2018

The Effect of the KONJAC1 and KONJAC2 Proteins on GDP-Glucose Pyrophosphorylase Activity of VTC1

Hafsah Jamil

Follow this and additional works at: https://commons.emich.edu/honors

Part of the Biology Commons

Recommended Citation
https://commons.emich.edu/honors/594

This Open Access Senior Honors Thesis is brought to you for free and open access by the Honors College at DigitalCommons@EMU. It has been accepted for inclusion in Senior Honors Theses by an authorized administrator of DigitalCommons@EMU. For more information, please contact lib-ir@emich.edu.
The Effect of the KONJAC1 and KONJAC2 Proteins on GDP-Glucose Pyrophosphorylase Activity of VTC1

Abstract
Vitamin C Defective I (VTC I) is the major pyrophosphorylase involved in guanosine diphosphomannose (GDP-mannose) biosynthesis in the plant Arabidopsis thaliana and it catalyzes the conversion of mannose I-phosphate and guanosine triphosphate (GTP) to GDP-mannose and pyrophosphate (PPi). The GDP-mannose pyrophosphorylase (GMPPase) activity of VTCI is enhanced by the proteins KONJACI (KJC1) and KONJAC2 (KJC2), which are homologs of VTCI. The purpose of this study was to analyze the effect of the KJC proteins on the GDP-glucose pyrophosphorylase (GGPPase) activity of VTCI. We hypothesized that the GGPPase activity of VTCI may also be enhanced due to interactions with KJC1 and/or KJC2 due to the structural similarities between mannose I-phosphate and glucose I-phosphate. Enzyme assays were conducted to analyze the GMPPase and GGPPase activity of VTCI. We found that while in vitro assays indicated that VTC I was able to catalyze both GMPP and GGPP reactions, they did not conclusively demonstrate the effect of the KJC proteins on the GMPPase and GGPPase activity of VTCI. Replicates of the assays will need to be conducted to conclude whether or not the KJC proteins enhance the GGPPase activity of VTCI.

Degree Type
Open Access Senior Honors Thesis

Department
Biology

First Advisor
Aaron Liepman

Second Advisor
Krish Judd

Third Advisor
Marianne Laporte

Keywords
Glucomannan, Enzymes, Plant Cell Wall Polysaccharides

Subject Categories
Biology

This open access senior honors thesis is available at DigitalCommons@EMU: https://commons.emich.edu/honors/594
THE EFFECT OF THE KONJAC1 AND KONJAC2 PROTEINS ON GDP-GLUCOSE PYROPHOSPHORYLASE ACTIVITY OF VTC1

By
Hafsah Jamil

A Senior Thesis Submitted to the
Eastern Michigan University
Honors College
in Partial Fulfillment of the Requirements for Graduation
with Honors in Biology

Approved at Ypsilanti, Michigan, on this date May 4, 2018
# TABLE OF CONTENTS

COVER PAGE ...................................................................................................................... 1

TABLE OF CONTENTS ........................................................................................................ 2

LIST OF TABLES .................................................................................................................. 4

LIST OF FIGURES ................................................................................................................ 5

ABSTRACT .......................................................................................................................... 7

INTRODUCTION .................................................................................................................. 8

Nucleotide diphosphate sugars: function and importance .................................................. 8

Synthesis of glucomannan ................................................................................................. 11

GDP-mannose synthesis ................................................................................................. 12

Proposed GDP-Glucose synthetic pathway ..................................................................... 14

MATERIALS AND METHODS ........................................................................................... 18

1. Expression of recombinant proteins in E. coli via autoinduction .................................. 18
   1.1 Transformation and autoinduction of recombinant proteins .................................. 18
   1.2 Analysis of recombinant protein induction following auto-induction ................. 21
   1.3 Lysis and fractionation of samples using glass beads .......................................... 22
   1.4 Lysis of E. coli cells using French Press .............................................................. 23

2. Purification of recombinant proteins ............................................................................. 23
   2.1 Purification of recombinant proteins by Three Phase Partitioning ..................... 23
   2.2 Desalting interfacial precipitate for SDS-PAGE analysis using Sephadex G-25 ... 25
   2.3 Desalting interfacial precipitate for SDS-PAGE analysis using dialysis .......... 25
3. GMPP and GGPP in vitro assays ................................................................. 25
   3.1 GMPP and GGPP time course assays ...................................................... 25
   3.2 Assay product purification ..................................................................... 26
   3.3 HPLC analysis of NDP-sugars .............................................................. 27
4. Testing the effect of KJC proteins on GMPPase and GGPPase activity of VTC1 .... 28

RESULTS ............................................................................................................. 29
1. Optimization of expression of recombinant proteins .................................... 29
2. Purification of recombinant proteins by three phase partitioning ................. 31
3. HPLC analysis of GDP-mannose and GDP-glucose standards .................... 33
4. GMPP and GGPP time course assays ........................................................... 34
5. Effect of KJC proteins on GMPPase and GGPPase activity of VTC1 .......... 36

DISCUSSION ....................................................................................................... 41

REFERENCES ................................................................................................. 45

APPENDIX ......................................................................................................... 48
LIST OF TABLES

Table 1. Examples of major NDP-sugars found in plants .................................................. 9
Table 2. ZYM-5052 auto-induction media ....................................................................... 20
Table 3. Breaking buffer formulation ............................................................................... 21
Table 4. Amount of ammonium sulfate needed for each saturation ................................. 24
Table 5. Pyrophosphorylase assay formulation ................................................................ 26
Table 6. Expected molecular weight of proteins .............................................................. 30
Table 7. Area of GDP-mannose peaks produced by VTC1 on its own and with other proteins .............................................................................................................................. 37
Table 8. Area of GDP-glucose peaks produced by VTC1 on its own and with other proteins ........................................................................................................................................ 37
LIST OF FIGURES

