Review

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Review of Current Approaches for Implementing Metabolic Reconstruction

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Abstract

Background: Metabolic modeling has been an essential tool in metabolic reconstruction, which has dramatically advanced in the last decades as a part of systems biology. At present, the protocol for metabolic reconstruction has been systematically established, and it provides the basis for the analysis of complex systems, which has been limited in the past. Therefore, metabolic reconstruction can be adapted to analyze agricultural systems whose metabolic data has been accumulated recently. **Purpose:** The aim of this review is to suggest the suitability of metabolic modeling for understanding agricultural metabolic data and to encourage the potential use of this modeling in the field of agriculture. **Review:** We reviewed the procedure of metabolic reconstruction using computational modeling with applicable strategies and software tools. Additionally, we presented the initial attempts of metabolic reconstruction in the field of agriculture and proposed further applications.

Keywords: Agricultural systems, Metabolic data, Metabolic modeling, Metabolic reconstruction, Modeling software tools

Introduction

Systems biology, which combines bioinformatics and computational biology, has provided an integrative insight of complex biological systems because of its potential for analyzing the accumulated data from independent experiments in the last decades (Kitano, 2002). As a field of systems biology, metabolic reconstruction plays a core role in solving the conundrums in our life, with over 2000 papers published in the last 10 years. Metabolic reconstruction is a computer-based analysis technique and is used to construct a schema of the data obtained from several components of biological systems, such as genes, enzymes, metabolites, and reactions (Feist et al., 2009). This powerful tool enables a new analysis that existing traditional methods cannot achieve, for e.g., searching dead ends and identifying missing reactions (Orth and Palsson, 2010).

As the first step in metabolic reconstruction, a metabolic database obtained from a target organism is required. Among various organisms, microbes, particularly *Escherichia coli*, have been widely used owing to their simple structure and easy cultivation properties. A notable metabolic reconstruction study using *E. coli* was published in 1994, in which a constraint-based mathematical model was developed and a flux balance analysis (FBA) was performed to explain its physiological behaviors (Varma and Palsson, 1994a; Varma and Palsson, 1994b). In 2000, the first genome-scale metabolic reconstruction was performed on *E. coli* (Edwards and Palsson, 2000), and thereafter, various methods in metabolic reconstruction have been gradually developed and used, such as flux variability analysis (FVA) and OptKnock (Mahadevan and Schilling, 2003; Burgard et al., 2003). With metabolic reconstruction, a new reaction that maintained the robustness of metabolism in *E. coli* was discovered (Nakahigashi et al., 2009), while meta-

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bolically engineered *E. coli* was developed to directly produce 1,4-butanediol (Yim et al., 2011). Moreover, a study used *E. coli* for synthesizing taxol, an anti-cancer component (Boghigian et al., 2012). In 2013, a comprehensive review, which summarized the direction of the current metabolic reconstruction performed on *E. coli*, was published by a leading group directed by Palsson (McCloskey et al., 2013). According to this study, metabolic reconstruction can be classified into six divisions depending on the study objective: (1) metabolic engineering, (2) model-driven discovery, (3) prediction of cellular phenotypes, (4) analysis of biological network properties, (5) studies on evolutionary processes, and (6) models of interspecies interactions.

One of the main advantages of metabolic reconstruction is the simultaneous analysis of a large amount of biological data from a target system. Hence, it is inevitable to use computational devices and languages, and some specialized tools for metabolic reconstruction have been developed to embed metabolic information into the model systems. The systems biology markup language (SBML) is a notable computational language widely used in systems biology (Hucka et al., 2003), contributing to establish web-based databases such as KEGG, Biocyc, and Reactome. In addition, some analytical methods (i.e., data managing, analyzing, and visualizing) have been discussed for analyzing the metabolic database in the computational analysis field. At the same time, various tools have been developed and utilized in the field of metabolic engineering depending on the purpose of the studies (Copeland et al., 2012). For example, constraint-based reconstruction and analysis (COBRA) has been broadly used, and it provides various functions such as FBA, gap filling, and knock out analysis (Becker et al., 2007; Schellenberger et al., 2012). OptFlux is used for identifying the target of metabolic engineering through the OptKnock algorithm (Rocha et al., 2010), and FBA-SimVis is a specialized program that interactively combines the results of COBRA and visualization (Grafahrend- Belau et al., 2009a). Further, computational tools for showing comprehensive outlines or visual results have been developed, e.g., VANTED (Junker et al., 2006), GLAMM (Bates et al., 2011), and Arcadia (Villéger et al., 2010).

