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Metabolic Network Modeling for Rational Drug Design against *Candida albicans*

Rashi Verma, Dibyabhaba Pradhan, Harpreet Singh,
Arun Kumar Jain and Luqman Ahmad Khan

Abstract

The growing evidences of *Candida albicans* (*C. albicans*) infections are slowly becoming a threat to public health. Moreover, prevalence of antifungal resistant strains of *C. albicans* has emphasized the need for identification of potent targets for rational drug designing. In this aspect, traditional methods for target identification with validation have been found to be expensive and time-consuming. To overcome the concern, genome scale metabolic model construction provides a promising platform that allows novel target identification in combination with subtractive genome analysis. Thus, the chapter details current advancement in model construction, target identification and validation. In brief, it elucidates the overall strategies of *C. albicans* metabolome draft preparation, gap filling, curation of model, simulation followed by model validation, target identification and host pathogen interaction analysis. Finally, several examples of successful metabolic model construction and their utility in rational drug designing also have been discussed.

Keywords: Genome Scale Metabolic Model, Target Identification, Drug Designing, Host-Pathogen Interaction, *In-Silico* Gene Knockout

1. Introduction

Candida albicans (*C. albicans*) is an opportunistic fungal pathogen that lives in equilibrium with normal microbial flora of healthy individual [1]. As commensal, it colonizes on the mucosal surface of oral, respiratory, gastrointestinal and genitourinary tract. But on transformation into pathogen, it breaches the protective barrier in immunocompromised patients and cause candidiasis [2, 3]. Over 90% patients of cancer and HIV endure with oropharyngeal candidiasis whereas vulvovaginal candidiasis distressed 138 million women per year [4, 5]. Candidemia is the most recurrent nosocomial infection acquiring up to 15% infections of blood with mortality rate from 40 to 70% [6]. Consequently, candidiasis has become the most common fungal infections responsible for increased mortality and morbidity worldwide.

For the treatment of candidiasis, limited number of antifungals has been approved for clinical use. These antifungals categorize into four major classes - azoles, polyenes, echinocandins, and pyrimidine analogs [7]. Azole and

echinocandins precisely target the enzymes liable for synthesis of cell membrane or cell wall while polyenes directly bind to membrane proteins that maintain the osmolarity of the cell. In addition, Pyrimidines analogs are the sole antifungals that target the pathogen's genome rather than proteome [8, 9]. Consequently, the antifungals disturb the integrity of cell directly or indirectly which ultimately leads to the death of the pathogen (**Figure 1**).

C. albicans still represents itself as an emergent pathogen due to the side effects associated with such as RBCs toxicity, nephrotoxicity, hepatotoxicity, arrhythmias, cardiotoxicity and genitointestinal disturbances [10, 11]. Moreover, the drug resistance has also increased the complexity of the disease. The reason behind resistance is the prolonged or discriminated use of antifungals. The resistance mechanism involves the hyperactivity of efflux pumps, mutation in targeted genes and metabolites bypass [12, 13]. Thus, *C. albicans* have different resistance pattern against the diverse antifungals that lift up the difficulty level during the management of