Figure 1. Nucleotide sugar interconversion pathways ...................................................... 10
Figure 2. Glucomannan synthesis ..................................................................................... 11
Figure 3. GDP-mannose synthesis .................................................................................... 12
Figure 4. Phylogeny of pyrophosphorylases from *Arabidopsis* (At) and rice (Os) ...... 13
Figure 5. Structures of glucose 1-phosphate and mannose 1-phosphate ....................... 15
Figure 6. Proposed synthesis of GDP-glucose through GGPPase activity of VTC1 ...... 16
Figure 7. Expression vectors for recombinant protein expression of amino-terminal
epitope tagged VTC1 ........................................................................................................ 19
Figure 8. Separation of phases during three phase partitioning ........................................ 24
Figure 9. Immunoblot analysis of recombinant, T7-tagged KJC1 and KJC2 from 25°C
auto-induction .................................................................................................................. 30
Figure 10. Immunoblot analysis of recombinant, His6-tagged mCherry and KJC2 proteins
from 10°C auto-induction .................................................................................................... 31
Figure 11. SDS-PAGE analysis of recombinant VTC1 and mCherry desalted through
dialysis .............................................................................................................................. 32
Figure 12. SDS-PAGE analysis of recombinant VTC1 and mCherry desalted through
Sephadex G-25 buffer column .......................................................................................... 33
Figure 13. HPLC analysis of NDP-sugar standards .......................................................... 35
Figure 14. HPLC analysis of GMPPase and GGPPase activity of VTC1 at various time
points .................................................................................................................................. 36
Figure 15. HPLC analysis of GMPPase activity of recombinant proteins ......................... 38
Figure 16. HPLC analysis of GGPPase activity of recombinant proteins ....................... 39
Figure 17. HPLC analysis of GMPPase and GGPPase activity of VTC1 with KJC1
included in assay ........................................................................................................ 40

Figure A1. HPLC analysis of GMPPase and GGPPase activity of KJC proteins .......... 48

Figure A2. HPLC analysis of GMPPase and GGPPase activity of VTC1 with KJC2
included in assay ........................................................................................................ 49

Figure A3. HPLC analysis of GMPPase and GGPPase activity of VTC1 with mCherry
included in assay ........................................................................................................ 50
Abstract

Vitamin C Defective 1 (VTC1) is the major pyrophosphorylase involved in guanosine diphosphomannose (GDP-mannose) biosynthesis in the plant *Arabidopsis thaliana* and it catalyzes the conversion of mannose 1-phosphate and guanosine triphosphate (GTP) to GDP-mannose and pyrophosphate (PPi). The GDP-mannose pyrophosphorylase (GMPPase) activity of VTC1 is enhanced by the proteins KONJAC1 (KJC1) and KONJAC2 (KJC2), which are homologs of VTC1. The purpose of this study was to analyze the effect of the KJC proteins on the GDP-glucose pyrophosphorylase (GGPPase) activity of VTC1. We hypothesized that the GGPPase activity of VTC1 may also be enhanced due to interactions with KJC1 and/or KJC2 due to the structural similarities between mannose 1-phosphate and glucose 1-phosphate. Enzyme assays were conducted to analyze the GMPPase and GGPPase activity of VTC1. We found that while in vitro assays indicated that VTC1 was able to catalyze both GMPP and GGPP reactions, they did not conclusively demonstrate the effect of the KJC proteins on the GMPPase and GGPPase activity of VTC1. Replicates of the assays will need to be conducted to conclude whether or not the KJC proteins enhance the GGPPase activity of VTC1.
Introduction

Nucleotide Diphosphate Sugars: Function and Importance

Nucleotide diphosphate sugars (NDP-sugars) are activated monosaccharides used by all organisms to synthesize polysaccharides through glycosyltransferase (GT) reactions (Bar-Peled and O'Neill, 2011). NDP-sugars are substrates in biosynthetic reactions catalyzed by a group of enzymes known as glycosyltransferases (GTs), which transfer the monosaccharide of the NDP-sugar to another molecule, such as a protein, lipid, or carbohydrate (Bar-Peled and O'Neill, 2011). At least 12 different types of NDP-sugars have been identified in humans (Bar-Peled and O'Neill, 2011), some of which play important roles in growth and development (Liu and Hirschberg, 2013). For example, glycogen synthesis and glycoprotein glycosylation pathways in humans rely on sugar chains that are constructed by glycosyltransferases that use NDP-sugar substrates (Liu and Hirschberg, 2013). In plants, at least 30 NDP-sugars have been identified (Bar-Peled and O'Neill, 2011; see Table 1 for examples); they are used as substrates for many GTs, including those involved in the synthesis of plant cell wall carbohydrates, glycoproteins, starch, and vitamin C (Table 1; Bar-Peled and O'Neill, 2011). While vitamin C synthesis is essential for plant growth (Lukowitz et al. 2001), it is also relevant to humans, because humans are unable to produce this nutrient. Therefore, adequate synthesis of NDP-sugars in plants is important because it plays a role in providing the vitamin C humans consume to stay healthy (Sawake et al. 2015).
Table 1. Examples of major NDP-sugars found in plants (Bar-Peled and O’Neill, 2011)

<table>
<thead>
<tr>
<th>NDP-Sugar(s):</th>
<th>Incorporated Into:</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP-Glucose</td>
<td>Starch</td>
</tr>
<tr>
<td>GDP-Fucose</td>
<td>Pectin, xyloglucan, glycoproteins</td>
</tr>
<tr>
<td>GDP-Galactose</td>
<td>Ascorbic acid (Vitamin C)</td>
</tr>
<tr>
<td>GDP-Glucose</td>
<td>Glucomannan</td>
</tr>
<tr>
<td>GDP-Mannose</td>
<td>Mannan, glucomannan, galactomannan, glycoproteins</td>
</tr>
<tr>
<td>UDP-Apiose</td>
<td>Pectin</td>
</tr>
<tr>
<td>UDP-Galactose</td>
<td>Pectin, glycoproteins, xyloglucans, galactomannans</td>
</tr>
<tr>
<td>UDP-Galacturonic Acid</td>
<td>Xylan and pectin</td>
</tr>
<tr>
<td>UDP-Glucose</td>
<td>Cellulose and xyloglucan</td>
</tr>
<tr>
<td>UDP-Glucuronic Acid</td>
<td>Xylan</td>
</tr>
<tr>
<td>UDP-N-Acetyl-α-D-Glucosamine</td>
<td>Glycoproteins</td>
</tr>
<tr>
<td>UDP-Rhamnose</td>
<td>Xylan</td>
</tr>
<tr>
<td>UDP-Xylose</td>
<td>Xylan, pectin, glycoproteins</td>
</tr>
<tr>
<td>UDP-β-L-Arabinopyranose and UDP-β-L-Arabinofuranose</td>
<td>Xylan and pectin</td>
</tr>
</tbody>
</table>

NDP-sugars are synthesized and interconverted via a complex and interconnected series of reactions (Figure 1; Bar-Peled and O’Neill, 2011). The synthesis of these NDP-sugars relies on a variety of enzymes, such as pyrophosphorylases, epimerases, dehydrogenases, kinases, isomerases, and decarboxylases (Reiter, 2008). Although many of the reactions of these pathways have been identified, there is still much to learn about the proteins involved. NDP-sugars play a variety of roles in plant cell wall polysaccharide synthesis, and it is important to understand their biosynthetic pathways because plant cell
walls are the most abundant source of renewable biomass on Earth (Reiter, 2008). Additional knowledge of the proteins involved in these pathways can help us better understand how to access and utilize the biomass in plant cell walls.

