Prediction of Vanillin Production in Yeast Using a Hybrid of Continuous Bees Algorithm and Flux Balance Analysis (CBAFBA)

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Abstract. Most food and beverage is containing artificial flavor compound. Creation of artificial flavors is not an easy step and it is hardly ever completely effective. In this paper, we introduce an *in silico* method in optimization of microbial strains of flavor compound synthesis. Previously, there are several algorithms such as Genetic Algorithm, Evolutionary Algorithm, OptKnock tool and other related techniques are widely used to predict the yield of target compound by suggesting the gene knockouts. The used of these algorithms or tools is able to predict the yield of production instead of using try and error method for gene deletions. Nowadays, without using *in silico* method, the direct experiment methods are not cost effective and time consumed. As we know, the cost of chemical is expensive and not all flavorist able to afford the cost. However, the main limitations of previous algorithms are it failed to optimize the prediction of the yield and suggesting unrealistic flux distribution. Therefore, this paper proposed a hybrid of continuous Bees algorithm and Flux Balance Analysis. The target compound in this research is vanillin. The aim of study is to identify optimum gene knockouts. The results in this paper are the prediction of the yield and the growth rate values of the model. The predictive results showed that the improvement in term of yield which may help in food flavorings.

Keywords: Bees Algorithm, Flux Balance Analysis, Yeast, Optimization.

1 Introduction

Vanillin is normally used as food ingredient or flavor compound. In order to get vanillin by traditional method of obtaining vanillin is from cured seed pods of the Vanilla

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planifolia (natural vanillin) and via chemical synthesis. As we know, yeast is considered to be a workhorse of the biotechnology industry for the production of many value-added chemical, alcoholic beverages and biofuels [1]. Thus, in this research, *S. cerevisiae* model is used in vanillin. Figure 1 showed the de novo biosynthetic pathway in *S. cerevisiae* for vanillin production. In order to meet the aim of prediction of the vanillin yield by *in silico* method, the algorithm introduced in this paper is a hybrid of Continuous Bees Algorithm and Flux Balance Analysis which able to predict a set of gene knockouts. The contributions of this paper are three fold. First, up to our best knowledge, this method is first used in prediction of biochemical production where no other researchers used this method before. Second, this prediction algorithm implemented in this research is able to predict the gene knockouts in the large number of reactions in *S. cerevisiae* model. Third, the experimental results shown that the prediction algorithm in this research had given a set of relatedness deletion whereby the experimental technique in wet lab can be avoided before the expected result is confirmed. This will contribute in term of cost efficiency where the materials for experiment are expensive.

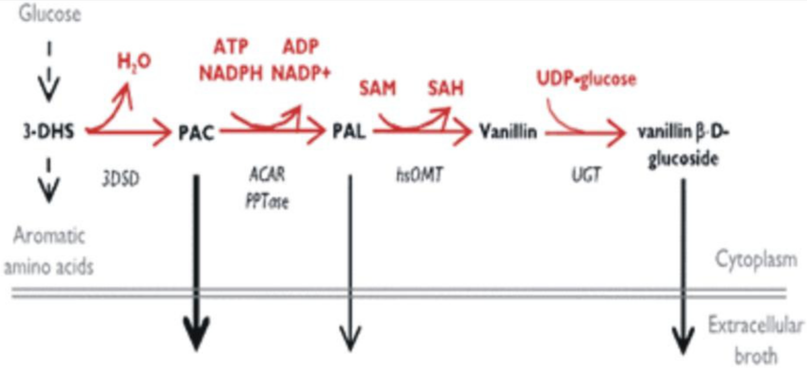


Fig. 1. The *de novo* biosynthetic pathway in *S. cerevisiae* for vanillin production.

Basically, the prediction of biochemical compounds is predicted by several algorithms such as Genetic Algorithm, Evolutionary Algorithm, OptKnock tool and OptGene which are widely used. Unfortunately, there is some limitation of those techniques. In this paper, the limitation of binOptGene is identified. In binOptGene, the representation of population is in binary variable where representation will form a set of “individual” representing a particular mutant. However, in this method the main problem is number of invalid individuals in population is larger and consequently negatively affects the convergence. It happened due to use of penalty functions after evaluation of individuals. Besides that, binOptGene also will suffer of several problems which causes by used of Genetic Algorithm in binOptGene or OptGene itself. One of the limitation of Genetic algorithm is stop criterion of the algorithm is not clear in every problem. In addition, it is tendency to converge towards local optima rather than global optimum of the problem. In the measurement of the fitness in a single right/wrong problem, Genetic algorithm is failed to solve the problem efficiently.