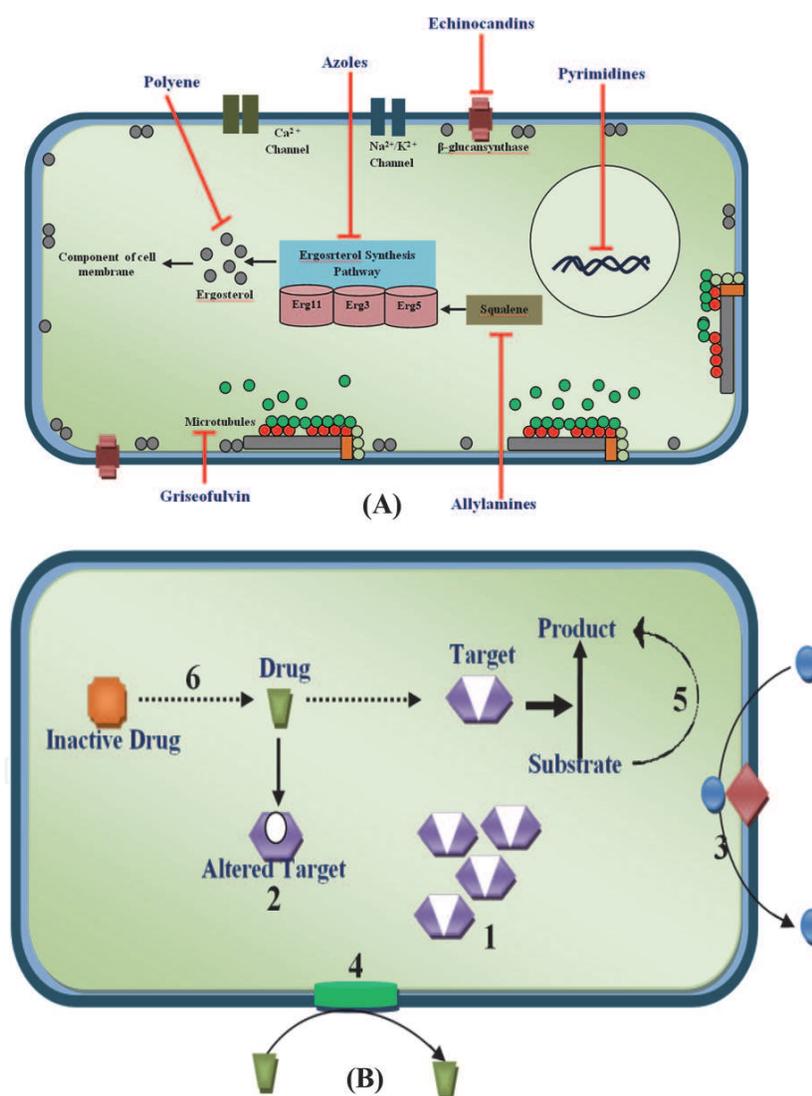


Figure 1. Antifungal Drug Discovery and Resistance. A) Since 1990s, polyenes, pyrimidine analogs, azoles, echinocandins, and allylamines, morpholines, thiocarbamates has been approved for treatment of *C. albicans* infection. Nystatin and 5-flucytosine binds to membrane ergosterol and thymidylate-synthetase, respectively that leads to the leakage of osmotic constituents. Azoles inhibit the synthesis of ergosterol by interrupting the activity of lanosterol- α -demethylase. Echinocandins halts the participation of (1,3) β -D-glucan synthase in glucan synthesis. Allylamines and thiocarbamates block the oxidation of squalene. Consequently, leads to the death of cell. B) Now, drug resistance has come into picture. Mechanism of resistance involves (1) overexpression of target product, (2) modification of target enzyme, (3) Hyperactivation of multi-drug pump, (4) Production of cell wall Barrier, (5) Adaption to stress response or metabolic bypass, (6) Inactivation of Drug.

infection. On the other aspect, significant homology of drug targets with human genes/protein, fitness traits and survival strategies such as secretion of hydrolytic enzymes, morphogenetic switch, adhesion to surface and formation of biofilm make this pathogen hard to kill. Thus, the scenario emphasizes the need of the novel drug designing *i.e.* effective against resistant strain of *C. albicans* and easily accessible with less or no side effects [14–18].

Effective drug designing could be possible only after the (i) identification and (ii) validation of potential target. *In silico* and *in vitro* approaches have been attempted for the identification and validation of novel targets of *C. albicans* followed by drug designing. Traditional methods of target identification with experimental validation (growth assay, enzyme inhibition assay, gene knockout, yeast to hybrid system, RNA interference) have been expensive, time-consuming and more focused towards few genes instead of whole genome. To reduce the time and cost, various *in silico* approaches such as subtractive genomics, comparative genomics, machine learning and inverse docking has been performed for novel target identification [19–21]. But a reliable approach is still required for proposed drug target validation.

The present chapter introduces the advancement in reconstruction and analysis of genome scale metabolic model (GSMM) which provides a platform that offers the opportunity to mimic the biological environment of pathogen into a machine to validate the essentiality of the target for the survival of pathogen. “Gene-Protein-Reaction” association in GSMM establishes its importance as a hub for validation of targets while stoichiometry matrix of model helps to depict the linkage information of metabolites to each reaction. If the inhibition of a particular metabolite shows a negative effect on growth of pathogen, it ensures the essentiality of the gene. Additionally, the approach also allows identification of the effect of inhibition on whole metabolome of pathogen. The GSMM can be used independently or in combination with different approaches (high throughput transcriptome profiling and subtractive genomics approach) (Figure 2) [19, 20, 22, 23].

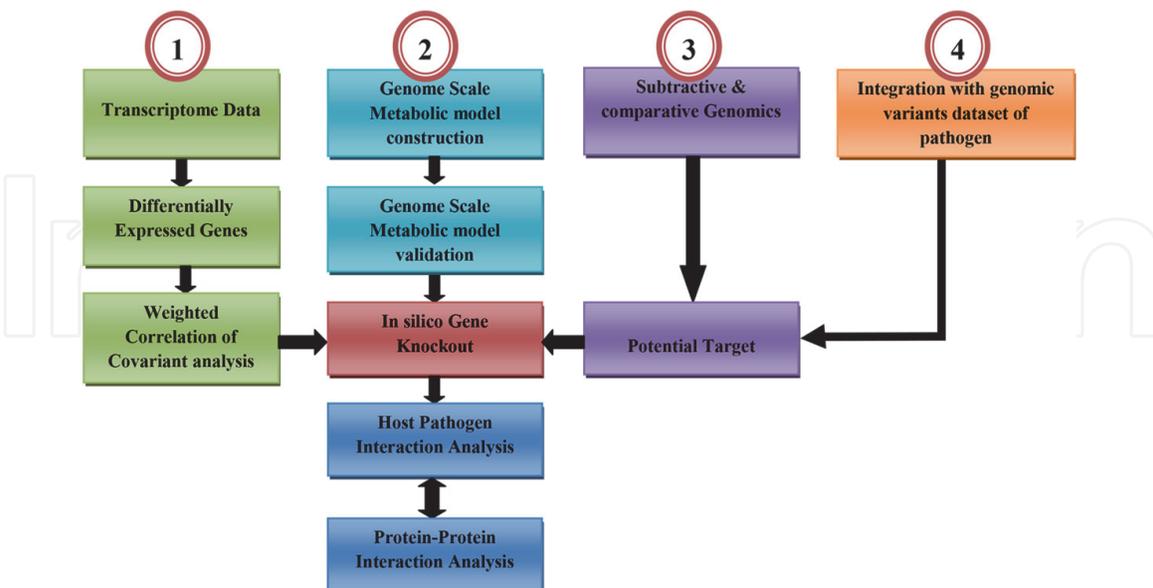


Figure 2.