Figure 1. Nucleotide sugar interconversion pathways (Bar-Peled and O’Neill, 2011). The GDP-mannose pathway and a proposed GDP-glucose pathway are circled.
Synthesis of Glucomannan

Glucomannans are a type of plant cell wall polysaccharide whose biosynthetic pathway is not fully understood. These polysaccharides are synthesized by CELLULOSE SYNTHASE-LIKE A family proteins using two NDP-sugar substrates: guanosine diphosphate (GDP)-glucose and GDP-mannose (Figure 2; Liepman et al. 2005).

Glucomannans serve structural functions and act as energy reserves in some plants (Gille et al. 2011). Due to their ability to form gels, glucomannans are extensively used in the pharmaceutical, cosmetic, coating/packaging, and food industries (Zhang et al. 2005).

Glucomannans are also used as dietary supplements to help lower cholesterol and body weight (Sood et al. 2008). Understanding the pathways involved in glucomannan synthesis could eventually improve their production.

Figure 2. GDP-Mannose and GDP-Glucose (P=Phosphate, G=Guanosine) are NDP-sugar substrates used by CELLULOSE SYNTHASE-LIKE A (CSLA) proteins to synthesize glucomannan.
**GDP-Mannose Synthesis**

GDP-mannose is synthesized through a pyrophosphorylase reaction (Sawake et al. 2015). In the plant *Arabidopsis thaliana*, the major pyrophosphorylase involved in GDP-mannose synthesis is called VITAMIN C DEFECTIVE 1 (VTC1/At2g39770), which catalyzes the conversion of mannose 1-phosphate and guanosine triphosphate (GTP) to GDP-mannose and pyrophosphate (PPI) (Figure 3; Sawake et al. 2015). When VTC1 is mutated, it impairs GDP-mannose pyrophosphorylase activity, resulting in a deficiency of GDP-mannose (Conklin et al. 1999). This mutation has indirect effects on plant cell wall development, because GDP-mannose plays a role in N-glycosylation and is a precursor for GDP-fucose and Vitamin C production (Lukowitz et al. 2000). Therefore, *A. thaliana* vtc1 mutants exhibit less N-glycosylation of their glycoproteins and reduced quantities of mannose and fucose (Lukowitz et al. 2000).

![Figure 3. Conversion of mannose 1-phosphate and GTP to GDP-mannose and PPI, catalyzed by the GDP-mannose pyrophosphorylase VTC1.](image-url)
It was recently discovered that the proteins KONJAC1 (KJC1; At1g74910) and KONJAC2 (KJC2; At2g04650) enhance the GDP-mannose pyrophosphorylase (GMPP) activity of VTC1 in *A. thaliana*. The KONJAC protein sequences are closely related to VTC1 (Figure 4; Sawake *et al.* 2015).

**Figure 4.** Phylogeny of pyrophosphorylases from *Arabidopsis* (At) and rice (Os) (Sawake *et al.* 2015). The clades indicated by arrows contain the proteins of interest to this study (VTC1, KJC1, KJC2), which are in red.
Proposed GDP-Glucose Synthetic Pathway

Unlike GDP-mannose, genes encoding enzymes involved in the synthesis of GDP-glucose in plants have not been identified. It has been speculated that, like GDP-mannose, GDP-glucose is also synthesized via a pyrophosphorylase reaction (Reiter, 2008; Gille et al. 2011). Prior studies have offered biochemical evidence for GDP-glucose pyrophosphorylase activity extracted from peas (Barber and Hassid, 1964; Péaud-Lenoël and Axelos, 1968). Interestingly, there appears to be a dependence upon manganese for GGPPase activity of the purified pea protein. When manganese (Mn\(^{2+}\)) was included in the assay buffer, GGPPase activity was approximately 20-fold higher than when magnesium (Mg\(^{2+}\)) was included in the assay buffer, which was an unexpected result because GGPPase activity in animals is stimulated by Mg\(^{2+}\) (Péaud-Lenoël and Axelos, 1968). While the authors of these studies concluded that GGPPase and GMPPase were different enzymes (Barber and Hassid, 1964; Péaud-Lenoël and Axelos, 1968), there was not sufficient data in these papers to support this claim.

Due to the similarities in the structures of mannose 1-phosphate and glucose 1-phosphate (Figure 5), VTCl and its homologs may also biosynthesize GDP-glucose. There is precedence for such dual activity among GMPPases present in other organisms. For example, GMPPases from *Escherichia coli* and *Salmonella enterica* also possess GDP-glucose pyrophosphorylase (GGPP) activity (Watt et al. 1999; Yang et al. 2005; Zou et al. 2012;).
Figure 5. Structures of glucose 1-phosphate and mannose 1-phosphate highlighting the carbon (C2) that distinguishes these two molecules. (Image sources: https://commons.wikimedia.org/wiki/File:Alpha-D-Mannose-1-phosphat.svg, http://www.wikiwand.com/fr/Glucose-1-phosphate)

There is conflicting evidence about the ability of the Arabidopsis VTC1 protein to synthesize GDP-glucose. Sawake et al. (2015) did not report significant GGPPase activity of recombinant VTC1 expressed in E. coli, even in the presence of KJCl and KJC2. However, it is possible that Mn$^{2+}$ had not been present in the assay buffer, which may explain why only low GGPPase activity was detected (Sawake et al. 2015). On the other hand, there is preliminary evidence that VTC1 from A. thaliana expressed as a recombinant protein in E. coli catalyzes both GMPP and GGPP reactions (Habbas-Nimer, 2015; Pusod and Liepman, unpublished observations).
Figure 6. Proposed synthesis of GDP-glucose through GDP-glucose pyrophosphorylase activity of VTC1. Above, little GDP-glucose formation by VTC1 in the absence of KJCl and/or KJC2 and manganese (Mn\textsuperscript{2+}). Below, significant GDP-glucose formation by VTC1 in the presence of KJCl and/or KJC2 and Mn\textsuperscript{2+}.

