Drug target identification and virtual screening in pursuit of phytochemical intervention of 

*Mycobacterium chelonae*

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Abstract

*Mycobacterium chelonae* is a rapidly growing mycobacterium present in the environment. It is associated with skin and soft tissue infections including abscess, cellulitis and osteomyelitis. Other infections by this bacterium are post-operative/transplant-associated, catheter, prostheses and even concomitant to haemodialytic procedures. In this study, we employ a subtractive genomics approach to predict the potential therapeutic candidates, intended for experimental research against this bacterium. A computational workflow was devised and executed to procure core proteome targets essential to the pathogen but with no similarity to the human host. Initially, essential *Mycobacterium chelonae* proteins were predicted through homology searching of core proteome content from 19 different bacteria. Druggable proteins were then identified and N-acetylglucosamine-1-phosphate uridyltransferase (GlmU) was chosen as a case study from identified therapeutic targets, based on its important bifunctional role. Structure modeling was followed by virtual screening of phytochemical library (N > 2200), from 500 medicinal plants, against it. A biflavonoid daphnodorin G from *Daphne odora* was screened as having best potential for binding GlmU. Phytotherapy helps curb the menace of antibiotic resistance so treatment of *Mycobacterium chelonae* infection through this method is recommended.

**Key words**

*Mycobacterium chelonae*, drug design, phytotherapy, GlmU, virtual screening.
1. Introduction

Mycobacteria species are categorized into two major groups, tubercular and non-tubercular mycobacteria. Nontuberculous mycobacteria are further divided into two groups, rapidly growing and slow growing mycobacteria, depending upon duration of their reproduction in suitable medium (Tortoli, 2014). *Mycobacterium chelonae* is a member of rapidly growing group, which takes less than a week for its reproduction in/on medium. It is the most commonly isolated organism among all rapidly growing mycobacteria. It is mostly found in water sources and on medical instruments such as bronchoscopes (Gonzalez-Santiago and Drage, 2015), and has been isolated from environmental, animal and human sources. *Mycobacterium chelonae* infections in human hosts have increased over time, with reports of both haematogenous and localized occurrences in the recent past (Hay, 2009).

Outbreaks due to *Mycobacterium chelonae* contaminated water and compromised injections are a rising problem. Infections have been linked to cosmetic and surgical procedures, such as trauma, surgery, injection (botulinum toxin, biologics, dermal fillers), liposuction, breast augmentation, under skin flaps, laser resurfacing, skin biopsy, tattoos, acupuncture, body piercing, pedicures, mesotherapy and contaminated foot bath (Kennedy et al., 2012; Gonzalez-Santiago and Drage, 2015). *Mycobacterium chelonae* may also colonize skin wounds, as result of which patients form abscess, skin nodules and sinus tracts (Patnaik et al., 2013).

Recent era has observed a trend for search of drug targets in pathogens using computational methods, with a focus on genomic and proteomic data (Shanmugham and Pan, 2013). Comparative/differential and subtractive genomics along with proteomics has been used by many researchers for the identification of drug targets in various pathogenic bacteria like *Pseudomonas aeruginosa* (Sakharkar et al., 2004), *Helicobacter pylori* (Dutta et al., 2006), *Campylobacter fetus* (Moolhuijzen et al., 2009), *Brugia malayi* (Kumar et al., 2007), *Leptospira interrogans* (Amineni et al., 2010), *Listeria monocytogenes* (Sarangi et al., 2015), *Mycobacterium leprae* (Shanmugam and Natarajan, 2013) etc. Determination of potential drug targets has been made possible by the availability of whole genome and their inferred protein complement sequences in public domain databases (Sarangi et al., 2015).

Parenteral antibiotics against *Mycobacterium chelonae* include tobramycin, amikacin, imipenem, and tigecycline, but it has demonstrated resistance to antibiotics and disinfectants (Brown-Elliott
et al., 2012; Jaén-Luchoro et al., 2016). This property assists *Mycobacterium chelonae* in colonizing water systems and allows its access to humans (Jaén-Luchoro et al., 2016). Till now, no specific guidelines for the treatment of *Mycobacterium chelonae* have been defined in the literature (Gonzalez-Santiago and Drage, 2015). This clearly illustrates the need to search out drug targets in the *Mycobacterium chelonae* for design of better therapies against infection by this bacterium, especially using naturally existing metabolites from microbes and plants. In the current study, subtractive proteomics was applied to identify essential druggable proteins in *Mycobacterium chelonae*. Docking of the selected protein GlnU with phytochemicals was carried out for identification of a candidate which might bind it and stop its normal cellular function, leading to bacterial lysis/death.