As mentioned before, metabolic reconstruction is a powerful effort to solve the mysteries in life by figuring out the mechanisms of organism, and adequate utilization of computational tools allows effective analysis of the target systems. There are notable reviews regarding the metabolic reconstruction procedures (Ruppin et al., 2010) and software that are widely used in the field (Wiechert, 2002; Copeland et al., 2012). Owing to its advantages in analyzing complex living systems and large data sets, other scientific fields besides systems and synthetic biology are trying to employ the methods of metabolic reconstruction. Especially, with the recent spotlight on the field of agriculture, the application of the tools of metabolic reconstruction has been proposed for comprehensively analyzing the agricultural and food systems because they have produced and stored large amounts of data, and they are as complex as the biological systems (Kim et al., 2016a). In this paper, therefore, we present the current opinions in metabolic reconstruction and review the metabolic modeling, the main technique of metabolic reconstruction. Finally, we also review the contemporary uses of metabolic modeling and reconstruction in the agricultural field to propose the application of metabolic reconstruction in agricultural research.

Review of Modeling Approaches in Metabolic Reconstruction

Models in metabolic reconstruction are built by the combination of the genome sequence and biochemical information, and they are used to predict metabolic responses under perturbations, either in genes or in metabolic reactions. A comprehensive protocol for metabolic reconstruction was proposed by Thiele and Palsson (2010) and assembled into five steps by Liu et al. (2010): (1) creation of a basic model from genome sequencing, (2) reconstruction of a metabolic model, (3) conversion to a mathematical model, (4) model analysis and evaluation, and (5) visualization of a model. This is the generally used protocol in metabolic reconstruction when using both genomic and metabolic data. There are also other studies that focus on the mathematical model itself for analyzing biological systems, but the basic procedures are mostly similar among the studies; Voit (2012) and Haefner (1996) proposed five steps similar to each other (Table 1). In this review, we propose a 4-step procedure for computational modeling specific to metabolic reconstruction based on a comprehensive review of both the mathematical modeling of biological systems and metabolic reconstruction (Figure 1).

Table 1. Basic modeling procedure

Procedure	Voit, 2012	Haefner, 2012
Step 1	selection of goals and objectives	selection of objectives and hypothesis
Step 2	model selection	mathematical formulation
Step 3	model design	verification
Step 4	model analysis and diagnosis	calibration
Step 5	model use and application	analysis and evaluation

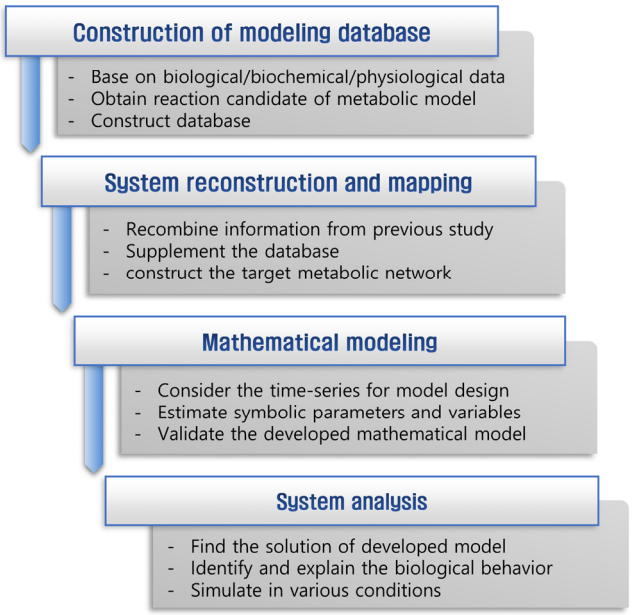

Figure 1. Application of modeling approaches in metabolic reconstruction procedure

Step 1: Construction of a modeling database

Modeling starts with obtaining the available information, i.e., quantitative and qualitative data, and constructing a database. The initial step in modeling is actually to determine the basic requirement of the model for its development, such as modeling target, modeling purpose, modeling scale, and model types and structures. Once the modeling direction is decided, the available data should be explored in terms of types of data required and amount of available data sets. Because computational models in biological systems are representative of available information (e.g., genetic, biochemical, and physiological data), empirical data (both qualitative and quantitative) is the basis of the model developed. Generally, computational modeling in metabolic reconstruction is a genome-scale model, which identifies the metabolism in organisms in conjunction with genes (Durot et al., 2009). Thus, the typical data required are genetic information, enzymatic data derived from the genes, metabolite affected by the enzyme, and cell organelle where the biochemical reaction occurred. The information of a gene can be experimentally obtained by genome sequencing, and some available data are built in a specific program and website (e.g., http://www.ebi.ac.uk/). The enzymatic information and metabolites in a biochemical reaction are defined as an EC number, a commission number of enzyme reactions (Bairoch, 1994), systemically linked to a biochemical reaction database, such as KEGG (Kanehisa and Goto, 2000) and BRENDA (Schomburg et al., 2002). Ultimately, the obtained data enable to list the biochemical reactions, which constitute the computational model.