GSMM as a central approach for target identification and validation. *In silico* approaches such as (1) Transcriptome data analysis, (2) Genome Scale Metabolic Reconstruction (GSMM), (3) Subtractive & Comparative Genomics and (4) Integration of genomics variants dataset of pathogen are widely used for target identification and validation. Among these, GSMM serves as a central approach which can be used independently or in combination with these approaches. Gene-protein-reaction association and *in silico* gene knockout feature of GSMM make it a reliable and standard approach to validate putative targets via monitoring the impact of gene deletion on biomass of the system. Further, prioritization of proposed genes can be done by host-pathogen interaction analysis and protein-protein interaction analysis.

2. Genome scale metabolic model reconstruction

A genome scale metabolic model (GSMM) is a computationally designed framework of microorganism that allows an efficient and comprehensive annotation of the metabolic functions of an organism, integrated with large-scale omics datasets and the study of microbe-host interactions [24, 25]. In brief, it describes gene-protein-reaction association of organism that mimics the biological condition in a machine to understand the genetic engineering, protein-protein interaction and evolutionary traits of organism [26]. Consequently, it generates forecast ranging from lethality of pathogen's gene to the dynamics engaged in defense mechanism of host towards infection.

As genome-scale metabolic model reconstruction become the more standard approach, the requirement of *in-silico*, automated tool turn out to be more perceptible to design and analyze these kinds of networks [27, 28]. Furthermore, availability of the whole genome of the pathogen also encourages the construction of *in silico* models. The Recent examples also have shown the potential of these models in the quest for novel drug targets in pathogenic organisms [29–33]. Kim et al., 2009 emerged a model of multi drug resistant *A. baumannii* and find the essential novel targets for therapeutic implications. Abdel-Haleem et al. in 2018, described the reconstruction of genome-scale metabolic models for five life cycle stages of *Plasmodium falciparum*, enabling the identification of potential drug targets that could be used as both, anti-malarial drugs and transmission-blocking agents [34]. Reinksma et al., 2019 developed combine model of *M. tuberculosis* and human to understand the metabolic state of pathogen during infection. Subsequently, Reinksma and team also assessed the effect of increasing dosages of drugs targeting metabolism on the metabolic state of the pathogen and predict resulting metabolic adaptations and flux rerouting through various pathways [35]. Similarly, Nouri et al., 2020 designed a comprehensive model of *Z. mobilis* to find the target for metabolic engineering applications [36]. Thus, design of *C. albicans* would also be the strong platform to understand its metabolic state in distinct adverse conditions that helps to identify and validate the target for novel drug design even against the resistant strains.

3. Experimental design for *C. albicans* model

Construction of a model involved 4 major steps: 1) Preparation of Draft; 2) Manual curation; 3) Generation of mathematical model; 4) Network evaluation and analysis [37]. In brief, draft preparation (50%) consist gene annotation of pathogen's genome that further map with data reported in literature. Manual curation (20%) considers the manual refinements and re-evaluation of draft due to the presence of annotations having low confidence score retrieved from organism unspecific biochemical databases that may affect the behavior of pathogenic model. Collection of data for growth condition and biomass composition also is the part of this stage. Generation of mathematical model (10%) is fully automated and includes the conversion of refined draft into mathematical model. Fourth stage comprises the verification, evaluation and validation of model that leads to the identification and fulfillment of network gaps by repeating stages 2 & 3 until the gap fill is accomplished.

The complete protocol of the genome scale metabolic model reconstruction of *C. albicans* is shown in **Figure 3**. The protocol consists of a set of methods that are introduced in sequence but can be combined in a multitude of ways.

3.1 Hardware and software

A 64 bit computer of 8 GB RAM with stable internet connection is desired for drafting a model till analysis. MATLAB vR2014b (<https://www.mathworks.com/>