In order to solve limitations, the usage of Bees algorithm is as an optimization algorithm. The Bees algorithm is known as a new population-based search algorithm [2]. This algorithm is able to search optimum solutions in large search space. However, in Bees algorithm the representation method of population is difference from binOptGene where it represented in integer number. In this way, number of genes to be deleted can be directly imposed by changing the size of the individuals. Besides that, Flux Balance Analysis (FBA) is used as an approach that widely used for studying and analyzing biochemical networks, in particularly the genome-scale metabolic network constructions that have been built in the past decade [3]. Flux Balance Analysis also known as a constraint-based modeling approach in which the stoichiometry of the underlying biochemical network constrains the solution [4]. Constraints applied in Flux Balance Analysis are represented in two (2) ways: Firstly, as equations that balance reactions input and secondly output and as inequalities that impose bounds on the system. Basically, this approach is used a mathematical modeling approach for analyzing the flow of metabolites in metabolic network.

2 A Hybrid of Continuous Bees Algorithm and Flux Balance Analysis (CBAFBA)

In this section, we describe the details of the proposed a hybrid algorithm, hybrid of CBAFBA. In CBAFBA algorithm, there are 3 main parts is explained in next subsections: initialization, neighborhood search, and assignment of the remaining bees for random search and obtained the solution of CBAFBA. The Figure 2 shows the flow chart of CBAFBA.

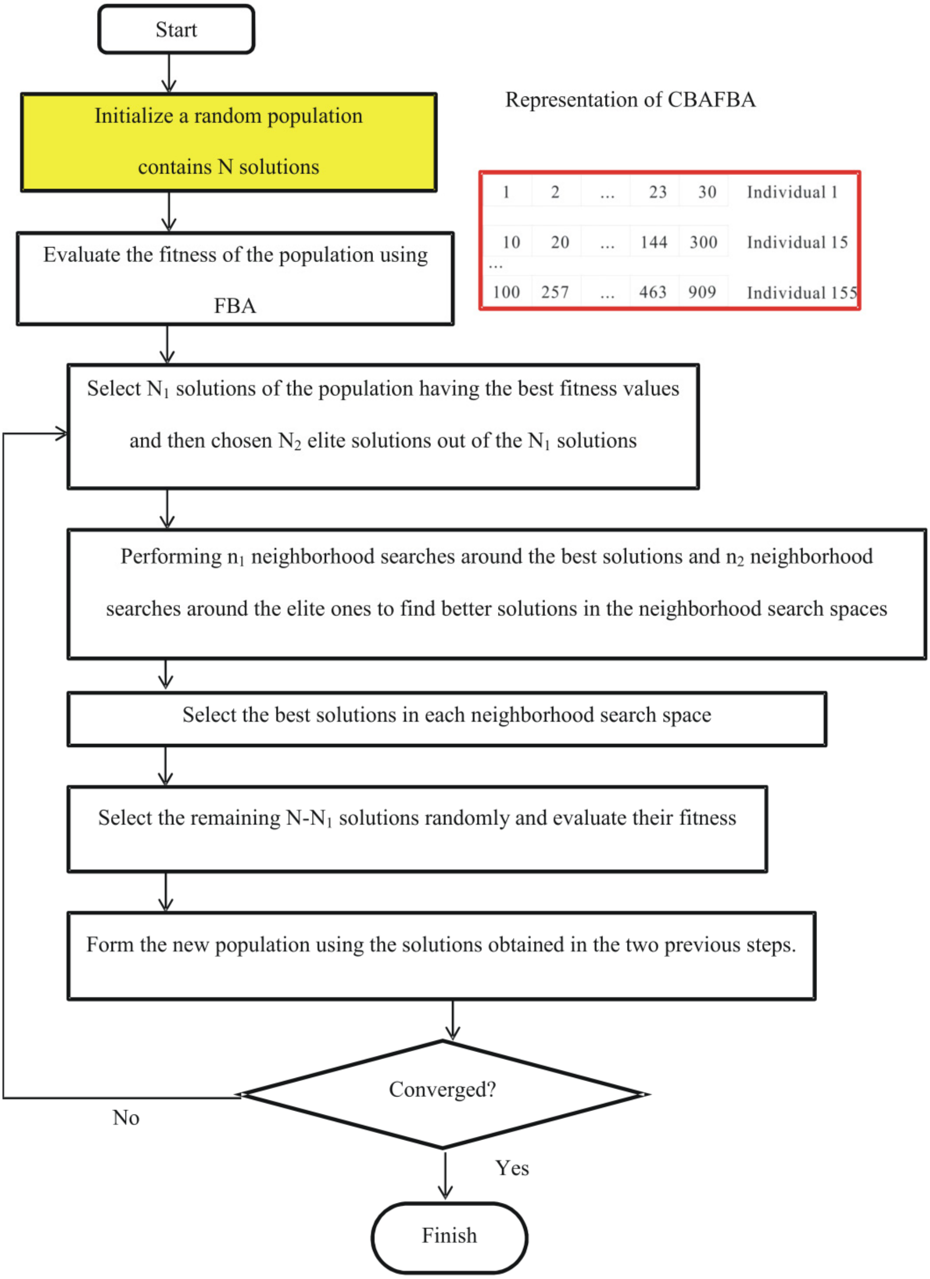
In next subsection, we describe the dataset used in this proposed. Measurement of the evaluation of the result obtained is the optimization of metabolic production method used to determine the growth rate is included in Flux Balance Analysis. The function is defines as below:

Maximize Z
Subject to $$\sum_{j=1}^{N} S_{ij} v_j = 0,\ i = 1, \dots M \qquad (1)$$

Thermodynamic and capacity constraints can be added as below:

α_(j)≤v_j≤β_j,j = 1,…N (2)

The Z is the linear objective function which to be minimize or maximize from particular metabolic engineering design objective to maximization of cellular growth of vanillin. The v_j corresponds to the rate of reaction j and S_{ij} is the stoichiometric coefficient. The different optimal solution obtained when different objective function is applied in optimization function.


Start
Initialize a random population contains N solutions
Evaluate the fitness of the population using FBA
Select N_1 solutions of the population having the best fitness values and then chosen N_2 elite solutions out of the N_1 solutions
Performing n_1 neighborhood searches around the best solutions and n_2 neighborhood searches around the elite ones to find better solutions in the neighborhood search spaces
Select the best solutions in each neighborhood search space
Select the remaining N-N_1 solutions randomly and evaluate their fitness
Form the new population using the solutions obtained in the two previous steps.
Converged?
No
Yes
Finish

Representation of CBAFBA

1	2	…	23	30	Individual 1
10	20	…	144	300	Individual 15
…					
100	257	…	463	909	Individual 155

Fig. 2. The flow chart of CBAFBA

The difference of CBAFBA compare to binOptGene [5] and BAFBA [6] is the representation strategies in initialization of population. In binOptGene, the represented in binary which may cause the population become larger and consequently negatively affect the convergence. In CBAFBA, the population is represented in integer number [7]. The representation is showed in next section. Figure 3 shows the overall of OptGene algorithm which the representation of population is in binary variable. Besides that, the CBAFBA is able to search in global population where it formed a new population of each iteration had been completed. However, in binOptGene which applied Genetic algorithm is tendency to convert into local optimal rather than search for global optimal of the problem [8].

2.1 Initialization

Initialization of CBAFBA is the first step which used to create a random set of list which represent as population. The size of population can be set according to the size of dimension or search space needed. The bigger search space will cause the computational time increases. Therefore the search space is reducing by model pre-processing phase. In this initialization of population, the representation of individual is in integer number. The individuals are composed of integer numbers representing only the genes to be deleted. Therefore, it is based on the relative order in of metabolic model.

2.2 Neighborhood Search

In neighborhood search stage, there are 3 steps of Bees algorithm is executed. The selection of sites which has higher fitnesses, recruitment of bees for selected sites and selection of fittest bee from each search are the step in Bees algorithm. In order to select the sites with higher fitnesses, the sorting function is created whereby it sorts the fitnesses and positions of the population. The sorted list is in descending order.

After the population is sorted according to it fitness, the recruitment stage is begin. This recruitment stage is sending the bees around the fittest site and evaluated the fitness. The fitness of this research is based on the Flux Balance Analysis. In order to prevent very small values of the production at set growth rate, there is a comparison between minimum productions of population with a fixed minimum production.

2.3 Assignment of the Remaining Bees for Random Search and Obtained the Solution of CBAFBA

In this stage, CBAFBA is assigned the remaining bees for random search. The remaining bees are used to find the potential new solutions. The searched of potential new solutions is done around the search space. Again, in this stage the fitness by Flux Balance Analysis is used which will used the minimum production compare with fixed value. After the calculation of the fitness, the list of production is sorted in order to identify the best production. At final of each run, the prediction of the gene knockout list will be generated after the CBAFBA algorithm is completely computed.