The second phase of the first step is to construct a database from the obtained data so that it can be used for model development. That is, it is necessary to convert the obtained data into a suitable form so that it is directly applicable and suitable for modeling. This is because most of the empirical data are recorded with a form determined by the study objectives and do not consider their further usage in modeling. Typically, there could be a large difference in the units of metabolites among different research articles. For solving these inconsistency, pre-processing of data for image processing (Hwang and Fu, 1983), data integration for central composite design in response to the surface methodology (Montgomery, 2008), and unit consistency in time-series data (Sasidharan et al., 2012) are examples that we can easily observe. Therefore, the first step would be to make the various scales in the obtained data consistent. This process includes not only unit consistency but also correction of the experimental environments. Then, we need to apply simple statistics to the obtained data to eliminate noise and outliers, to derive their representative values in the case of large groups of data, and sometimes to normalize the data into a dimensionless form. Finally, the complete modeling database can be constructed by arranging the processed data according to the main variables (e.g., sorting data by metabolite types and by time). This database now becomes available for modeling, particularly for parameter estimation and model validation (herein, the sub-processes in step 3).

Step 2: System reconstruction and mapping

The second step is to reconstruct the target system

based on the collected information. In this process, we aim to construct a map or network of metabolic/genetic pathways to be modeled by integrating the partial information collected from relevant previous studies and data. This mapping step requires the evaluation of previous models, uncommon chemical reactions (i.e., biomass reaction), and gene expression (Palsson, 2006). Ultimately, we have to determine which pathways would be included and excluded, and to develop a metabolic map, which clearly represents our modeling target. For example, transport systems, which are caused by osmotic pressure or diffusion in the organism, such as extracellular transport reactions, intracellular transport reactions, and exchange reactions, are sometimes not coded in gene annotations, and thus, they are not included in the database developed in step 1. Therefore, it is required to add some necessary reactions that improve network connectivity and reduce the dead-end metabolites (Durot et al., 2009). In addition, biomass-related reactions containing information regarding the energy required for cell growth and maintenance need to be added. This type of information can be generally obtained from empirical data (Liu et al., 2010). The complexity of biological systems, generally caused by the numerous interactions among their components, limits the realization of comprehensive experiments. This means that most of the available empirical data exist in a piecewise form, and thus, it is difficult to integrate them into a complete metabolic map, which requires intensive review of literature for constructing a database to be used in modeling. Moreover, large variation even in the same domain makes this step more difficult. For example, the lignin biosynthesis in Populus xylem and that in switchgrass are different (Lee and Voit, 2010; Faraji et al., 2015). Compared to the available information on populous xylem, that on lignin biosynthesis in switchgrass is relatively insufficient. For this reason, we need to reconstruct the process by combining information obtained from similar species (Faraji et al., 2015).

Step 3: Mathematical modeling

Once the target metabolism is reconstructed, its map is converted into a mathematical formulation. In other words, in the third step, a mathematical equation is developed wherein the mathematical formulation defines the model structure, which is determined by the purpose of modeling and desired outcomes. This process corresponds to steps 2∼4 presented in Table 1, and its practical modeling procedures, which are carried out through the following process, are reviewed by Kim et al. (Kim et al., 2016a).

Model design

Generally, mathematical modeling is classified by several standards (Kim et al., 2016a). Voit et al. (2012) categorized the mathematical models into two classes: correlative and explanatory models. Based on their textbook, a correlative model uses statistics to develop an equation for predicting the target by the correlated factors, but it cannot explain the mechanism underlying the target system. In contrast, the explanatory model explains the mechanism of a biological process while simultaneously identifying the target phenomenon, and it is generally expressed as a series of differential equations (Voit et al., 2012). The mechanistic model (or explanatory model) can be divided into steady-state (the system property is not changed by time) and dynamic (the system property changes over time) models.