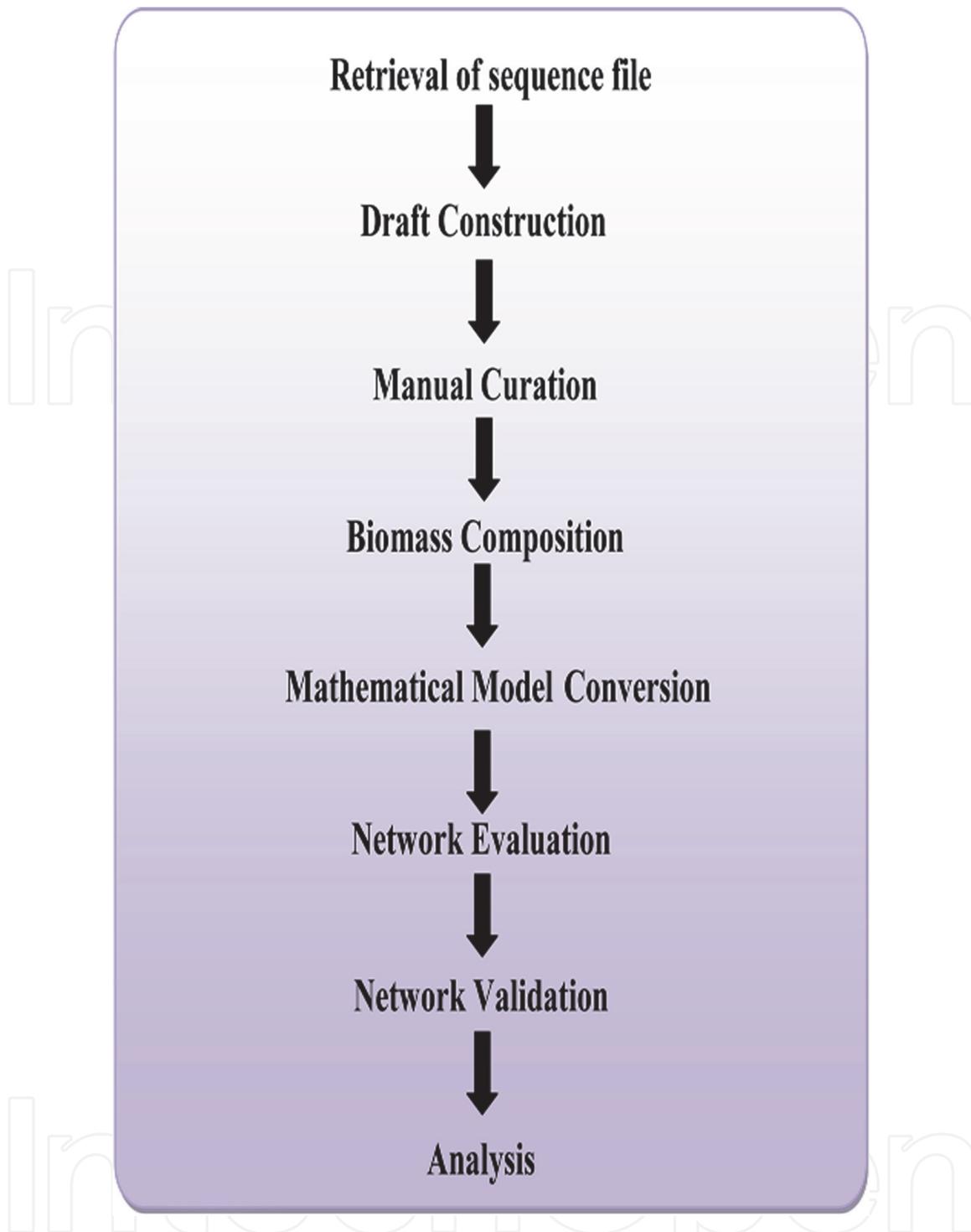


Figure 3.
A flow diagram of Genome Scale Metabolic Model Reconstruction.

products/matlab.html) or above, COBRA Toolbox v3.0 or above, Pathway Tools v 22.5 are required to accomplish the reconstruction [38–40].

Steps:

Matlab Installation (v2014 or above)

>> Download → Extract → Click on Setup → Install with or without Internet → Next → Accept license agreement → Next → Provide installation key → Next → Choose Installation Type → Next → Specify installation folder → Next → Provide license file location → Next → Select installation options → Confirm the Installation → Finish

CobraTool box Installation

>> Download i) git ii) curl (v7.0 or above) and iii) CobraTool box (v3.0 or above)
>> First Install git: extract → Click on Setup → choose default settings except adjusting your PATH environment (select use git and optional unix tool from window command prompt) and configuring

```

the line ending conversion (choose checkout as -is, commit Unix-style line ending).
>> Install curl: Select default settings → Just click next → Finish
>> Install CobraTool box: Open git bash → Run command “git clone -depth = 1 https://github.com/
opencobra/cobratoolbox.git cobratoolbox” (it will install the setup in C:
/user/username/cobratoolbox) → open matlab → click on set path → select the Toolbox folder
# SBML installation
>> Download → Extract → Open Matlab → Navigate to SBML toolbox folder → Run script “run
(install.m)”.
# Pathway tool Installation
>> Download → Click on Setup.exe → Select the location of installation (same as cobratool
box) → Next → Choose location to store configuration and data file → Next → Verify location of
installation → Next → uninstall older version (if present) → Click finish to continue → Ok → Create
desktop icon (optional) → Finish

```

3.2 Preparation of draft

The draft reconstruction can be done manually or automatically. On manual mode, it is very tedious and time taking process. Thus, the software such as metaShark and PathwayTools are available which automate the draft by using genome database (CMR, GOLD, SEED, TIGR and NCBI Entrez Gene), biochemical database (KEGG, BRENDA, Transport DB, TCDB and PubChem) and organism-specific database (EcoCyc, BioCyc, Metacyc and Gene Cards) [38, 41]. First, the chapter described the draft construction with PathwayTools followed by manual curation and biomass composition. Further, *in silico* activities and model analysis illustrated using COBRA Toolbox in MATLAB [38–40].

