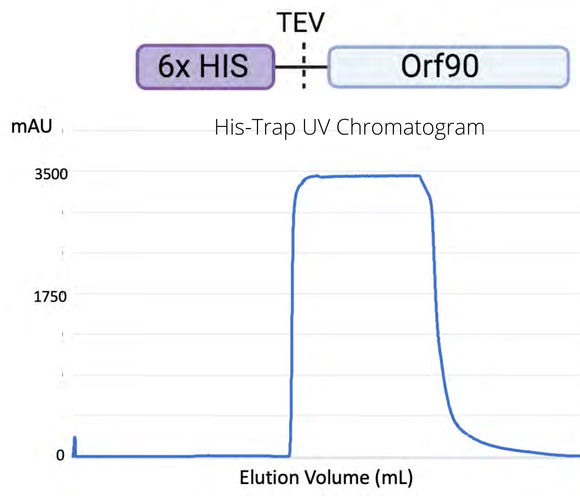
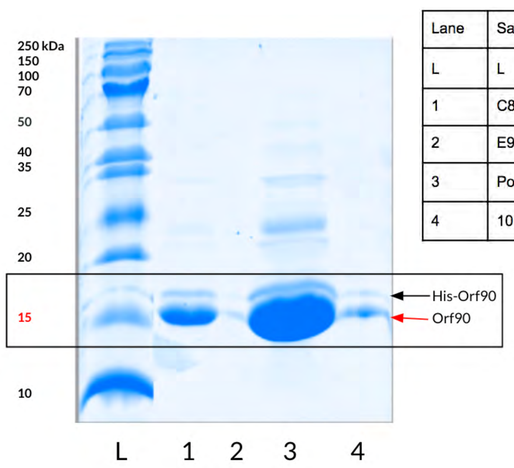


Fig 4. Ni2+ affinity chromatograph. 3 joined peaks indicate contamination.

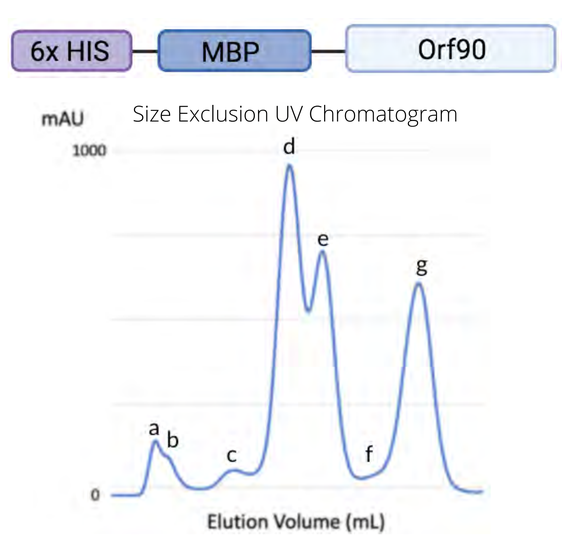


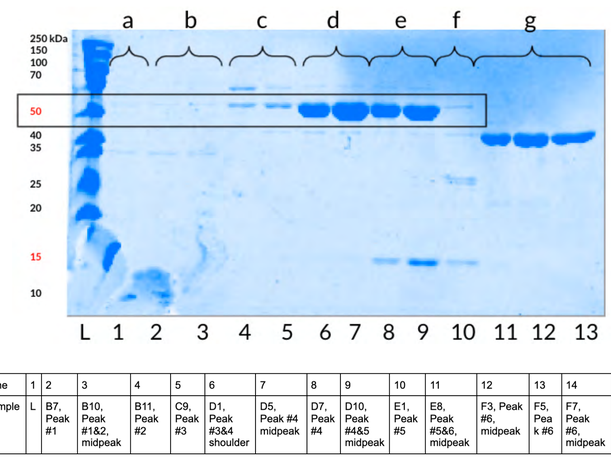
Lane	Sample
L	L
1	C8
2	E9
3	Post GF flow through
4	10 uL final protein sample

Fig 5. 15% SDS PAGE. His-Orf90 is at ~15 kDa. Image cropped to display relevant lanes.

- Expressed in *E. coli* Strain BL21 DE3 plysS
- Purified using Ni2+ affinity, subtractive affinity (Fig.4), and size exclusion chromatography
- Obtained purity of 95.5% (Fig 5.)
- 2nd Purification trial: ran linear gradient during Ni2+ affinity to separate peaks
- Obtained 2nd purity of 100% Orf90:
  - 71.4% Orf90, 28.6% His-Orf90

HIS-MBP-ORF90 PURIFICATION





Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sample	L	B7, Peak #1	B10, Peak #1&2, midpeak	B11, Peak #2	C9, Peak #3	D1, Peak #3&4 shoulder	D5, Peak #4 midpeak	D7, Peak #4	D10, Peak #4&5 midpeak	E1, Peak #5	E8, Peak #5&6, midpeak	F3, Peak #6, midpeak	F5, Pea k #6	F7, Peak #6, midpeak	F10, Peak #6, shoulder

Fig 11&12. Size exclusion chromatography and corresponding 15% SDS PAGE, His-MBP-Orf90 is at ~50 kDa. Cleaved Orf90 is at 15 kDa. Concentrated lanes 6-7 (Peak D)

- Purified using method from 2nd His-Orf90 purification, excluded subtractive affinity
- MBP tag kept intact to improve protein purity by enhancing protein solubility
- Obtained purity of 85.85%
- First experiment to successfully purify His-MBP-Orf90

CRYSTALLOGRAPHY TRIALS

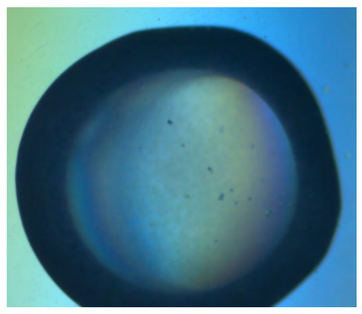


Fig 9. Drop composed of 1 µL His-ORF90: 2µ well contents (3.50M Ammonium Sulfate, 1.0M Bicine pH 9.0)

- His-Orf90 purification: No Crystals
- Second His-Orf90 purification: Microcrystals and Precipitation, no macromolecular crystals (Fig. 6)

EMSA

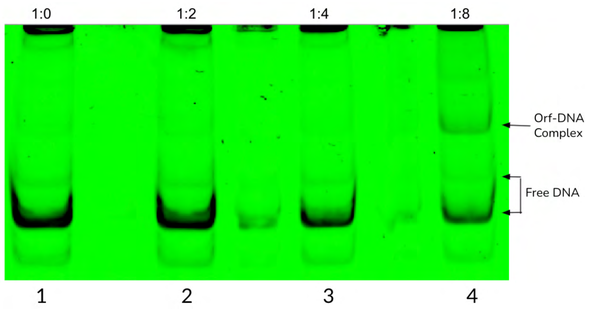


Fig 12. 12% Native Polyacrylamide Gel. Orf90 incubated with 205 bp pSK41 oriT DNA at 1:0, 1, 4, and 1:8 DNA: Protein ratio. A shift in lane 4 indicates binding.

- EMSA - Electrophoretic Mobility Shift Assay
- Detects nucleic acid-protein interactions
- Confirmed Orf90 bound OriT DNA

CONCLUSIONS & NEXT STEPS

- Developed successful purification method for His-Orf90 and His-MBP Orf90
- Unable to crystallize His-Orf90 since contaminants remained
- Affinity tag not fully cleaved, will increase TEV protease concentration
- Run a optimization screen for MBP-Orf90 and conduct crystallography trials

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