Parameter estimation

In the initial formulation, the equations are coded by symbolic parameters and variables, which represent properties of biochemical reactions (e.g., constant rate), and metabolites (e.g., affinity of enzyme and substrate, and kinetics). In the next formulation, numeric values are assigned to the symbolic parameters, and this is called parameter estimation. The estimating parameters and the formation vary with model state depending on whether it is divided as a dynamic model or a static model.

A steady-state model, which means it does not change with time, is mainly used for calculating the flux at a steady state or measurement of gene expression degree, within the assumption of constraint modification, to explain a behavior close to that of a living organism. If the constraint modifications are not applied, it would not reflect the behavior of living organisms at steady-state because of the error caused by the absence of constraint modifications. Commonly, four types of environmental constraints are supposed, as follows: flux balance ($S \cdot v = 0$), energy balance ($\Delta E = 0$), enzyme or transporter capacity ($v_i \leq v_{max}$), and thermodynamics ($0 \leq v_{min}$) (Shlomi et al., 2005). The mathematical format is a tabulation consisting of metabolites and reaction, which is called

S-matrix. This formula is simpler than the formula of a dynamic model, and less data are required for the parameter.

On the contrary, a dynamic model reflecting a significant change in time is composed of a series of differential equations. It requires time-series data as the response values of each parameter depends on the time change, which requires a huge amount of quantitative data and modeling work. Therefore, it is hard to estimate the available mathematical parameter because of its complexity, but this could be improved by some techniques, such as smoothing overly noisy data and estimating slopes of time series (Chou and Voit, 2009). Although the acquiring process of the parameter estimation is relatively difficult, a dynamic model can explain the variance or response under a particular environment, such as changes in immunized, infected, and susceptible individuals over time with respect to the rate of birth, immigration, death, vaccination, and initial number of individuals (Kanehisa and Goto, 2000; Voit et al., 2012).

Model validation

After completing the parameter estimation, the computational model is built using a mathematical formulation with numeric parameters, indicating that the model is ready to be solved. Before solving the model, model validation is conducted i.e., testing whether the adequate developed model is required. In other words, it is necessary to confirm the similarity between the predicted values and practical values of the developed model, which shows that the followed steps based on this model are valid. Model validation requires experimental data of the same target and it can be acquired through two ways. One is to obtain it from previous studies and the other is to separate out the validation data in the parameter estimation step. Various experiments have been carried out and published, and if an experimental study on the same target could be found, it would be easy to acquire from it the validation data. However, it is rare to find completely suitable data, and the units should be checked and converted when gathering the data. For example, Lee and Okos performed validation of the established model in their bone cell modeling paper by using data from previous studies before simulating the action of IGF-1 (Lee and Okos, 2016). Validation can also be applied for testing the accuracy of the scenarios in the last step of the following stage through the process of characteristic verification.

Step 4: System analysis

The main objective of this step is to resolve the developed model and to conduct its analysis. This means that we need to carry out the test of the hypothesis set earlier and identify the characteristics of system response under various conditions. Most biological models are established using a series of differential equations, called system equations, and thus, a numerical analysis is necessary along with the determination of initial values. Once the solution is found, further analysis is available by conceiving various scenarios that we would like to simulate. These simulations may give an insight about the target system, and they can sometimes be used for discovering the mechanisms under a specific situation. As aforementioned, the numerical analysis is inevitable for solving and analyzing the model. In other words, to apply the numerical analysis, a computational tool is required, and some software is available that includes internal algorithms coding fundamental mathematical principles. For example, the COBRA toolbox developed for MATLAB and python language is one of the most widely used open source *in silico* modeling software.

Tools and Software for Metabolic Modeling in Metabolic Reconstruction

Contemporary metabolic modeling is actively utilized in metabolic reconstruction, and systems and synthetic biology has a highly complex structure requiring computational software in most of the steps (Copeland et al., 2012). In the past decades, various tools have been developed to facilitate the analysis of the target system and to increase the model accuracy (Copeland et al., 2012). Herein, we categorize these software tools according to three modeling steps: 1) metabolic database construction (tools for constructing the database, including genetic, biochemical, and physiological data from the target system), 2) computational solver (tools for solving the reconstructed model), and 3) systematic analysis (tools for performing a system-level analysis, including visualization of the reconstructed network) (Copeland et al., 2012). In this section, we largely focus on introducing some useful sources and their applications through the

aforementioned computational modeling steps, based on a literature review.