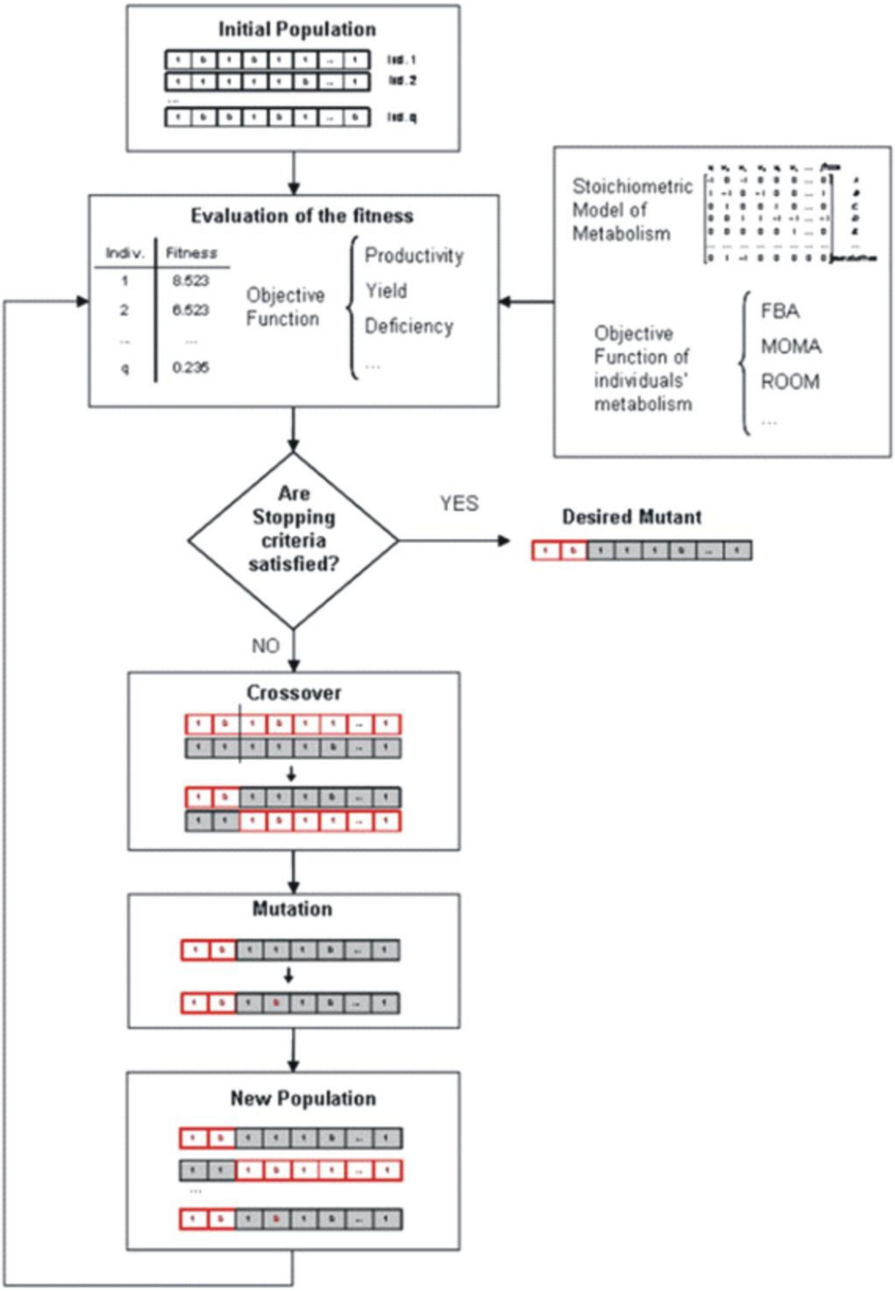


Fig. 3. The overall of OptGene algorithm

3 Results

3.1 Dataset

A model of *S. cerevisiae* dataset is used in the computational algorithms. Basically, *S. cerevisiae* is a type of baker's yeast. This dataset is originally obtained from Kyoto Encyclopedia of Gene and Genomes (KEGG). Yeast dataset from KEGG is then converted into System Biology Markup Language (SBML) format. In the model of *S. cerevisiae* dataset, all the pathways in Baker's yeast are included. From the abundant of pathways included in the dataset, several pathways is excluded or removed to reduce unrelated pathways and minimized the computational time during prediction process in on going. Thus, the model pre-processing is needed. The model pre-processing is include reduce dead-end reactions whereby problem size considerably small compare to initial model.

In this paper, the stoichiometric simulations provide an estimation of possible range of flux values for every reaction in the network. Due to the existence of a large number of alternatives flux routes or path-ways in genome-scale metabolic models require the use of optimization or computational methods to predict the alternative deletion of genes which will help to improve the production. The used of FBA are guaranteed to be optimal, but not necessarily unique due to the existence of a large number of pathways involves [8]. In this paper, the vanillin is the product to be predicted.

3.2 Vanillin Production

Selection of Target Reaction in Vanillin Prediction by Bees Algorithm and Flux Balance Analysis

Here, a core substrate which can contribute to vanillin production is L-phenylalanine which shows in Figure 4. In Figure 4, the formation or production of vanillin is known as biotransformation of aromatic acids. Generally, the production of vanillin increased when the production of L-phenylalanine increased. In the key reaction, phenylalanine is deaminated to transcinnamic acid, which catalyzed by phenylalanine ammonia lysase [9]. The transcinnamic acid then undergoes a chain reaction until reached the vanillin production which show in Figure 4.