3.2.1 Input file format

PathoLogic plugin of PathwayTool is dedicated for automated draft construction that accepts FASTA file (.fasta), genetic-elements.dat (.dat), GenBank (.gbk) or PathoLogic (.pf) format as input. FASTA and GenBank file formats are easily accessible and can be retrieved from RefSeq and GenBank database while genetic-elements.dat and PathoLogic must be prepared that defines the annotation for each genetic element of organism. Each input file comprises at least the basic attributes such as unique ID, name, start base, end base, function, EC number and gene ontology.

Steps:

```

>> Retrieved the .fasta file and .gbk file of each chromosome of C. albicans from RefSeq (https://www.ncbi.nlm.nih.gov/genome/?term=candida+albicans).

```

3.2.2 Creation of new database

Database creation is the first step of draft model construction that requires the information like unique identifier, database name, taxonomy of organism and database storage type *etc.* Consequently, the provided data is saved into organism.dat and organism-init.dat files that indicate the initialization of new database. Once the database has been initialized, specify the replicons of your organism *i.e.*, the input files of each chromosome that can be .fasta, .dat, .gbk or .pf. Thereafter, specify the reference database of closest organism that will add the missing entities (reactions, enzymes and metabolites) which are absent in databases linked to pathway tool. Trial Parse operation parse the input file(s) to correct the errors present in input file before to automate the building of new database. Finally, the removal of

errors allows building the model. This is the focal phase of PathoLogic plugin that perform the parsing again and generate a database for each chromosome, gene, proteins, enzymes, metabolites of organism. Now save the organism database that will take several minutes to complete (**Figure 4, steps: 1–6**).

Steps:

- >> Pathway Tool → PathoLogic (New window popup)
- >> Database → Specify Reference PGDB (*S. cerevisiae* or *C. glabrata*)
- >> Database → Create New → Organism ID (CanCyc) → NCBI Taxonomy ID (237561)
- >> Build → Trial Parse
- >> Build → Automated Build.
- >> Database → Save KB

3.2.3 Refinement

Refining of database includes the inferences and manual operations: **1) Probable Enzymes** involved the additional enzyme-to-reaction assignments; **2) Name Matcher** add the additional name; **3) Rescore Pathways** performs the addition of new pathways and deletion of un-established pathways; **4) Create Protein Complex** permit to stipulate protein complexes that involuntarily link to appropriate reactions; **5) Assign Modified Proteins** allocate the modified substrate within the reaction encoded by gene product within the database; **6) Predict Operons** allow to choose genetic elements on the whole genome; **7) Transport Inference Parser** finds transport reaction catalyzes proteins to construct their protein complexes and enzymatic reactions; **8) Pathway Hole Filler** seal the gaps using candidate enzymes arise during the construction; **9) Update Cellular Review** draw the cellular outline of database; **10) Consistency Checker** automatically rectify the disturbances of data constraints. Among the refinement, hole filler play the major role which can be done mechanically or manually (**Figure 5A and B**). Now, resave the database and

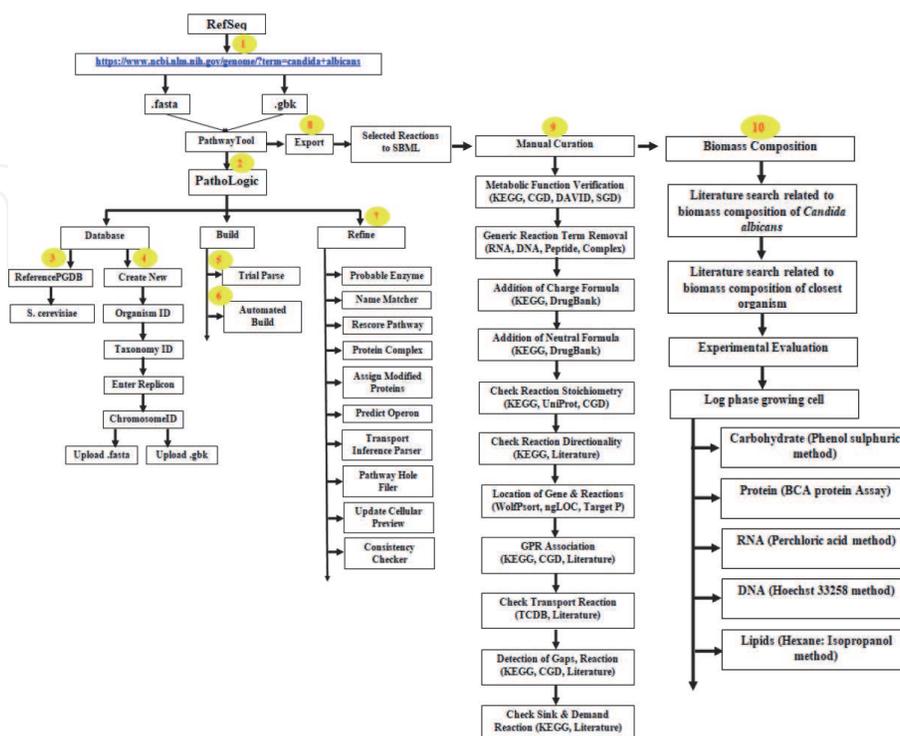


Figure 4.
 A detailed Protocol of Genome Scale Metabolic Reconstruction.

export it for further analysis in .sbml format using the command **File** → **Export** → **Selected reactions to SBML file**. For further processing, convert the sbml model into “CanCyc.xls” file for manual curation (**Figure 4, step: 7–8**).