Sources useful for metabolic database management: for steps 1 and 2

Metabolic reconstruction is a study on identifying the characteristics and functions of metabolic components in organisms by connecting metabolic data with genomic data. Therefore, construction of metabolic data is the important first step for subsequent model development, and it affects the model accuracy and reliability. This first step of data acquisition can be divided into two categories: 1) database construction, and 2) target data screening and selection. Because database management is a large field and serves as the basis of bioinformatics, this study focuses on the construction and use of databases for computational modeling. Computational modeling requires data for parameter estimation and model validation, as previously suggested. Thus, it is necessary to collect experimental data of a previous study or to find a public database, and then process them to be in a suitable form for modeling. In general, the applicable tools for database construction depend on the size of data. For example, universal tools, such as EXCEL and ACCESS, can be used for small-sized data, which is required for small-scale modeling. In addition, text files have been used to store data, but data importing is required to insert it into the modeling software. In contrast, in the case of high-throughput omics data or raw spectrum data, it is more efficient to use tools specialized for database construction (e.g., SQL and MATLAB) because they have better processing speed and ability to manage large-volume data. For example, hyperspectral image data, which has been applied to develop a model for detecting biochemical and physical differences between target products, exceeds 4 GB in size (Bajwa et al., 2004). Sometimes, we may not experimentally obtain the relevant information or may have limited access to the previous data. For example, BLAST, a tool for comparative analysis strategy, attempts to identify a new pathway by using the local sequence alignment method, which finds the statistically significant match by comparing the query sequence and database (Altschul et al., 1990). Hence, it is necessary to access the sequence data, such as nucleotide or protein, and information is important for developing a genome-scale metabolic model. In case a part of the necessary data is not accessible due to the complexity of the system or scarcity of available data, there are useful public sources, such as the KEGG PATHWAY (Kanehisa and Goto, 2000) and MetaCyc (Caspi et al., 2007). These public open databases provide reaction maps and show the interactions among metabolites (e.g., substrates and enzymes). Besides the above tools, various data sources were developed, but differences among the sources in terms of representing the information caused errors in data mining for constituting the metabolic network. For this reason, there was a demand for comprehensively integrated databases, and this was solved by consistent data representation schema. As a result, biochemical, genetic, and genomic (BiGG) (Schellenberger et al., 2010) and MetRxn (Kumar et al., 2012) were developed. BiGG includes information about 7 manually curated reconstructions, while MetRxn integrates 8 databases and 44 metabolic models.

Tools used for system solution and simulation: for steps 3 and 4

Common software is used for solving the computational model, while specialized tools exist for simulating a specifically written model. MATLAB, Mathematica, and MathCAD are the most notable software tools that can be used for finding a solution for a computational model, particularly one written using differential equations. Additionally, there is a commercial software for solving partial differential equations, which has been applied to model the metabolite diffusion into or within cells (Hengenius et al., 2011). COBRA toolbox is a free metabolic reconstruction platform coded on MATLAB and Python (Ebrahim et al., 2013; Becker et al., 2007; Schellenberger et al., 2012). This COBRA toolbox is specialized in developing a tissue-specific model by matching gene expression data with metabolic reactions (Becker and Palsson, 2008). In addition, there are other specific-type software, such as WinSAAM, for physiological modeling (Stefanovski et al., 2003; Wastney et al., 1999), and PLAS, a software specialized in solving a model by the biochemical systems theory (BST) (Voit, 2013). Certainly, there are too many software tools to be introduced, and that is not the scope of our study. Instead, we select a powerful and commonly used analysis tool, metabolic flux analysis (MFA), and review it with the relevant computational tools.

Metabolic flux is a significant determinant of cell physiology because it is used for predicting cell growth

and function by evaluating the core part of cell function, the target pathways, where the fluxes and regulatory point in a metabolic process are involved. For this reason, a goal of metabolic engineering is to optimally produce the desired metabolite and biomass by controlling flux distribution (Stephanopoulos et al., 1998). MFA has been applied to identify the flux distribution maximizing the desired products in an *in vivo* study. The basis of MFA is stoichiometry, which originates from chemistry, and is used for calculating the quantitative relationship between products and substrates (Gombert and Nielsen, 2000). The constraint is another requirement for MFA, particularly in constraint-based modeling. There are widely used constraints, such as stoichiometric constraints (mass balance), thermodynamic constraints (reversibility of a reaction), and enzymatic capacity constraints (Reed and Palsson, 2003). Based on the constraint-based stoichiometric matrix, mathematical equations explaining the relationship among substrates and products can be developed, and it is possible to calculate the solution space and range of a flux. As the solution space varies with the purpose of metabolic engineering, specific methodologies have been studied, such as FBA, which optimizes the flux distribution to maximize or minimize the target products (Orth et al., 2010), and extreme pathway and elementary model analysis frameworks, which define an eigenvalue in a given solution space (Schilling et al., 2000; Schuster et al., 1999). Especially, FBA is the most frequently used platform, and has been reviewed regarding its applications in metabolic engineering (Edwards et al., 2002; Bonarius et al., 1997; Ruppin et al., 2010; Orth et al., 2010).