In addition, the L-phenylalanine used as precursor to vanillin production in biosynthesis of alkaloid de-rived from shikimate pathway. The L-phenylalanine is synthesis from prehenate where prephenate dehy-dratase is an enzyme involved in the process. Next, the L-phenylalanine is involved in the synthesis of caffeoyl-CoA. There are series of process and enzymes involved in the synthesis of vanillin from caffeoyl-CoA. One of the enzymes involved is caffeoyl-CoA O-methyltransferase which catalyze the conversion of caffeoyl-CoA into feruloyl-CoA.

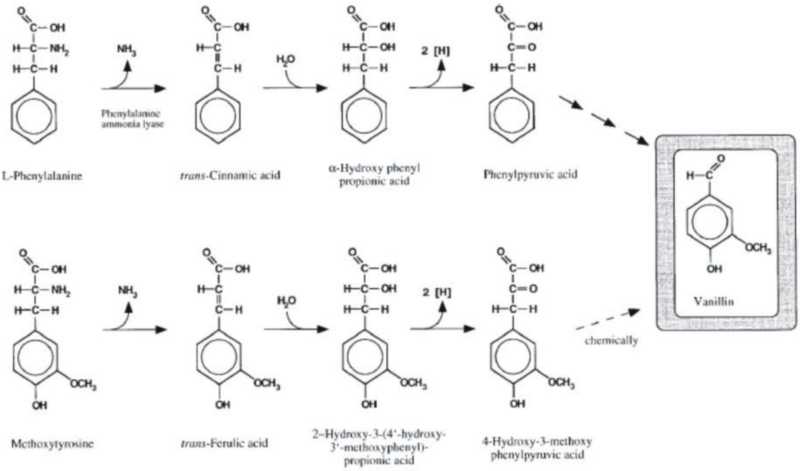

NH3
Phenylalanine ammonia lyase
H2O
2 [H]
L-Phenylalanine
trans-Cinnamic acid
α-Hydroxy phenyl propionic acid
Phenylpyruvic acid
Vanillin
NH3
H2O
2 [H]
chemically
Methoxytyrosine
trans-Ferulic acid
2-Hydroxy-3-(4'-hydroxy-3'-methoxyphenyl)-propionic acid
4-Hydroxy-3-methoxy phenylpyruvic acid

Fig. 4. Main contribution of vanillin production is L-phenylalanine

Therefore, the selection of synthesis of L-phenylalanine is contributed to vanillin production. In order to select the substrate and target reaction, glucose and prephenate dehydratase had been selected, respec-tively. The purpose prephenate dehydratase been chosen is due to this enzyme will affect the production of L-phenylalanine, a substrate to produce vanillin. Besides that, the purpose of glucose reaction been chose is due to the main substance of mostly biosynthesis in any pathways.

Selection of Gene Knockouts List Based on Growth Rate Prediction Using Flux Balance Analysis

Prediction strategies described in this work are based on the assumption that microbial cells would evolve in higher growth rates and biochemical production. As we knows, knockout mutants that force the coupling between biomass and biochemical production allow researcher to use growth rate as a selective pressure and find adaptively evolved strains with improved growth rates and production capabilities [10].

Figure 5 shows Average of frequency for predicted result cases in each growth rate values from same biochemical product which is vanillin. Based on that result, the higher growth rate is 1.7023 which indi-cates the cellular cell is alive and able to produce the desired product at optimal rate. Basically, the bio-chemical production would increase along with cellular growth rate [10].

On the other hand in this research, the minimum growth rate is -5.4019e-013 where this set of genes knockout is eliminate. The reason of the elimination is due to the cellular cell is unable to live or cell is death. The elimination is also because of the deletion of lethal gene. This deletion may cause the cellular cell die and fail to produce desire biochemical production.

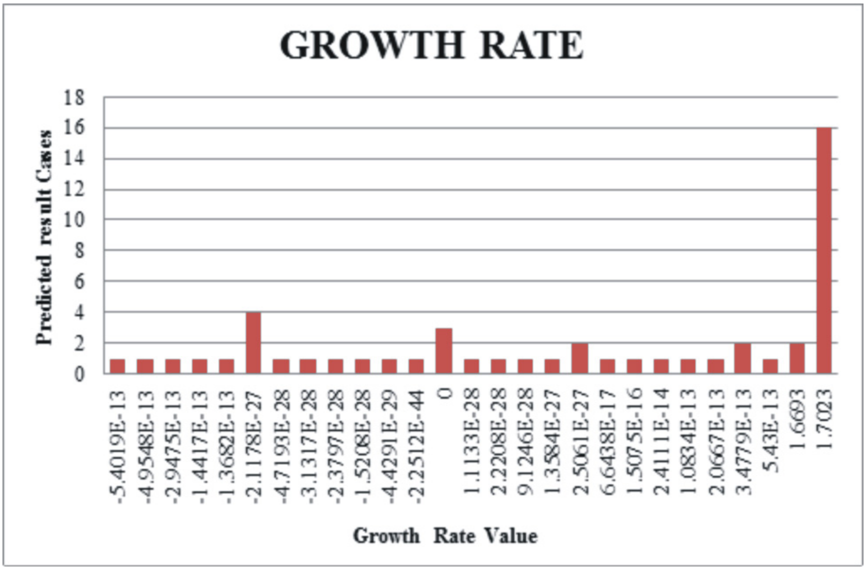


Fig. 5. Average of frequency for predicted result cases in each growth rate value