3.3 Manual curation

Curation of model is time taking and tedious task that require special consideration during performance. It concentrates on re-evaluation and refinement of model content manually instead of mechanically. The reason behind the manual curation is the absence of proper and complete annotation of gene and their functions. In addition, the available database provides the information which is not organism specific. Consequently, there is a chance of adding those genes or reactions or metabolites which might not be the part of organism’s metabolic network and affect the expected behavior of modeled organism. Thus, it is suggested to curate and assemble the draft model in pathway to pathway manners using KEGG, Gene Ontology, *Candida* Genome Database, UniProt and DrugBank that ultimately facilitate the detection of gaps of the model [42–45]. Moreover, the stage also includes the metabolic function verification, removal of generic reaction terms (protein, electron acceptor/donor, DNA, RNA *etc.*), addition of charged formula of each metabolite, inspection of reaction stoichiometry as well as directionality, localization of gene with its related reactions, association of gene-protein-reaction, append of transport reaction with literature support. Other than this, inclusion of sink reaction, demand reaction, growth associated and non-growth associated ATP maintenance reaction are also required in a model for *in-silico* growth of the organism (**Figure 4, step: 9**).

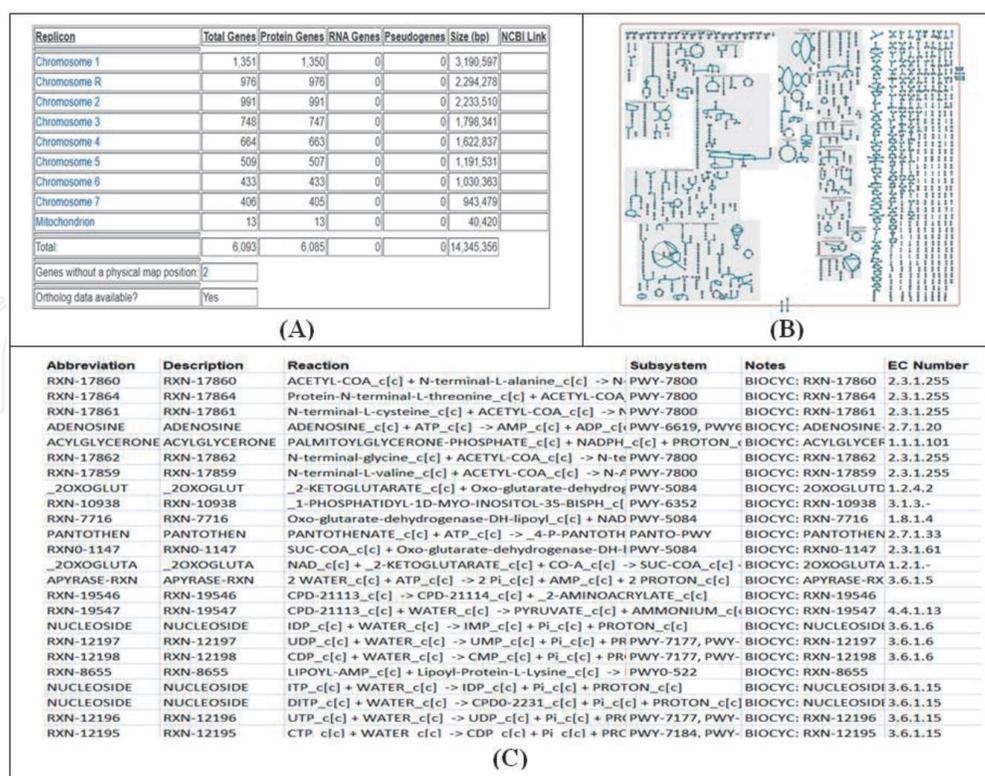


Figure 5. Genome-Scale Metabolic Model of *C. albicans*. (A) Construction of model using Pathway Tools generated the detailed chromosome-wise description and (B) cellular overview of the model that defines the linkage among the metabolic pathways present in model. (C) For manual curaion, sbml format of model is converted to mathematical model which further subjected for evaluation and validation.

3.4 Biomass composition

Biomass reaction is the engine of metabolic model as it shows the obvious effects on model validation and strain improvement. The biomass consist of cellular components (proteins, RNA, DNA, Lipids, Lipopolysaccharides, Peptidoglycan, Glycogen, Polyamines, *etc.*) with its fractional constituents [46]. Estimation of total biomass composition may not be feasible; still the determination of comparative fraction of all precursors can be possible experimentally for log phase growing cells. Among the biomass precursors, lipid extraction is quite tough due the presence of different fatty acid with diverse chain length, saturation and un-saturation. After the quantification of biomass components, data is subjected to normalization that computes the equation of biomass (**Figure 4, step: 10**).