The purpose of this study was to characterize the biosynthetic pathway of GDP-glucose. Because KJCl and KJC2 enhance the GMPP activity of VTC1 (Sawake et al. 2015), we hypothesized that the synthesis of GDP-glucose by VTC1 may also be enhanced due to interactions of VTC1 with KJCl and/or KJC2 when Mn\textsuperscript{2+} is included in the assay buffer (Figure 6). In this study, recombinant Arabidopsis VTCl, KJC1, and KJC2 were expressed in BL21(DE3) Escherichia coli cells via auto-induction and soluble fractions were purified by three phase partitioning (TPP). High performance liquid
chromatography (HPLC) was used to assess the GMPPase and GGPPase activities of the individual recombinant proteins and enzyme assays were used to determine whether the presence of recombinant KJC1 and/or KJC2 enhance VTC1 GMPPase and GGPPase activity.
Materials and Methods

1. Expression of recombinant proteins (VTC1, KJC1, KJC2, mCherry) in *E. coli* via auto-induction

1.1 Transformation and autoinduction of recombinant VTC1, KJC1, KJC2, and mCherry

Recombinant VTC1 (At2g39770), KJC1 (At1g74910), KJC2 (At2g04650), and the fluorescent mCherry protein (used as a control) were expressed in BL21(DE3) *Escherichia coli* cells, which are capable of *lac*-inducible protein expression. The pET28 vectors (Figure 7) containing the sequences encoding amino-terminal epitope-tagged VTC1 were already constructed and transformed into the *E. coli* cells (Habbas-Nimer, 2015).

pET28 expression vectors carrying the His6- and T7-tagged KJC1 and KJC2 transgenes were transformed using the CaCl2-heat shock technique. 50 µL of competent BL21(DE3) cells were thawed on ice and 5 µL of purified plasmid DNA was added to each and gently mixed. The samples were incubated on ice for 30 minutes, heat shocked at 42°C for 30 seconds, and then placed on ice for 5 minutes. 1 mL of Super Optimal Broth with Catabolite Repression (SOC) medium was added to each tube and they were incubated at 37°C at 250 RPM for 1 hour. 150 µL of the transformation reaction was plated on Luria-Bertani (LB) media plates containing 50 µg/mL kanamycin and incubated at 37°C overnight.
Figure 7. Expression vectors for recombinant protein expression of amino-terminal epitope tagged VTC1. (A) pET28-His6-VTC1, (B) pET28-T7-VTC1. The vectors for His6- and T7-tagged versions of KJC1 and KJC2 are similarly constructed.
Protein expression was induced using the auto-induction media ZYM-5052 (Table 2), a formulation that yields high levels of protein expression (Studier, 2005). Colonies from streaks started from glycerol stocks of BL21(DE3) cells containing pET28-His6-KJC1, pET28-His6-KJC2, pET28-T7-KJC1, pET28-T7-KJC2, pET28-His6-mCherry, and untagged pET28-VTC1 were added to auto-induction media and auto-induced at 25°C and shaken at 250 RPM for approximately 48 hours until a color change in mCherry indicated that auto-induction had occurred. Temperature was subsequently reduced to 10°C for another 24-30 hours.

Table 2. ZYM-5052 auto-induction media (Studier, 2005)

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Final Concentration</th>
<th>Volume Used for 50 mL Auto-induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZY Solution</td>
<td>1% w/v N-Z Amine AS 0.5% w/v yeast extract</td>
<td>47.9 mL</td>
</tr>
<tr>
<td>50X M</td>
<td>25 mM Na2HPO4 25 mM KH2PO4 50 mM NH4Cl 5 mM Na2SO4</td>
<td>1 mL</td>
</tr>
<tr>
<td>50X 5052</td>
<td>0.5% w/v glycerol 0.2% w/v α-lactose 0.05% w/v glucose</td>
<td>1 mL</td>
</tr>
<tr>
<td>1000X metals</td>
<td>0.2X Metals</td>
<td>10 µL</td>
</tr>
<tr>
<td>1 M MgSO4</td>
<td>2 mM MgSO4</td>
<td>100 µL</td>
</tr>
<tr>
<td>50 mg/mL Kanamycin</td>
<td>100 µg/mL kanamycin</td>
<td>100 µL</td>
</tr>
<tr>
<td>Bacterial culture</td>
<td></td>
<td>5 µL</td>
</tr>
</tbody>
</table>
1.2 Analysis of recombinant protein expression following auto-induction

Following auto-induction, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was used to analyze recombinant protein expression. Bacterial cell pellets were resuspended in breaking buffer (Table 3) volumes that corresponded to their OD<sub>600</sub> to achieve a final bacterial cell unit (BCU) per mL of 10 BCU/mL (Table 4). 25 µL of each sample was analyzed on gels made with 12.5% acrylamide in the resolving gel and 4% acrylamide in the stacking gel. Protein gels were stained by incubation in Coomassie fixative solution for 15 minutes followed by Coomassie staining solution for at least 1 hour. Excess stain was removed from protein gels using destain solution until the background of the gel was clear.

Table 3. Breaking buffer formulation

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Final Concentration</th>
<th>Volume Used for 10 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris-HCl pH 7.5</td>
<td>50 mM Tris-HCl pH 7.5</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>1 M NaCl</td>
<td>10 mM NaCl</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>100% (v/v) Glycerol</td>
<td>10% (v/v) Glycerol</td>
<td>1 mL</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td></td>
<td>8.4 mL</td>
</tr>
</tbody>
</table>

Immunoblot analysis was used to detect recombinant proteins. Protein samples were loaded and electrophoresed through SDS-PAGE, and transferred to a PVDF membrane in a stirred transfer chamber containing 25 mM Tris base, 192 mM glycine, 20% (v/v) methanol, and 0.005% (w/v) SDS at 4°C for 15-20 hours at 30-40 V. After 15-20 hours, a 1X-TBS-Tween solution containing 5% (w/v) nonfat dry milk was used to block the PVDF membrane. 10 mL of the blocking solution was used to dilute 2 µL of the anti-His<sub>6</sub>-HRP conjugated monoclonal antibody (MBL) for a final dilution of 1: 5,000 and 3 µL of anti-T7-HRP conjugated antibody (Millipore) was diluted in 15 mL of
blocking solution for a final dilution of 1: 5,000. Membranes were incubated in antibody solution for 1 hour and washed with 1X TBS-Tween four times for 10 minutes each, with vigorous agitation. BIO-RAD clarity ECL Western Blotting Substrates were mixed to make 5 mL of a 1:1 ratio of the reagents. ECL reagent mixture was poured onto membranes prior to imaging. Membranes were imaged using the western blot chemi application on the BIO-RAD ChemiDoc XRS+ imaging system and images were annotated using Adobe Photoshop.