Selection of Genes Knockout List Based on Production Rate

In general, the knockout strategy is consists of two approaches; reaction-based deletion and gene-based deletions. In this section, the genes-based deletion approaches are preferred compare to reaction-based deletion. As we know, the relationship between genes, proteins and reactions is not one-to-one. In other words, a metabolic reaction usually carries out by one or more enzymes where each of it can comprise to produce multiple gene products (proteins) [10]. The removal of multiple genes may affect the additional reactions by removal of additional reactions [10]. The worst scenario for removal of additional reactions is the reaction for desired product been removed incidentally.

After applied the proposed method in yeast model, the result shown that there are 3 mutants are obtained. Table 1 summarizes three of the identified gene knockout strategies for L-phenylalanine (i.e mutant A, B, and C). Based on the result obtained from CBAFBA, maximum yield is 0.19466 for three mu-tants in the phenylalanine target reaction. Here, the result for mutant A suggests that removal of shikimate pathway reaction from the network. In mutant A, ARO4 gene is being removed which it is encoded into 3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase (DAHPS). Experimentally from web lab, DAHPS is used for condensation of erythrose-4-phosphate and phosphoenolpyruvate to 3-deoxy-d-arabino-heptulosonate-7-phosphate (DAHP) [11].

The removal in mutant A is less intuitive strategy which focuses on inactivating phosphoenolpyruvate (PEP) consuming reactions rather than eliminating competing by product mechanism. With this strategy, some researches assuming that the maximum biomass yield could be attained. Note that the predicted yield in CBAFBA is assumed only by the theoretical maximum where further experiment from wet lab is needed for proofing purpose. Figure 6 reveals the flux distribution of the mutant A.

Table 1. List knockout for vanillin case study which control by L-phenylalanine compound

L-Phenylalanine

Mutant	Gene	Knockouts	Enzyme
A	ARO4	PEP + D-erythrose 4-phosphate + H_2O = DHAP + phosphate	3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase
B	BDH1	(R,R)-Butane-2,3-diol + NAD+ = (R)-Acetoin + NADH + H+	(R,R)-butanediol dehydrogenase
C	ARO10	Ehrlich pathway	2-isopropylmalate synthase

Second gene deletion is removal of butanoate metabolism pathway whereby CBAFBA suggested dele-tion of (R,R)-butanediol dehydrogenase. The gene involve in encoded (R,R)-butanediol dehydrogenase in S. cerevisiae is BDH1. This enzyme involve in formation of (R)-acetoin from (R,R)- butane-2,3-diol. In order to obtain the (R)-acetoin in this reaction, the (R,R)-butanediol dehydrogenase is dependent on NAD+ which is the co-enzyme for butanoate metabolism [12]. Theoretically, this reaction assumed to be affected the glycolysis pathway in order to produce PEP. In glycolysis pathway, NAD+ is used as co-enzyme in the conversion of glyceraldehyde-3-phosphate into 3-phospho-D-glyceroyl- phosphate. The following balanced equation shows that the oxidation of glucose to pyruvate [13].

$$\begin{aligned} &C_6H_{12}O_6 + 2NAD^+ + AADPM_g{}^- + 2H_XPO_4^{3-X-} \\ &\qquad \rightarrow 2CH_3COCO_2^- + 2NADH + 2ATPM_G^{2-} + 2H_2O + 2_XH^+ \end{aligned} \tag{3}$$

The strategy assumed by CBAFBA in mutant B shown that the production yield is 0.19466 and growth rate is 1.7023 respectively after BDH1 gene is deleted. Figure 7 shows the glycolysis pathway in *S. cerevisiae*.