3.5 Curated model conversion to mathematical model

In this stage, model is subjected to convert the curated draft into a condition-specific mathematical model *i.e.*, fully automated. MATLAB and COBRA Toolbox are widely used software for model conversion, evaluation and analysis. To convert the model initialize the COBRA toolbox using the command “**initCobraToolbox**” first, then set of optimization solvers such as Gurobi and LP. The optimization solvers provide commanding algorithms that improvise the programming of mathematical models, constraint models and constraint based scheduling models. The solver Gurobi is a default solver for LP, MILP and QP problems while GLPK is selected for LP and MILP problems. Read the model with “**xls2model**” command to verify and set the objective as well as simulation constraints to the model. Save it to “CanCyc.xml” format.

Script to load and save the model in mathematical format is provided in **Supplementary Data 1**.

3.6 Model evaluation and validation

The metabolic model designed in third stage may have some common errors: 1) wrong reaction constraints; 2) cofactor cannot be produced or consumed; 3) shuffling of compounds across compartments; 4) missing transport and exchange reactions. To rectify these issues network verification, evaluation and validation is needed. Verification and evaluation usually leads to the addition of transport reaction, exchange reactions and metabolic function that can be done by the repetitive process of stage 2 and 3. Thus, it is also known as iterative process that evokes the debugging to cure errors arising computationally. The major concern is to make a decision when to end this process which is based on the rationale of reconstruction.

The process starts with the test of unbalanced reaction that provides the list of unbalanced reaction in model to balance it manually. Next is to identify the dead end metabolites that are only consumed or produced and indicates about gaps present in the model. Removal of dead metabolites promote gap filling which is a manual process that can be done by using the published literature, genome pathway annotation tools (KEGG) and organism specific databases. During gap fill, all added reactions and metabolites must be connecting to each other. This step also includes the addition of exchange reactions and transport reaction as well. Thereafter, the upper and lower constraints to desire medium or environmental condition required for the growth of organism. Constraints must be varying according to the objective of study.

To practice the exercise of evaluation and validation, user can also download the published GSMM model of other organisms from BioModel Database (<http://www.ebi.ac.uk/biomodels/>).

Steps:

```
>> initCobraToolbox;
>> solverok = changeCobraSolver('glpk','LP');
>> model = xls2model(CanCyc.xlsx);
# Check biomass production
>> FBAsolution = optimizeCbModel(model,'max')
# Test unbalances mass and charge of C, N, P and S atoms and balances them.
>> [dE E] = checkBalance(model,'C');
>> ind = find(dE);
>> ImbalReacs_C = model.rxns(ind);
>> [dE E] = checkBalance(model,'N');
>> ind = find(dE);
>> ImbalReacs_N = model.rxns(ind);
>> [dE E] = checkBalance(model,'P');
>> ind = find(dE);
>> ImbalReacs_P = model.rxns(ind);
>> [dE E] = checkBalance(model,'S');
>> ind = find(dE);
>> ImbalReacs_S = model.rxns(ind);
# Identification of metabolic dead ends and document it in excel.
>> model = changeObjective(model,'BiomassReac_1');
>> [missingMets,presentMets] = AnalyzeGaps('model');
# Filling the gap of the model.
• Search the published literature first on metabolome of C. albicans
• Give the second priority to genome annotation tools (KEGG - https://www.kegg.jp/kegg-bin/show\_organism?menu\_type=pathway\_maps&org=cal) and organism specific database (Candida genome database - http://www.candidagenome.org/).

• Last precedence gives to closest organism database (Saccharomyces - https://www.yeastgenome.org/)
• Document all the gap fill reactions with references.
• Add gap fill reaction by repeating the stage 2 till the biomass production occurs. For addition or deletion use the command given below-
>> model_add = addReaction(model, 'ReactionName', 'Reaction');
>> model_del = removeRxnns(model, 'ReactionName', 'Reaction');
• After filling the gaps, check the production of biomass of the cell as commands given below:
>> initCobraToolbox;
>> solverok = changeCobraSolver('glpk','LP');
>> model = xls2model(CanCyc.xlsx);
>> model = changeObjective(model,'BiomassReac_1');
>> FBAsolution = optimizeCbModel(model,'max')
# Set the reaction constraints of modeled organism on minimal media
• For example-check the growth of C. albicans at Glucose and oxygen
>> model = changeRxnBounds(model, 'GLC_tx_c', -10, 'l');
>> model = changeRxnBounds(model, Oxygen_Molecule_tx_c', -10, 'l');
# Change the objective function according the study and simulate the model
>> model = changeObjective(model,'BiomassReac_2');
>> FBAsolution = optimizeCbModel(model,'max')
• Change the reaction and reaction bounds according to the aim of the study and validate the model.
  'u' = upper bound, 'l' = lower bound and 'b' = both bound
# Validation of the model
• On different carbon sources (Glucose for example).
>> GrowthRate = 0.10;
>> CarbonIntake = -10.0;
>> model = changeRxnBounds(model,'BiomassReac_c_test_2',GrwothRate,'b');
>> model = changeRxnBounds(model,'GLC_tx_c',CarbonIntkRate,'b');
>> fba = MinOptmz
```

-
- Similarly perform the validation of model on nitrogen and sulfur sources i.e., reported in the literature. Draw the flux map to check the flow of carbon throughout the model.
-

Once the model has been developed and validated, write the model in either .mat, .sbml or .xlsx format for further analysis (**Figure 5C**).