1.3 Lysis and fractionation of samples using glass beads

Following verification of recombinant protein expression, the E. coli cells were lysed and fractionated into crude, soluble, and insoluble fractions. For cell lysis, 3 g of glass beads were weighed into screw-capped 30 mL glass tubes and cooled on ice for 5 minutes. Cell pellets were resuspended in breaking buffer so that they each had a concentration of 10 OD₆₀₀/mL. 3 mL of resuspended cells were transferred into each tube of glass beads and vortexed for 30 seconds, then placed on ice for 1 minute. This was repeated for a total of eight cycles of vortexing and cooling. 200 µL of each crude sample was removed and stored at -20°C.

The remaining crude lysate was transferred into 15 mL Corex centrifuge tubes that were pre-cooled on ice. The 15 mL Corex centrifuge tubes were placed in rubber sleeves, balanced against each other, and placed in the pre-cooled SA-600 rotor in the Sorvall RCSB plus centrifuge. The samples were centrifuged at 17,000 x g at 4°C for 15 minutes. The supernatant following centrifugation was the soluble fraction. A volume of breaking buffer equivalent to that of the supernatant was added to re-suspend the pellets,
to prepare insoluble fractions. Soluble and insoluble fractions were stored at -80°C.

1.4 Lysis of *E. coli* cells using French Press

In preparation for French Press, 150 mL auto-inductions of untagged VTC1, T7-KJC1, and His6-KJC2 were carried out in ZYM-5052 media (Table 2). A 100 mL auto-induction of mCherry was used as a positive control. Following auto-induction, cell pellets were re-suspended in 25 mL of breaking buffer. A fraction of the samples was poured into a pre-cooled French Press apparatus pressure cell and the flow valve was gently tightened. The cell pressure was set at ~10,000 PSI, or a "high" setting of gauge pressure and lysed bacterial cells were collected. Once lysis was complete, samples were fractionated following the same procedure as described in part 1.3.

2. Purification of recombinant proteins

2.1 Purification of recombinant proteins (VTC1 and mCherry) by three phase partitioning (TPP)

Due to difficulty expressing recombinant proteins with N-terminal epitope tags in *E. coli* in this study and others (Pusod & Liepman, unpublished observations), untagged VTC1 was purified for GMPP and GGPP in vitro assays. Soluble fractions of VTC1 and mCherry were saturated with different concentrations of ammonium sulfate (Table 3) in 5 mL micro-centrifuge tubes at 25°C. 2 mL of tert-butanol was added to each tube, and tubes were agitated and then left to stand for 1.5 hours (Jain *et al.* 2004). The samples were centrifuged at 733 x g for 10 minutes and formed a bottom aqueous layer, an interfacial precipitate layer, and an upper organic layer (Figure 8). The interfacial
precipitate layer was removed using a spatula and placed in a separate 5 mL micro-
centrifuge tube where it was dissolved in 2 mL of breaking buffer and stored at -20°C.

**Table 4.** Amount of ammonium sulfate \((\text{NH}_4)_2\text{SO}_4\) needed for each saturation

<table>
<thead>
<tr>
<th>Saturation</th>
<th>Amount of ((\text{NH}_4)_2\text{SO}_4) added to 2 mL of Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>0.11 g</td>
</tr>
<tr>
<td>20%</td>
<td>0.23 g</td>
</tr>
<tr>
<td>30%</td>
<td>0.36 g</td>
</tr>
<tr>
<td>40%</td>
<td>0.49 g</td>
</tr>
<tr>
<td>50%</td>
<td>0.64 g</td>
</tr>
<tr>
<td>60%</td>
<td>0.79 g</td>
</tr>
</tbody>
</table>

**Figure 8.** Separation of phases into upper organic, interfacial precipitate, and aqueous during three phase partitioning.
2.2 Desalting interfacial precipitate for SDS-PAGE analysis using Sephadex G-25

Interfacial precipitate samples from TPP were desalted using Sephadex G-25. 3 mL syringe barrels were used to create the columns. 2 pieces of circular filter (Whatman 3MM) paper were placed at the bottom of each syringe barrel followed by a 1.5 mL mixture of Sephadex G-25 (fine) that had been swollen in breaking buffer. 180 µL of sample was added to the column and pushed through the barrel into a micro-centrifuge tube using the syringe plunger. This was followed with 200 µL of breaking buffer, and the second flow-through was collected into a separate tube. The samples were stored at -20°C.

2.3 Desalting interfacial precipitate for SDS-PAGE analysis using dialysis

Interfacial precipitate was also desalted using 0.5 mL Thermo Scientific Slide-A-Lyzer dialysis cassettes in order to compare their effectiveness to the Sephadex G-25 column. Each cassette was hydrated in breaking buffer for 2 minutes before adding sample. 350 µL of sample was added to each cassette and placed upright in a beaker containing breaking buffer and a stir bar. The samples were dialyzed for 15 hours at 4°C. Samples were removed using 3 mL syringes. 40 µL of sample was stored in micro-centrifuge tubes at -20°C to be used for SDS-PAGE analysis and the remainder was stored at -80°C.

3. GMPP and GGPP in vitro assays

3.1 GMPP and GGPP time course assays

50 µL assays were conducted in micro-centrifuge tubes containing reagents
required for the pyrophosphorylase reaction (Table 5). In order to establish the linearity
of the GMPPase and GGPPase activity of VTCl, time course assays were conducted.

GMPPase assay samples were incubated at 37°C for 0 minutes, 5 minutes, or 10 minutes.
GGPPase assay samples were incubated at 37°C for 0 minutes, 10 minutes, 20 minutes,
or 30 minutes. Following incubation, samples were boiled at 95°C for 2 minutes to stop
the reactions. Samples were then centrifuged at 12,000 RPM for 5 minutes and the
supernatant was stored at -20°C.