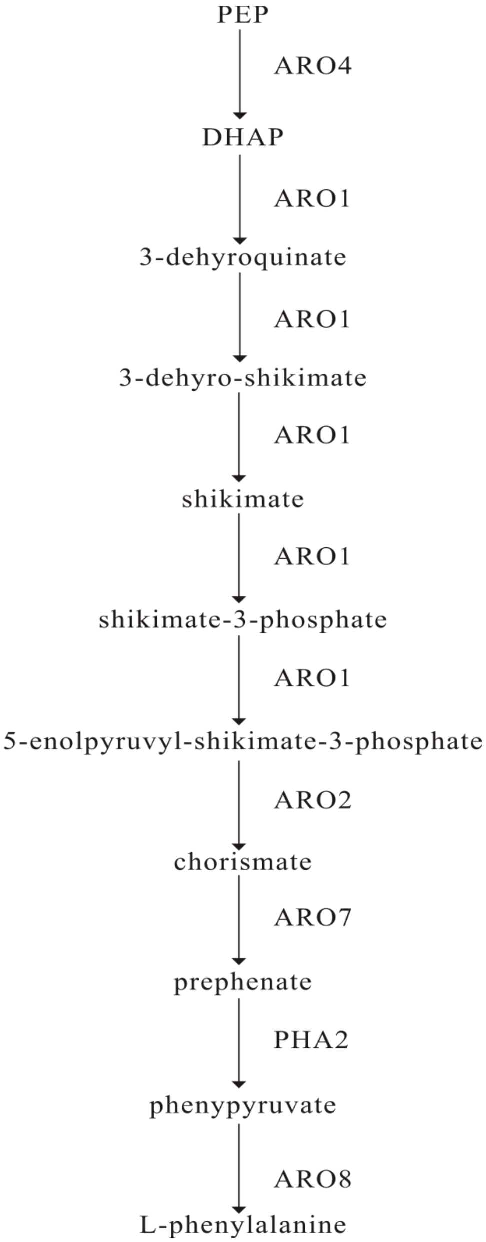


Fig. 6. The flux distribution of the mutant A

Third mutant (mutant C) suggests that deletion of ARO10 gene. The deletion of ARO10 gene is directly remove Ehrlich pathway of *S. cerevisiae*. The Ehrlich pathway is chemical reactions and pathways involved in the catabolism of amino acids to produce alcohols with one carbon less than the starting amino acid. The catabolism process is involving the breaking down of molecules (amino acids) into smaller units (fusel alcohols). In S. cerevisiae, amino acids that assimilated by the Ehrlich pathway is taken up slowly throughout the fermentation time [14]. The amino acids usually taken up from Ehrlich pathway are valine, leucine, isoleucine, methionine, and phenylalanine. This will affect the production of the L- phenylalanine, where the yield will decrease if the Ehrlich pathway fails to be removed.

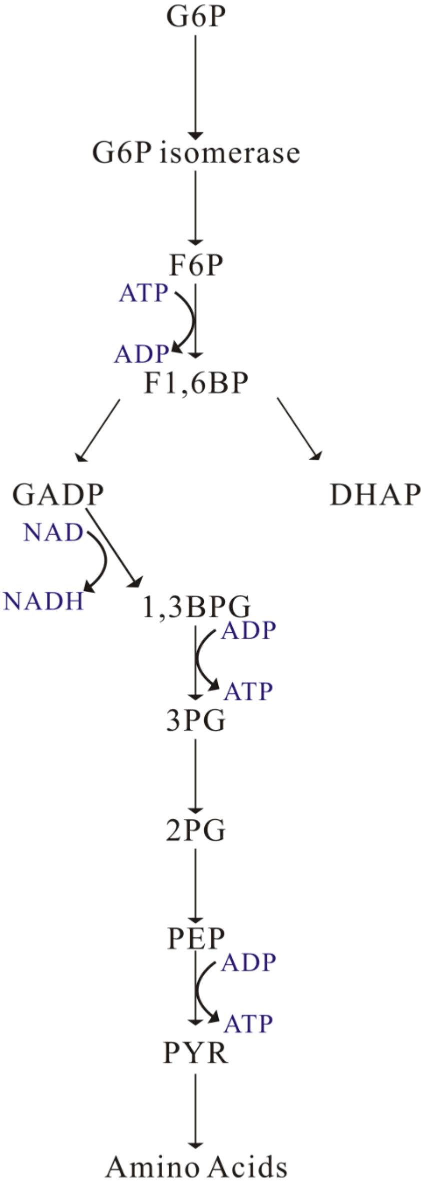


Fig. 7. The glycolysis pathway in *S. cerevisiae*

Besides that, in aerobic glucose-limited chemostat, phenylalanine is used as sole nitrogen sources, where phenylalanine is converted predominantly to fusel acids and only very low concentrations of fusel alcohols are formed [14]. However, without glucose-limited chemostat of *S. cerevisiae*, growth is pre-dominantly fermentative, and when phenylalanine is the sole nitrogen source, it is converted into a mix-ture of phenylethanol and phenylacetate [14]. This proves that the deletion of ARO10 is assumed to be increasing the phenylalanine yield. Figure 8 shows overall of the Ehrlich pathway.