2. Material and methods

2.1. Prediction of *Mycobacterium chelonae* essential proteome

Complete proteome sequence of *Mycobacterium chelonae* CCUG 47445 with accession no: [NZ_CP007220](https://www.ncbi.nlm.nih.gov/) was downloaded from the NCBI database. For the prediction of essential proteins, Geptop (Wei et al., 2015) was installed on computer and a search was carried out to align the *Mycobacterium chelonae* protein sequences against the essential or core protein sequences from defined set of 19 bacteria with an essentiality score cut-off value range of 0.15 (Wei et al., 2015). Results were saved and analyzed further.

2.2. Prediction of non-homologous host proteins

In order to find the bacterial proteins which do not have similarity with human host, the set of essential protein sequences of *Mycobacterium chelonae* was subjected to BLASTP against the human proteome database (Uniprot release 2014). The standalone BLAST software (Altschul et al., 1990) was used for this purpose. For identification of non-homologous proteins, an expectation (E-value) cut-off of $10^{-2}$, gap penalty of 11 and gap extension penalty of 1 was set as the standard. E-value cut-off ($10^{-2}$), based on reported research protocols (Perumal et al., 2007; Sarangi et al., 2015) was considered.

2.3. Identification of putative drug targets

There are several molecular and structural properties which have been explored by researchers for selecting suitable therapeutic targets in pathogenic microorganisms. These properties include determination of molecular weight, sub-cellular localization, 3D structure and druggability analysis (Hassan et al., 2015; Silverio-Machado et al., 2014; Uddin et al., 2015). These properties
were evaluated for selection of the therapeutic targets in *Mycobacterium chelonae*. Molecular weight was calculated by using computational tools and drug target-associated literature available in the Swiss-Prot database (Boeckmann *et al*., 2003). Subcellular localization of therapeutic targets was predicted using PSORTb (Nancy *et al*., 2010). It uses feature support vector machine-based method and suffix tree algorithm for downstream analysis. Predictions are grouped through a Bayesian scheme into one final (consensus) result. Druggable targets were identified with BLAST hits through unified protocol from the DrugBank. Parameters were: gap cost: -1 in case of extension or opening, mismatch penalty: -3, E-value: $1 \times 10^{-5}$, match: 1, filter algorithm: DUST and SEG (Azam and Shamim, 2014). The targets were subjected to KEGG blast for identification of associated pathways.

2.4. **Virtual screening of ligand against selected target**

Keeping in view the results of subcellular localization, molecular weight determination and druggability analysis, an essential protein (GlmU) was chosen for further downstream processing. Swiss Model was used for the prediction of 3D structure of the selected target protein (Biasini *et al*., 2014). This tool constructs structure model by recognizing structural templates from the PDB using multiple threading alignment approaches (Wang *et al*., 2016). The top structure used for structure prediction was that of GlmU from *Mycobacterium tuberculosis* (PDB ID: 3D8V). The structure was validated and analyzed for quality using SAVES (https://services.mbi.ucla.edu/SAVES/), consisting of ERRAT, VERIFY3D and Ramachandran plot analysis.

A phytochemical library consisting of 2266 phytochemical compounds was then docked with GlmU (Ashfaq *et al*., 2013; Mumtaz *et al*., 2016). Docking was carried out using Molecular Operating Environment (MOE) with the parameters: placement: triangle matcher, rescoring 1: London dG, refinement: forcefield, rescoring 2: affinity dG. MOE provides fast and accurate docking results based on dedicated algorithms and accurate scoring functions (Halim *et al*., 2015). Structural preparation program embedded in MOE added the missing hydrogen atoms, corrected the charges and assigned near precise hybridization state to each residue (Junaid *et al*., 2016).