Table 5. Pyrophosphorylase assay formulation

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Final Concentration</th>
<th>Volume Used for 50 µL Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris-HCl pH 7.5</td>
<td>50 mM Tris-HCl pH 7.5</td>
<td>2 µL</td>
</tr>
<tr>
<td>50 mM MgCl₂</td>
<td>4 mM MgCl₂</td>
<td>3.2 µL</td>
</tr>
<tr>
<td>50 mM MnCl₂</td>
<td>8 mM MnCl₂</td>
<td>6.4 µL</td>
</tr>
<tr>
<td>10 mM GTP</td>
<td>1 mM GTP</td>
<td>4 µL</td>
</tr>
<tr>
<td>10 mM DTT</td>
<td>1 mM DTT</td>
<td>4 µL</td>
</tr>
<tr>
<td>10 mM mannose-1-phosphate Or</td>
<td>1 mM mannose-1-phosphate</td>
<td>4 µL</td>
</tr>
<tr>
<td>10 mM glucose-1-phosphate</td>
<td>1 mM glucose-1-phosphate</td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td></td>
<td>16.4 µL</td>
</tr>
<tr>
<td>Crude soluble recombinant protein (mCherry or VTCl)</td>
<td></td>
<td>10 µL</td>
</tr>
</tbody>
</table>

3.2 Assay product purification

NDP-sugars from GMPP and GPPP assays were purified using Envi-Carb
columns as described in Rabina et al. (2001). To prepare Envi-carb powder, ~1 g of Envi-
Carb powder was washed with 80% (v/v) acetonitrile (ACN) /0.1% (v/v) trifluoroacetic
acid (TFA) and vortexed for 10 seconds. Once the Envi-Carb settled, the ACN/TFA
solution was aspirated. Envi-Carb was then rinsed with 20 mL H₂O and vortexed for 10
seconds. Once the Envi-Carb settled, the H₂O was aspirated, and another H₂O wash was
done. Envi-Carb was resuspended at a final concentration of ~100 mg/mL in H₂O.

Three mL syringe barrels with 2 pieces of circular filter (Whatman 3MM) paper placed at the bottom of each were used to create the columns. 1 mL of the 100 mg/mL Envi-Carb suspension was added to each column, which were then centrifuged at 2,000 RPM for 5 minutes. Following centrifugation, 3 circular filter papers were added to the columns above the Envi-Carb.

Ten mM ammonium bicarbonate (NH₄HCO₃) was added to each assay product to bring it to 1 mL. Assay products were added to Envi-Carb columns and sequentially washed with 4 mL H₂O, 4 mL 25% ACN, and 4 mL 50 mM triethylammonium acetate (TEAA). Purified NDP-sugars were eluted with a final wash of 1 mL 25% ACN/50 mM TEAA. The samples were frozen at 80°C and lyophilized to dryness.

3.3 HPLC analysis of NDP-sugars

High Performance Liquid Chromatography (HPLC) analysis using the Thermo Scientific DIONEX ICS-5000 SP was used to measure GDP-mannose and GDP-glucose production.

The solvents used for HPLC analysis were 20 mM TEAA (solvent A) and 20 mM TEAA/4% ACN (solvent B). The Rabina et al. (2001) HPLC parameters were followed so that after sample injection the HPLC column was washed with solvent A for 15 minutes, 25% solvent A / 75% solvent B for 20 minutes, and 100% solvent B for 7 minutes. 0.2 mM of a GDP-mannose standard and 0.4 mM of a GDP-glucose standard in TEAA were analyzed before and after the samples in order to measure their elution times.
Elution times of NDP-sugars and peak area integrations were recorded on the Thermo Fisher Scientific Chromeleon software.

4. Testing the effects of KJC proteins on GMPPase and GGPPase activity of VTC1

Recombinant T7-KJC1 and His6-KJC2 were added to GMPP and GGPP assays with recombinant VTC1 to analyze whether or not they enhanced its GMPPase and/or GGPPase activity. Assay mixtures were made according to Table 5 except 5 µL of either crude soluble T7-KJC1 or crude soluble His6-KJC2 was added to each micro-centrifuge tube in place of 5 µL of H2O. Assay mixtures were incubated at 37°C for 15 minutes and boiled at 95°C for 2 minutes. The mixtures were then centrifuged and stored as described in 3.1. Assay products were purified as described in 3.2 and NDP-sugars were analyzed as described in 3.3.
Results

1. Optimization of expression of recombinant proteins

Expression of recombinant His6-tagged and T7-tagged KJC1 and KJC2 and His6-tagged mCherry induced using the auto-induction media ZYM-5052 yielded low levels of expression for His6-tagged proteins (Figure 10), so it was hypothesized that His6-tagged proteins required additional time to fold properly.

The 25°C auto-induction yielded expression of soluble and insoluble T7-KJC1 at the expected molecular weight (MW) (Figure 9; Table 6). Low levels of insoluble T7-KJC2 were also detected following the 25°C auto-induction at the expected MW (Figure 9; Table 6). Because the 25°C auto-inductions yielded low levels of His6-tagged KJC1 and KJC2, the proteins were not detected on the immunoblot membrane (Figure 10). Lowering the temperature of auto-induction to 10°C in order to slow the rate of protein production yielded higher expression of His6-KJC2 at the expected MW (Table 6) compared to auto-induction at a constant temperature of 25°C (Figure 10).
Table 6. Expected molecular weight (MW) of proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Expected MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCherry</td>
<td>29 kDa</td>
</tr>
<tr>
<td>His6-KJC1</td>
<td>46.5 kDa</td>
</tr>
<tr>
<td>T7-KJC1</td>
<td>46.5 kDa</td>
</tr>
<tr>
<td>His6-KJC2</td>
<td>46.5 kDa</td>
</tr>
<tr>
<td>T7-KJC2</td>
<td>46.5 kDa</td>
</tr>
<tr>
<td>VTC1</td>
<td>40 kDa</td>
</tr>
</tbody>
</table>

Figure 9. Immunoblot analysis of recombinant, T7-tagged crude, soluble, and insoluble KJC1 and KJC2 expressed in *E. coli* from days 2 and 3 of 25°C auto-inductions. The sizes of MW standards (in kDa) are noted to the left of the image.
Figure 10. Left, immunoblot analysis of recombinant, His6-tagged mCherry and KJC2 proteins from 10°C auto-induction expressed in E. coli. from days 1, 2, and 3 of the auto-induction as well as from the crude, soluble, and insoluble fractions. Right, immunoblot analysis of recombinant, His6-tagged mCherry and KJC2 proteins from 25°C auto-induction, expressed in E. coli. from days 1, 2, and 3 of auto-induction. The sizes of MW standards (in kDa) are noted.