Consequently, various types of software implementing FBA have been developed. According to Lakshmana et al. (2012), there are over twenty FBA software applications, which can be categorized into three types: stand-alone, toolbox-based, and web-based tools. Typical examples of the stand-alone tools are OptFlux (Rocha et al., 2010) and FASIMU (Hoppe et al., 2011), which were developed as independent programs for FBA. Toolbox-based tools mean functional expansion tools based on an existing program. The most widely used tools in this category are FBA-SimVis (Grafahrend-Belau et al., 2009a) and the COBRA toolbox (Schellenberger et al., 2012), based on VANTED (Junker et al., 2006) and MATLAB, respectively. Especially, when compared to other tools, the COBRA toolbox contains diverse algorithms, such as FVA (Palsson, 2006), MOMA (Durot et al., 2009), ROOM (Shlomi et al., 2005), dFBA (Mahadevan et al., 2002), and OptKnock (Burgard et al., 2003), and thus, it is used in the metabolic engineering field with great efficacy. Lastly, the web-based tools operate online, without installation of certain programs in the computer; thus, they require a web browser with an internet connection. For instance, GSMN-TB (Beste et al., 2007) and Model SEED (Henry et al., 2010) are broadly used web-based tools.

Additional useful tools for system visualization: for steps 2 and 4

Sometimes, visualization of a target metabolic pathway and network is effective when reconstructing a metabolic map or reporting a result of computational modeling. In the visibly reconstructed metabolic map, the general reactions and metabolites, linked using interconnected edges and nodes, constitute the so-called reaction visualizing network (Palsson, 2006). For example, specialized tools for visualizing the network map based on experimental data are the web-based GLAMM (Bates et al., 2011) and JAVA-based VANTED (Junker et al., 2006). In GLAMM, data import from other data sources is limited as it requires tool-specific types of input. In contrast, VANTED uses SBML as a standard format (Hucka et al., 2003), and it can even import data from KEGG, one of most widely used metabolic databases on the web (Kanehisa and Goto, 2000), thus allowing high usability. Besides, Arcadia focuses on showing the biochemical system with nodes and layers representing the metabolic molecules (Villéger et al., 2010), and Omix is specialized in the constraint-based flux using carbon-13 (Droste et al., 2013). One of the useful tools is FBA-simVis, which is particularly developed for FBA and FVA by expanding VANTED (Grafahrend-Belau et al., 2009a). This tool immediately reflects modifications in the parameters, so we can see on-time changes in flux distribution on the visualized map. Recently, a web-based visualizing application, named Escher, was developed. Escher provides three key functions and is operated on web technologies; it designs a new pathway map semi-automatically and visualizes the gene data on the related reactions (King et al., 2015).

Visualization has the unique advantage that the pattern unpredicted by using a computer algorithm can be found through the human's intuition and insight, because visualization provides the overall structure of

the target metabolism (Copeland et al., 2012). At the same time, we should note that it only displays the network structure itself, and thus researchers should be able to integrate the visualized target with the results from computational modeling.

Application of Metabolic Modeling in Agricultural Systems

Recently, genome-scale metabolic reconstruction and computational modeling has been used to identify complex mechanisms in living organisms, and thus, it essentially requires experimental data. For this reason, microorganisms have been the main target because they have a relatively shorter biological cycle and are easier to grow than high-level organisms. In particular, most of the studies have been focused on the microorganisms that produce high industrial and commercial value products (Yim et al., 2011, Klanchui et al., 2012, Bodor et al., 2014). Nevertheless, metabolic reconstruction and computational modeling is, to some extent, limited in the field of agriculture because of the characteristics of the target system, which has relatively longer life cycle and higher complexity compared to microorganisms. There is a notable study that targeted *Sinorhizobium meliloti*, a microorganism related to nitrogen fixation in soil (Zhao et al., 2012). As the recent trend in sustainable alternative energy increased the importance of biofuel production from plants, metabolic reconstruction and modeling have been applied to agricultural crops such as rice (Dharmawardhana et al., 2013, Lakshmanan et al., 2013), maize (Saha et al., 2011; Simons et al., 2014), and barley