3. **Results and Discussion**

3.1. **Essential proteome prediction**

Initially, total proteome of *Mycobacterium chelonae* was subjected to core or essential proteins prediction. Geptop identified these proteins by screening against 19 bacteria (Fig. 1), based upon
orthology and phylogeny features. In the process of essential proteome mining through homology-based methods, a query protein is considered as essential if it is also present in another bacterium and experimentally identified as essential for survival. There are various methods for the prediction of essential proteins, for example single-gene knockout, transposon mutagenesis and RNA interference but all these methods are time consuming and laborious. A good alternative is high-efficiency computational methods designed specifically for this type of work (Cheng et al., 2013; Wei et al., 2013). Predicted essential proteins were 305 in number (Supplementary File 1), linked with significant metabolic pathways in the pathogen life cycle and necessary for its survival. In order to disrupt the function and existence of pathogen it is most important to attack those bacterial proteins which regulate important functions (e.g. nutrient uptake) in the host environment (Butt et al., 2012). Latest antimicrobial drugs are designed on the principle of the inhibition of the pathogen’s metabolic pathway (Lemaitre and Girardin, 2013; Uddin et al., 2015). Therefore, such protein sequences may be considered as possible therapeutic targets.

3.2. Identification of non-host proteins

Non-host proteins refer to those bacterial proteins which do not have homology with human proteins. If the homologous proteins are targeted, they could badly affect the metabolism of host due to similarity with host proteins. Therefore, non-host proteins could be preferred better drug targets, as side effects and cross-reactivity caused by the use of antibiotics could be evaded for harming host (Azam and Shamim, 2014; Sarangi et al., 2015). Among the core proteome of Mycobacterium chelonae, 117 proteins (Supplementary File 2) indicated ‘no hit’ against the human proteome according to the set criteria. These proteins were then used for subsequent analysis.

3.3. Drug Target mining and analysis

BLASTp was performed to identify significant drug target from newly selected essential proteins. Only 17 proteins had significant hits against druggable proteins present in the DrugBank (Table 1).

Molecular weight determination and druggability analysis could improve the screening process for therapeutic targets, as observed previously for numerous pathogenic bacteria and fungi (Abadio et al., 2011). The molecular weight for each potential drug target was calculated (Table 2) and based upon previous studies, it is suggested that smaller proteins are readily soluble and easier to purify (Duffield et al., 2010).
Sub-cellular localization is a critical factor as it helps in accessing the target gene. Cellular functions are compartment specific, so if the location of unknown protein is predicted then its function could also be known which help in selection of proteins for further study. Membrane proteins are reported as more useful target and more than 60% of the currently known drug targets are membrane proteins (Arinaminpathy et al., 2009; Tsirigos et al., 2015). Cytoplasm is the site of proteins synthesis and most of these proteins remain there to carry out their specific functions after synthesis. However, some proteins need to be transported to different cellular compartments for their specific function (Strzyz, 2016). The subcellular localization of non-host proteins of *Mycobacterium chelonae* was predicted and majority were demarcated as cytoplasmic (Table 1).

### 3.4. GlmU analysis and phytochemical screening
After the characterization of all druggable proteins of *Mycobacterium chelonae*, GlmU was selected for further analysis (out of 305 essential and 117 non-homologous proteins). It is a bifunctional enzyme, exhibiting both acetyltransferase and uridylytransferase activities (Moraes et al., 2015; Sharma et al., 2016).

GlmU has been analyzed for various bacterial species (Patin et al., 2015; Rani et al., 2015), including *Escherichia coli* (Brown et al., 1999), *Streptococcus pneumonia* (Kostrewa et al., 2001), *Haemophilus influenzae* (Mochalkin et al., 2008) and *Mycobacterium tuberculosis* (Zhang et al., 2009). Predicted structure of GlmU estimated RMSD of 0.5 Å. Structure was found to be a homotrimer. Ramachandran plot showed 97.2% residues in favored regions (90.4% in core, 9.4% in allowed, 0.3% in generously allowed) and no residue in disallowed region. According to VERIFY3D program, at least 80% of the amino acids should have value >= 0.2 in the 3D/1D profile and 96.55% of predicted GlmU residues had an averaged 3D-1D score >= 0.2, thus passing the quality check. An ERRAT score of 90.66 was obtained.