2. Purification of recombinant proteins by three phase partitioning (TPP)

Protein purification was used to isolate recombinant VTC1 and mCherry from E. coli to reduce contaminating E. coli proteins. SDS-PAGE analysis of purified recombinant mCherry and VTC1 showed that recombinant VTC1 precipitated at saturations of 10%-50% (Figure 11 and Figure 12).
SDS-PAGE analysis showed that desalting through the dialysis method provided a clearer image of what proteins were expressed, while desalting using the Sephadex G-25 column provided a blurrier image and protein expression was less evident (Figure 12).

Figure 11. SDS-PAGE analysis of recombinant VTC1 (~40 kDa) from 10%, 20%, 30%, 40%, 50%, and 60% ammonium sulfate saturation and mCherry (~29 kDa) from 60% ammonium sulfate saturation. The samples were desalted via dialysis. The sizes of molecular weight standards (in kilodaltons) are indicated to the left of the gel image. Gel stained using Coomassie brilliant blue R250.
Figure 12. SDS-PAGE analysis of recombinant VTC1 (40 kDa) from 10%, 20%, 30%, 40%, 50%, and 60% ammonium sulfate saturation and mCherry (29 kDa) from 60% ammonium sulfate saturation. The samples were desalted using a sephadex G-25 buffer column. The sizes of molecular weight standards (in kilodaltons) are indicated to the left of the gel image. Gel stained using Coomassie brilliant blue R250.

3. HPLC analysis of GDP-mannose and GDP-glucose standards

High Performance Liquid Chromatography (HPLC analysis) effectively separated GDP-mannose and GDP-glucose. HPLC analysis showed that the GDP-mannose standard eluted at 28.649 minutes and the GDP-glucose standard eluted at 31.166 minutes (Figure 13); these elution times were highly reproducible.
4. GMPP and GGPP in vitro time course assays

Time course assays were conducted to establish the linearity of the GMPPase and GGPPase activity of VTC1. HPLC analysis showed that the GMPPase activity of VTC1 increased for 10 minutes but began to plateau after 5 minutes (Figure 14). The GGPPase activity of VTC1 increased steadily for 30 minutes (Figure 14).
Figure 13. HPLC analysis of NDP-sugar standards. Above, GDP-mannose eluted at 28.649 minutes. Below, GDP-glucose eluted at 31.166 minutes.
5. Effect of KJC proteins on GMPPase and GGPPase activity of VTC1

To determine the effect of KJC proteins on pyrophosphorylase activities of VTC1, a series of in vitro assay was used. HPLC analysis showed that the assays containing only KJCI or only KJC2 resulted in no peaks at the elution times for GDP-mannose and GDP-glucose (Figure A1), which was expected because they are not known to have enzymatic activity (Sawake et al. 2015). Similarly, the assay containing mCherry did not result in any peaks at the elution times for GDP-mannose and GDP-glucose (Figure 15; Figure 16), which verified that crude soluble protein fractions of E. coli did not possess GMPP or GGPP activity.

HPLC analysis showed the area of the GDP-mannose peak produced by VTC1, which was unexpectedly higher than the area of the peak produced by VTC1 when KJCI or KJC2 were included in the assay (Table 7).
HPLC analysis showed the peak area for GDP-glucose produced by VTC1 alone was lower than the peak area for GDP-glucose produced by VTC1 when KJC1 or KJC2 were included in the assay (Table 8). However, HPLC analysis of the assay including mCherry, which was used as a negative control, showed a GDP-glucose peak that was greater than the others (Table 8). Replicates of the assays will need to be conducted to conclude whether or not the KJC proteins enhance the GGPPase activity of VTC1.

**Table 7.** Area of GDP-mannose peaks produced by VTC1 on its own and with other proteins included in the assay

<table>
<thead>
<tr>
<th>Protein(s) included in GMPPase assay</th>
<th>Peak area (mAU x minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTC1</td>
<td>25.2776</td>
</tr>
<tr>
<td>VTC1+KJC1</td>
<td>16.2314</td>
</tr>
<tr>
<td>VTC1+KJC2</td>
<td>11.4518</td>
</tr>
<tr>
<td>VTC1+mCherry</td>
<td>13.2590</td>
</tr>
</tbody>
</table>

**Table 8.** Area of GDP-glucose peaks produced by VTC1 on its own and with other proteins included in the assay

<table>
<thead>
<tr>
<th>Protein(s) included in GGPPase assay</th>
<th>Peak area (mAU x minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTC1</td>
<td>3.6004</td>
</tr>
<tr>
<td>VTC1+KJC1</td>
<td>6.9856</td>
</tr>
<tr>
<td>VTC1+KJC2</td>
<td>5.7004</td>
</tr>
<tr>
<td>VTC1+mCherry</td>
<td>9.9278</td>
</tr>
</tbody>
</table>
Figure 15. HPLC analysis of GMPPase activity of recombinant proteins. (A) mCherry, (B) VTC1.
Figure 16. HPLC analysis of GGPPase activity of recombinant proteins. (A) mCherry, (B) VTCl.
Figure 17. HPLC analysis of (A) GMPPase activity of VTC1 with KJC1 included in assay, (B) GGPPase activity of VTC1 with KJC1 included in assay.
Discussion

In this study, we hypothesized that KJC1 and KJC2 would enhance the GGPPase activity of VTCl, because: 1) the KJC proteins stimulate the GMPPase activity of VTCl (Sawake et al. 2015), and 2) glucose 1-phosphate is structurally similar to mannose 1-phosphate (Figure 5). While the GGPPase activity of VTCl was shown using GGPP in vitro assays (Figure 16 and Pusod & Liepman, unpublished observations), the effect of the KJC proteins on this activity remains unclear. Although preliminary HPLC analysis showed that GDP-glucose production increased when either KJC1 or KJC2 was added to the GGPPase assay (Figure 17; Figure A2), there was a similar increase in GDP-glucose production following the addition of the mCherry protein to the assay (Figure A3). Because crude soluble VTCl was used in the assays, we suspect that proteins found in E. coli may have interfered with the pyrophosphorylase activity of VTCl. Replicate assays are needed to show whether KJC1 and KJC2 have a significant effect on the GGPPase activity of VTCl. Similarly, GMPP in vitro assays provided unclear results on the effect of the KJC proteins on the GMPPase activity of VTCl (Figure 17; Figure A2) and additional assays will need to be conducted to replicate the results of Sawake et al. (2015).