Table 2. Studies related to metabolic modeling in agricultural systems

Reference	Target	Description
Soil microorganism		
Resendis-Antonio et al., 2007	*Rhizobium etli*	Reconstructs metabolic network for analyzing nitrogen fixation
Zhao et al., 2012	*S. meliloti*	Provides overview of symbiotic nitrogen fixation process
Simons et al., 2014	Maize	Demonstrates application of metabolic models for nitrogen-use efficiency
Plant		
Ouyang et al., 2006	Rice	Annotates TIGR rice genome
Dharmawardhana et al., 2013	Rice	Develops metabolic pathway network database for rice
Poolman et al., 2013	Rice (*Oryza sativa*)	Develops genome-scale model of rice metabolism and examines its responses to light availability
Grafahrend-Belau et al., 2013	Barley (*Hordeum vulgare*)	Develops multi-scale metabolic model for studying yield stability and crop improvement
Bombarely et al., 2011	Solanaceae	Constructs clade-oriented database containing biological data for species in Solanaceae and their close relatives
Livestock		
Waghorn and Baldwin, 1984	Cow	Calculates uptakes of glucose, acetate, and beta-hydroxybutyrate through metabolic flux model
Hanigan and Baldwin, 1994	Cow	Constructs model of mammary gland metabolism
Martin and Sauvant, 2007	Cow	Simulates digestive flows, metabolic pathways, and milk composition during lactation through dynamic model
Lemosquet et al., 2009	Cow	Analyzes effect of propionate and casein on whole-body mammary metabolism of energetic nutrients
Kim and Seo, 2012	Cattle	Describes sequencing analysis of cattle genome and presents information about feed efficiency of cattle
Seo et al., 2013	Cattle (*Bos taurus*)	Describes unique genomic features of cattle biology
Kim et al., 2016b	Cattle	Reconstructs genome-scale cattle-specific metabolic pathways
Hillier et al., 2004	Chicken	Drafts genome sequence of red jungle fowl, *Gallus gallus*
Kim et al., 2010	Chicken	Reconstructs metabolic pathway of the chicken genome and chicken-specific pathway genome database

(Grafahrend-Belau et al., 2009b). Another study suggested that metabolic modeling in plants was challenging owing to the unrevealed gene and genome content (Seaver et al., 2012). In this section, we review previous studies on metabolic modeling and reconstruction approaches and discuss the possibility of their application in the field of agriculture (Table 2).

Soil microorganisms

Nitrogen fixation in the nitrogen cycle, a symbiotic association between plant and microorganisms, has been highlighted as it plays an important role in plant growth (Resendis-Antonio et al., 2007). In a 2007 study, the symbiotic microorganism *Rhizobium etli*, related to nitrogen fixation, was identified (Resendis-Antonio et al., 2007). In addition, the metabolic reconstruction and computational modeling approach have been applied to *S. meliloti* to study the symbiotic nitrogen fixation (SNF) as a plausible method for sustainable agriculture and ecosystems (Zhao et al., 2012). In 2014, a review reporting the application of metabolic modeling and discussing the importance of nitrogen-use efficiency (NUE) in maize was published (Simons et al., 2014). This study also proposed an outline for utilizing the transcriptome, proteome, and metabolome datasets for comprehensive understanding of the nitrogen regulation. Future studies regarding agriculturally suitable microorganisms are expected to be advanced so that the metabolic reconstruction and modeling can constitute an *in silico* model, which predicts the interaction between the biological cycle of soil microorganisms and crops.

Plant

Plants are the most fundamental and major food source for all living organisms. Recent food shortage problems, generally observed in underdeveloped and developing countries, have created a need for increasing crop yield. To accomplish this task, it is inevitable to understand the plant metabolism. This requires a metabolic reconstruction through computational modeling, which allows us to comprehend the metabolic networks in the plants (Grafahrend-Belau et al., 2013). The gene annotation of rice, which is the staple food of people in Asia, was completed in 2007 (Ouyang et al., 2006). In 2013, the pathway gene database specialized for rice, riceCyc, was built, enabling us to perform full-scale reconstruction modeling for rice (Dharmawardhana et al., 2013). Further, the first rice metabolic reconstruction was accomplished using the riceCyc database for interpreting the physiological characteristics of two types of tissues from sprouted seeds and photorespiration leaves (Poolman et al., 2013). As a result, this research revealed a part of the vital regulation in photorespiration and explained the usage trait of glycolysis and ethanol fermentation when utilizing oxygen in photorespiration. Besides riceCyc, the specific 'cyc,' as a pathway gene database specialized for particular species, was developed for diverse plants. For example, the Sol Genomics Network clade-oriented database (COD), which targets the Solanaceae family plants, was constructed by integrating the sequence information of several wild tomato and wild potato species (*Solanum phureja*) as hosts (Bombarely et al., 2011). This study simultaneously added microarray data measuring RNA-sequence and gene expressions to basic information of the host plant, and resulted in the pathway/genome database (PGDB) for various plants, such as Lycocyc (tomato), Solacyc (eggplant), Nicotianacyc (tobacco), Petuniacyc (petunia), Capcyc (capsicum), and Potatocyc (potato). This database is open to the public, and it has contributed to the activation of metabolic reconstruction in plants.