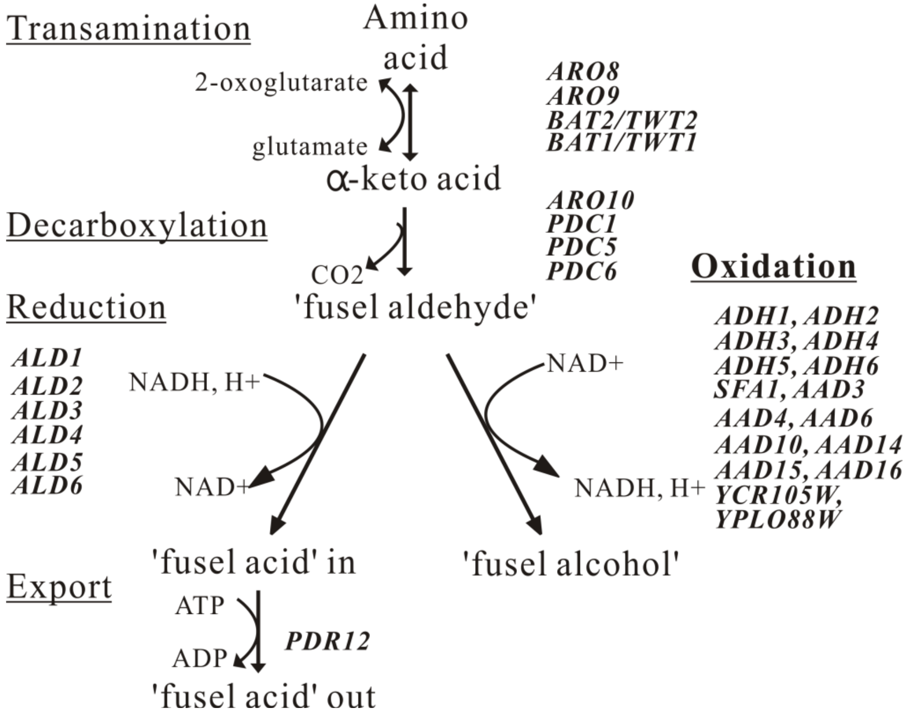


Fig. 8. Overall of the Ehrlich pathway

Table 2 shows the biomass overall yield of vanillin in batch cultivation after simulated by OptGene tool. The production of vanillin β-D-glucoside (VG) in table 2 shows the minimum yield (μmax) is 0.10 (VG1) and the maximum yield is 0.2 (VG2). All stains (VG0, VG1, VG2, VG3, and VG4) are involved in either the removal or overexpression of pyruvate decarboxylase (PDC) and glutamate dehydrogenase (GDH). Based on Brochado and his colleagues, they believe that by in silico analysis, PDC was found as a target to increase formation of VG considering both respiratory and respire-fermentation reference flux distribution. By comparing with the proposed method in this research, the formation of vanillin is acceptable. The μmax of this research is reached 0.19466 where slightly less than μmax for VG2 in Table 2. However, the μmax of this research is higher than μmax of VG0, VG1, VG3, and VG4 in table 2. Based on Brochado and his colleagues [8], they agreed that VG4 strain showed significantly improved in cellular fitness compare VG2 strain. This is due to the yield of biomass on substrate (glucose) is higher compare other strains.

Table 2. Biomass Overall Yield of Vanillin in Batch Cultivation [7]

Strains	Engineered Genotype	μ_{max}	$Y_{S\,X}$	$Y_{S\,Etoh}$	$Y_{S\,gly}$
VG0		0.14	0.10	0.23	0.05
VG1	gdh1Δ	0.10	0.07	0.25	0.03
VG2	pdc1Δ	0.20	0.14	0.23	0.07
VG3	pdc1Δ gdh1Δ	0.11	0.10	0.27	0.05
VG4	pdc1Δ gdh1Δ ↑GDH2	0.17	0.17	0.25	0.07

Overall, suggested genes deletion by proposed method are included ARO4, BDH1 and ARO10 genes which can contribute to formation of L-phenylalanine, precursor for biotransformation of aromatic amino acids to produce vanillin. In theory, the more L-phenylalanine compound is produced, the more vanillin.

4 Conclusion

As a conclusion, our proposed CBAFBA which predicts the gene knockouts by *in silico* method showed to perform better in terms of time and cost-effective. The strategies applied in CBAFBA could lead to chemical production in *S. cerevisiae*. This is done by ensuring that the drain towards the metabolites/compounds necessary for growth resources such as carbons and energy must be accompanied. However, it should be noted that our proposed is deal with the reactions in the model not the real experiment. Therefore, the experiments are needed to carrier out to validate the deletion technique suggested by *in silico* technique. Specifically, CBAFBA is pinpoints which reactions needed to remove from a metabolic network, which can realized and contribute to yield the product by gene deletions where it associated with the identified the functionality. Reminder, it is important to note that the suggested gene deletion strategies must be interpreted carefully. For instance, in many cases the deletion of gene in one branch of a branched pathway is equivalent to the significant up regulation in the other [15]. Lastly, the suggested set of gene(s) deletions is not always uniquely specified. Thus, the technique of identification of most economical gene set accounting for enzyme and multifunctional enzyme needs to be made.

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