3.7 Target identification through essentiality analysis and gene knockout

Developed model can be used for novel drug target identification by prioritizing essential genes through *in silico* gene knockout of single or double genes. Reaction deletion command lists out the reactions that help the pathogen model to cultivate too fast. On the other hand, gene knockout inhibit the particular gene of metabolome which act as chokepoint in pathogen. Consequently, it provokes the survival of pathogen that establishes the necessity of that gene as drug target for therapeutic use.

```
# Reaction essentiality analysis
>> [EssentialReactions,NonEssentialReactions,Reactions] = ReacEssentiality(model);
# Gene essentiality analysis
• Single gene deletion
>> [EssentialGenes,NonEssentialGenes,Genes] = SingleGeneEssentiality(model);
• Double gene deletion
>> [EssentialGenes,NonEssentialGenes,Genes] = DoubleGeneEssentiality(model);
# Gene Knockout
• Single gene knockout
>> geneList = "GeneName1";
>> [grRatioDble, grRateKO, grRateWT] = singleGeneDeleion(model, MOMA, geneList);
• Double gene knockout
>> geneList1 = "GeneName1";
>> geneList2 = "GeneName2";
>> [grRatioDble, grRateKO, grRateWT] = doubleGeneDeleion (model, MOMA, geneList1,
geneList2);
```

3.8 Host-pathogen interaction (HPI) analysis

HPI analysis comprised of five central stages: 1) reconstruction of high quality host and pathogen model, 2) check the common reaction and metabolites of both the model, 3) integration of the model, 4) integration testing and 5) simulation [47].

HPI model development and analysis is presented in the **Figure 6**. As the protocol for high quality model development has been described above, this section will exclude the first stage i.e., reconstruction of model. Next is pre-integration check to remove the violation of mass conservation. Thus, it checks the mass balance and stoichiometric consistency on prior basis followed by the identification of overlap features of both the models [48]. These regions are expected to be the region of host-pathogen interaction. Thereafter, assign the unique metabolite and reaction identifier to common metabolites and reaction respectively that promotes the integration of both the model successfully.

Once the model has been merged, detailed integration testing required to be carried out that ensure the linkage among the merged models. Integrity testing is divided into functionality test suits and independence suit. Functionality suits include the check mass balance, flux variability analysis and literature based boundaries verification while independence suit needs to find objective function



Figure 6.

Host-Pathogen Interaction (HPI) Analysis using GSMM. Host-pathogen interaction analysis using GSMM includes draft construction, pre-integration model check, integration of host-pathogen models, test of integration and simulation. Consequently, the build mathematical model can be used to evaluate the interactions of individual components and highlight potential targets for drug development.

that assumed to be influence by the host pathogen coupling. Further, the evaluation of function is performed to proceed towards simulation of the integrated model. Through simulation, one can envisage the characteristics of disease or validate the experimental data in infection circumstances using HPI model. In addition, Gene knockout of the HPI model can potentially predict virulent genes of the pathogen with better accuracy than the individual model. The identification of lethal gene and knockout can be performed as similar as mentioned in the section of 3.7.

4. Advantages of GSMM of *C. albicans*

Genome Scale metabolic models of several pathogens have been designed and available at Biocompare database. The potential of these models in studying whole

metabolite organization in living cell/organism widen significant attention in system medicine. Nevertheless, GSMM of *C. albicans* would provide a platform for target identification and validation. Till date, single metabolic model (iRV781) of *C. albicans* has been developed [49]. The published model, designed on the GUI platform of merlin, found to be non-compatible with widely used system biology platforms such as Matlab and CobraToolbox. On comparison, model of present study explained the complete set of gene-protein-reaction associations based on genome annotation data and experimentally obtained information. Consequently, it allows the production of flux value for entire set of reactions. The model also provides the opportunity to integrate the omics and kinetic data that contributed to better understanding of metabolism of pathogen. Such progression in model development of *C. albicans* permits the context-specific simulation. Additionally, the model would be beneficial in prediction of enzyme functions, pan-reactome analysis, modeling interaction among multiple cells or organism and understanding the colonization of pathogen and disease progression in human. In future prospect, proposed model could be used as a reference template to design the model for resistant strain of *C. albicans*.

5. Conclusion

Despite the presence of distinct antifungals, current situation demands the discovery of novel antifungal(s) against resistant strain of *C. albicans*. A successful drug design method is reliable on when a potent target is present. Thus, a potential strategy, approach, pipeline and tools are required to identify the druggable target. System biology and genome scale metabolic reconstruction of infectious pathogen offer a novel and effective approach that positively impel the research towards the identification of drug target that could help to design a novel antifungal against all kind of pathogenic strains of *C. albicans*.

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Declaration of interest

No issues of conflicting interest have been declared or identified.

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