Livestock

As a major source of milk and meat, the dairy cattle metabolism has been studied using metabolic reconstruction and computational modeling. The first study on cattle metabolism using computational modeling was published in 1984, which calculated the flux of metabolites, such as glucose, acetate, and β- hydroxybutyrate, in mammary glands (Waghorn and Baldwin, 1984). Through this study, the initial mathematical model for the milk synthetic pathway was developed. Another study attempted to develop a mammary gland metabolism model by using both *in vivo* and *in vitro* data obtained from a previous study and experiment (Hanigan and Baldwin, 1994). In 2007, a study reported the lactating dairy cow metabolism in the whole-animal model, and this dynamic model, which was considered as intermediate between simple and sophisticated models, explained the main metabolic flux and lactate creation according to the consumption of dry matter (Martin and Sauvant, 2007). Similarly, a research has also computationally analyzed the effect of casein and propionate on the mammary

metabolism of energetic nutrients in the whole body (Lemosquet et al., 2009). Meanwhile, a reconstruction of the metabolic pathway based on the cattle genome sequence was performed in 2013 (Seo et al., 2013). The genome sequencing data allowed species-specific reconstruction, compared to that based on metabolic reactions only, and was used for finding efficient feeding (Kim and Seo, 2012). Recently, the genome-scale cattle-specific metabolic pathway based on the updated cattle genome build was reconstructed, and the cattle PGDB was also developed (Kim et al., 2016b).

Poultry farming is another large field, and chicken has been an important livestock as a protein source for humans. In addition, it has a high academic value as a model of embryology and vertebrates (Seo et al., 2009). In 2005, its genome sequencing and analysis was completed. For example, the sequence and annotation of the *Gallus gallus* species, published in 2006, have been widely used in recent times (Hillier et al., 2004). A study on constructing the species-specific PGDB of chicken was carried out in 2010 (Kim et al., 2010). Specifically, the database for this model was constructed from gene data in the National Center for Biotechnology Information (NCBI) (Maglott et al., 2004) and pathway information in KEGG (Kanehisa and Goto, 2000). Then, gene annotation was implemented to the chicken pathway using pathway tool software. This study is interesting because it developed a chicken-specific reconstruction model and suggested a new trend in computational biology that integrates gene and metabolic data.

Conclusions

In this review, we presented the current opinions on metabolic reconstruction in three parts: a review of metabolic reconstruction in relation to modeling, the presentation of tools and software according to the modeling process of metabolic reconstruction, and the contemporary usage of metabolic modeling and reconstruction in the agricultural field. Comprehensively, the utilization of metabolic reconstruction in agricultural research is in a basal phase compared to that in the biological field because of the difficulty in achieving an integrated analysis. This is caused by the complexity of the target and the tendency in conventional agriculture to focus on accumulating data. However, the fundamentals for comprehensive analyses through metabolic modeling have been lately established by constructing metabolic databases such as the species-specific PGDB and metaCyc. Based on the accumulated databases, the next step for applying metabolic modeling into the agricultural field is to develop a complete metabolic model for each plant and species. In a similar way to that followed in microbiology and pharmacy, which use metabolic engineering to produce a desired target, a complete metabolic model for a specific crop may provide the most effective regulatory method for producing functionally optimized crops. Moreover, a model for predicting the phenotype based on genetic information may become important as phenotyping has been recently highlighted for producing a functionally modified plant. For livestock, information derived from the linkage between whole-genome information and metabolism can be utilized for selecting cattle-feeding strategies. This review contributes to encourage the applicability of metabolic modeling in the agriculture field by suggesting the modeling procedure for metabolic reconstruction and presenting diverse useful computational tools for the analysis. Therefore, we expect that this study will help accelerate its utilization in the analysis of agricultural complex systems.

Conflict of Interest

The authors have no conflicting financial or other interests.

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