Each monomer of GlmU consists of two domains: N and C-terminal domains. N-terminal domain has α/β like fold, similar to dinucleotide-binding Rossmann fold topology. C-terminal domain exhibits a regular left-handed β-helix conformation and a long α-helical arm connecting both domains (Sharma et al., 2016). N-terminal domain is essential for uridylytransferase activity as it catalyses the transfer of uridine monophosphate from uridine-triphosphate to N-acetylglucosamine-1-phosphate (GlcNAc1P). C-terminal domain has acetyltransferase activity as it catalyzes the transfer of an acetyl group from acetyl-CoA coenzyme to GlcN1P, in order to produce GlcNAc1P (Moraes et al., 2015).
GlmU plays fundamental role in the formation of bacterial cell wall by carrying out catalysis of uridine-diphospho-N-acetylglucosamine, an important precursor in bacterial peptidoglycan cell wall (Sharma et al., 2016). Activity of acetyltransferase of eukaryotic cells differ from the activity of GlmU in the way that, eukaryotic acetyltransferase occurs on GlcN6P and not on GlcN1P. These properties make GlmU suitable as the drug target (Moraes et al., 2015) and it has been used for drug targeting in bacteria such as Haemophilus influenza (Mochalkin et al., 2007) apart from designing inhibitors against it for Mycobacterial species (Li et al., 2011; Tran et al., 2013; Rani and Khan, 2015; Mehra et al., 2016). It is also known that proteins that are involved in more than one pathway of pathogen, in addition to that they are non-host proteins, could be more effective drug targets (Sarangi et al., 2015). Inactivation of bifunctional GlmU enzyme leads to loss of mycobacterial viability (Zhang et al., 2008; Rani and Khan, 2015), therefore GlmU was used for docking against phytochemicals. The possible interaction between protein and the ligand is understood computationally through molecular docking. Docking results of GlmU with compounds from 500 medicinal plants (a phytocchemical library of 2266 compounds (Mumtaz et al., 2016), revealed that daphnodorin G was the top scoring compound showing affinity for GlmU (Fig. 3; Table 2). It has molecular weight of 558.495 g/mol and is a metabolite of the plant Daphne odora (Taniguchi and Baba, 1996).

Receptor centric docking approach was employed for screening of prospective phytocchemical library of compounds from more than 500 medicinal plants against GlmU of Mycobacterium chelonae. We focused on phytocchemical screening against Mycobacterium chelonae GlmU because plant derived/natural compounds could be used as antibacterial therapeutics for treatment of bacterial infections (Aparna et al., 2014). A comparative analysis of structural shape and chemical complementarity to GlmU was ranked, based on S value in MOE and the one with least score i.e. Daphnodorin G was obtained as best inhibitor. Analogues of Daphnodorin have been reported previously to show antibacterial and nematicidal activities (Zhuo et al., 2015). Huang et al. (2010) reported Daphnodorin analogs as inhibitor of respiratory syncytial virus while Hu et al. (2000) reported its analog as moderately active against HIV-1. Further Lab testing is proposed to know about minimum inhibitory concentration value and other parameters for Daphnodorin G against Mycobacterium chelonae.
4. Conclusion
In this study, we proposed GlmU as one of the important therapeutic drug targets for *Mycobacterium chelonae* as it is bifunctional, essential protein for pathogen and has no homology with human proteome. We have provided putative model for phytotherapy against *Mycobacterium chelonae* through virtual screening-based identification of potent metabolite from a database of more than 2000 compounds. This study could be taken as an initiative for screening and quick designing of phytotherapy against microbes using a computational *modus operandi*. It is expected that our study will also facilitate selection and screening of other *Mycobacterium chelonae* therapeutic target proteins against phytochemical and other relevant compounds for Lab testing and successful entry into drug design pipeline.

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Declaration of interest
The authors declare that they have no conflict of interest.

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Figure/Table legends

Fig. 1. Workflow describing subtractive proteomics with respective number of obtained sequences. *In silico* parameter analysis included hydrophobicity, sub-cellular location prediction, molecular weight etc.

Fig. 2. (A) Docked GlmU with daphnodorin G (3D conformation) and (B) 2D conformation of docked complex showing residue interactions.

Table 1. Predicted druggable proteome of *Mycobacterium chelonae*. GRAVY is average hydrophobicity of the protein measured by Kyte-Doolittle algorithm. Hydrophobicity value below 0 are indicates globularity (means protein is hydrophilic), while score value of above 0 indicates protein to be membranous (hydrophobic).