Expression of amino-terminal epitope tagged KJC proteins

While Sawake et al. (2015) used N-terminal epitope tagged proteins to characterize interactions between VTCl and KJC proteins, we experienced significant difficulties expressing adequate quantities of such tagged proteins. A commonly used temperature for inducing recombinant protein production in bacteria is 37°C (Studier,
2005), but we reduced the auto-induction temperature for production of His6-KJC1 and His6-KJC2 to 25°C to enhance production of these proteins. However, even at 25°C, His-tagged proteins were expressed at levels too low to detect using immunoblot (Figure 10). Sawake et al. (2015) reported inducing protein production at 10°C, which implied that they may also have experienced difficulty expressing the recombinant N-terminal epitope tagged KJC proteins. In order to enhance protein folding conditions, the auto-induction temperature was reduced to 10°C. Following this auto-induction, His6-KJC2 was still expressed at low levels, but was detected using immunoblot (Figure 10). We also experienced difficulty expressing T7-KJC2 at 25°C but were able to achieve adequate expression of crude soluble T7-KJC1 (Figure 9) to be included in GMPP and GGPP in vitro assays. We speculate that the low ratio of N-terminal epitope tagged KJC1 and KJC2 protein to the amount of untagged VTC1 included in assays contributed to the unclear results. Earlier studies had used a 1:1 ratio of VTC1: KJC protein, which may explain why the effect of KJC1 and KJC2 on the GMPPase activity of VTC1 was more evident (Sawake et al. 2015).

TPP purification of VTC1

Because preliminary data showed that N-terminal epitope tagged VTC1 was difficult to express, we attempted to purify untagged VTC1, which is produced at much higher levels (Pusod & Liepman, unpublished observations). Untagged recombinant VTC1 and mCherry were purified using three phase partitioning (TPP) to reduce unwanted proteins found in E. coli. TPP has been shown to be a simple and efficient method of purification that resulted in high yields of protein recovery (Jain et al. 2004;
Sen et al. 2011; Venal and Rathod, 2015). For example, recombinant green fluorescent protein, α-galactosidase, and peroxidase were all successfully purified via TPP (Jain et al. 2004; Sen et al. 2011; Venal and Rathod, 2015). However, TPP did not appear to be an effective method for purifying VTC1, because VTC1 precipitated at a wide range of ammonium sulfate concentrations with many other contaminant proteins (Figure 11 and Figure 12). Due to the abundance of protein that was expressed and because TPP purified protein was no purer that the crude soluble protein, we used crude soluble VTC1 for the GMPP and GGPP in vitro assays. However, the use of crude soluble protein meant that enzymes found in *E. coli* may have also reacted with GTP, mannose 1-phosphate, or glucose 1-phosphate, which may have interfered with our ability to accurately measure the pyrophosphorylase activity of VTC1.

**Future Directions**

Future studies on the effect of KJC1 and KJC2 on the GGPPase activity of VTC1 should focus on replicating in vitro GGPP assays in order to determine whether the KJC proteins have a significant effect on this activity of VTC1. This study and others found that N-terminal epitope tagged VTC1 was produced at much lower levels than the untagged VTC1 protein (Pusod & Liepman, unpublished observations), suggesting that the N-terminal tag on the KJC proteins may have decreased their expression. Therefore, we predict that the use of a different N-terminal tag or a C-terminal tag may improve the expression of recombinant VTC1 and KJC proteins in *E. coli*. In fact, another report describes the crystallization of C-terminal His6-tagged VTC1 (Zhao and Liu, 2016).
Finally, it is unclear why earlier studies reported insignificant GGPPase activity of VTC1 (Sawake et al. 2015). We speculate that we may have used more VTC1 protein for assays than earlier studies, which reported the use of a 1:1 ratio of VTC1: KJC protein (Sawake et al. 2015). Our results indicate that the GMPPase activity of VTC1 was approximately eight times higher than its GGPPase activity (Table 7; Table 8), so if previous studies had used low amounts of protein they may not have been able to detect much GGPPase activity. Additionally, we are unsure if Sawake et al. (2015) included Mn$^{2+}$ in their assay buffer. A prior study noted that adding Mn$^{2+}$ to the GGPP assay buffer stimulated the GGPPase activity of purified plant protein (Péaud-Lenoèl and Axelos, 1968). It is not clear whether the purified pea protein characterized by Péaud-Lenoèl and Axelos (1968) was a pea ortholog of VTC1 or another type of protein. Future studies aimed to determine whether the GGPPase activity of VTC1 is stimulated by Mn$^{2+}$ are needed.

It is important to continue studying the biosynthetic pathways of GDP-mannose and GDP-glucose, because they are substrates used by CELLULOSE SYNTHASE-LIKE A (CSLA) proteins to synthesize glucomannan, a plant cell wall polysaccharide (Liepman et al. 2005). In the plant A. thaliana, VTC1 is the major pyrophosphorylase involved in GDP-mannose synthesis and appears to play a role in GDP-glucose synthesis as well. The KJC proteins, which are homologs of VTC1, may enhance this pyrophosphorylase activity and in doing so, improve production of glucomannan. Glucomannans are extensively used in a variety of industries due to their ability to form gels (Zhang et al. 2005), so understanding the biosynthetic pathway of GDP-mannose and GDP-glucose could improve production of glucomannan for industrial use.
References


Figure A1. HPLC analysis of (A) GMPPase activity KJC1, (B) GGPPase activity of KJC1, (C) GMPPase activity of KJC2, (D) GGPPase activity of KJC2.
Figure A2. HPLC analysis of (A) GMPPase activity of VTc1 with KJC2 included in assay, (B) GGPPase activity of VTc1 with KJC2 included in assay.
Figure A3. HPLC analysis of (A) GMPPase activity of VTC1 with mCherry included in assay, (B) GGPPase activity of VTC1 with mCherry included in assay.