Table 2. Top 5 compounds obtained with best inhibition score against GlmU of *Mycobacterium chelonae*.

Supplementary File 1. List of bacterial species used for essential proteome prediction of *Mycobacterium chelonae*.

Supplementary File 2. Essential proteome of *Mycobacterium chelonae*.

Supplementary File 3. Non-homologous core proteome of *Mycobacterium chelonae*. 
Complete proteome of *Mycobacterium chelonae* obtained from NCBI

(4762 proteins)

Prediction of essential genes

(305 proteins)

Non-homologous proteins to host

(117 proteins)

Druggable proteins

(17 proteins)

In silico parameter analysis

Virtual screening via docking

Fig. 1.
Fig. 2.
Table 1. Predicted druggable proteome of *Mycobacterium chelonae*. GRAVY is average hydrophobicity of the protein measured by Kyte-Doolittle algorithm. Hydrophobicity value below 0 are indicates globularity (means protein is hydrophilic), while score value of above 0 indicates protein to be membranous (hydrophobic).

| Serial No. | Accession No. | Protein name | No. of Matches | Mol. Wt. | Length (amino acids) | GRAVY | Subcellular localization | KEGG Pathway | Experimental evidence for druggability in bacteria |
|------------|---------------|--------------|----------------|----------|----------------------|-------|--------------------------|--------------|-----------------------------------------------|
| 1          | WP_030093515 | 6,7-dimethyl-8-ribityllumazine synthase/riboflavin synthase | 1              | 1677.0   | 0.173                | Unknown | Riboflavin metabolism, biosynthesis of secondary metabolites | Cushman et al., 2002; Cushman et al., 2005; Kaiser et al., 2007; Zhao et al., 2009 |
| 2          | WP_030095371 | acyl carrier protein | 1              | 10805.0  | 0.207                | Cytoplasmic | Fatty acid biosynthesis | Madluli et al., 1998; Moche et al., 1999; Heath et al., 2000; Wang et al., 2006; Luckner et al., 2010 |
| 3          | WP_030097637 | replicative DNA helicase | 1              | 5037.2   | 0.323                | Cytoplasmic membrane | DNA replication | McKay et al., 2006; Aiello et al., 2010 |
| 4          | WP_046251970 | DNA topoisomerase IV subunit B | 3              | 74147.8  | 0.371                | Cytoplasmic | DNA repair and recombinatio n | Hoshino et al., 1994; Khodursky and Cozzarelli, 1998; Pan and Fisher, 1998; Anderson et al., 1999; Werner et al., 2000 |
| No. | Accession | Description | Fold Change | p-Value | Localization | Function/Pathway |
|-----|-----------|-------------|-------------|----------|--------------|------------------|
| 5   | WP_046252 | dihydropterate synthase | 2           | 37365.3  | -0.165       | Cytoplasmic Folate biosynthesis | Roland et al., 1979; Hammoudeh et al., 2014; Zhao et al., 2016 |
| 6   | WP_046252 | 2-amino-4-hydroxy-6-hydroxymethylidihydropteridine diphosphokinase | 1           | 19332.9  | -0.054       | Cytoplasmic Tetrahydrofolate biosynthesis | Shi et al., 2001 |
| 7   | WP_046252 | pantoate - beta-alanine ligase/ pantothenate synthetase | 1           | 33527.3  | -0.057       | Cytoplasmic Pantothenate biosynthesis | White et al., 2007; Ciulli et al., 2008; Velaparhi et al., 2008; Hung et al., 2009; Sledz et al., 2010; Yang et al., 2011 |
| 8   | WP_046252 | 3-oxoacyl-ACP synthase/beta-ketoacyl acyl carrier protein synthase | 2           | 35485.1  | 0.070        | Cytoplasmic Lipid synthesis | He and Rynold, 2002; He et al., 2004; Nie et al., 2005; Lee et al., 2012 |
| 9   | WP_046252 | bifunctional N-acetylglucosamine-1-phosphate uridylyltransferase (GlmU)/glucosamine-1-phosphate acetyltransferase | 1           | 50208.3  | -0.026       | Cytoplasmic Amino sugar and nucleotide sugar metabolism, Biosynthesis of antibiotics | Pereira et al., 2009; Buurman et al., 2011; Stokes et al., 2012; Min et al., 2012; Mehra et al., 2015 |
| 1   | WP_046253 | cell division protein FtsI | 1           | 68842.9  | -0.300       | Cytoplasmic membrane Peptidoglycan biosynthesis, beta-Lactam resistance | Slayden and Belisle, 2009 |
| 1   | WP_046253 | cell division protein FtsZ | 1           | 38795.6  | 0.076        | Cytoplasmic Chromosome partition, cytoskeleton, | Wang et al., 2003b; Ito et al., 2006; |
| WP_046253_835 | enoyl-[acyl-carrier-protein] reductase (InhA) | 3 | 28724.0 | 269 | 0.152 | Cytoplasmic membrane | Lipid biosynthesis | Park et al., 2014 |
| WP_046254_273 | DNA ligase (NAD(+) | 1 | 74430.9 | 684 | -0.226 | Cytoplasmic | DNA replication, Base excision repair, nucleotide excision repair, mismatch repair | Brötz-Oesterhelt et al., 2003; Srivastava et al., 2005; Mills et al., 2011 |
| WP_046254_500 | thymidylate kinase | 1 | 23403.1 | 212 | -0.394 | Cytoplasmic | Pyrimidine metabolism | Wang et al., 2000; Haouz et al., 2003; Fioravanti et al., 2005; Kosinska et al., 2005 |
| WP_046254_518 | biotin--[acyetyl-CoA-carboxylase] ligase | 1 | 27934.5 | 264 | -0.084 | Cytoplasmic | Biotin metabolism | Freiberg et al., 2004; Polyak et al., 2012; Soares da Costa et al., 2012 |
| WP_046254_614 | alanine racemase | 1 | 41861.3 | 397 | -0.008 | Cytoplasmic | D-Alanine metabolism, Vancomycin resistance | Badet and Walsh, 1985; Flynn et al., 1985; Anthony et al., 2011 |
| WP_046255_923 | ribonucleotide-diphosphate reductase subunit beta/ribonucleoside diphosphate reductase-β | 2 | 36899.2 | 320 | -0.437 | Unknown | Purine and pyrimidine metabolism | Moore, 1969; Tholander and Sjöberg, 2012 |
| Serial No. | Compound                                                                 | S-value | Metabolite forming plant                                                                 |
|-----------|--------------------------------------------------------------------------|---------|----------------------------------------------------------------------------------------|
| 1         | Daphnodorin G                                                             | -7.67   | Daphne odora                                                                            |
| 2         | Durantanin I                                                              | -6.90   | Duranta repens                                                                          |
| 3         | 5,7-Dihydroxy-4'-methoxy-6,8-di-C-methylflavanone 7-(4,6-digalloylglucoside) Matteucinol-7-O-[4",6"-di-O-galloyl]-beta-D-glucopyranoside | -6.15   | Miconia myriantha                                                                      |
| 4         | Chebulinic acid                                                           | -5.89   | Terminalia chebula, Caesalpinia coriana, Phyllanthus emblica                           |
| 5         | Myricitrin                                                                | -5.85   | Pistacia weinmannifolia J.Pisson ex.Franch, Rhus parviflora, Haplopappus baihlauen, Warburgia stuhlmannii, Cornus kousa, Davidsonia pruriens, Doliocarpus spraguei, Acacia aroma, Acacia saligna, Caesalpinia pulcherrima, Desmanthus illinoensis, Flemingia congesta, Quercus rubra, Patersonia spp., Juglans mandshurica, Leea thorelii Gagnep, Myrica rubra, Lysimachia spp., Eugenia edulis, Luma chequen, Plinia pinnata, Nymphaea caerulea, Nymphaea lotus, Phyllanthus emblica, Abies amabilis, }
| Armeria sp.        |
|--------------------|
| Ceratostigma willmottianum, |
| Limonium spp.,     |
| Eskemukerjea megacarpum HARA, |
| Chondropetalum spp., |
| Elegia capensis,   |
| Diploknema butyracea, |
| Manilkara zapota cv.Tikal, |
| Leptolaena diospyroidea, |
| Leptolaena pauciflora, |
| Heuchera spp.,     |
| Lithophragma spp., |
| Metasequoia glyptostroboides, |
| Hedychium spp.,    |
| Peltiphyllum peltatum, |
| Sarcolaeana multiflora |