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Research article

Sustainable biosynthesis of lycopene by using evolutionary adaptive recombinant *Escherichia coli* from orange peel waste

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ABSTRACT

This study aimed to evaluate the hydrolysates from orange peel waste (OPW) as the low-cost carbon source for lycopene production. Initially, the dilute acid pretreatment combined with enzymatic hydrolysis of OPW resulted in a total sugar concentration of 62.18 g/L. Meanwhile, a four-month adaptive laboratory evolution (ALE) experiment using a d-galacturonic acid minimal medium resulted in an improvement in the growth rate of our previously engineered *Escherichia coli* strain for lycopene production. After evolutionary adaptation, response surface methodology (RSM) was adapted to optimize the medium composition in fermentation. The results obtained from RSM analysis revealed that the 5.53 % carbon source of orange peel hydrolysate (OPH), 6.57 g/L nitrogen source, and 30 °C temperature boosted lycopene production in the final strain. Subsequently, the optimized treatment for lycopene fermentation was then conducted in a 5 L batch fermenter under the surveillance of a kinetic model that uses the Logistic equation for strain growth ($\mu m = 0.441\ h^{-1}$), and Luedeking-Piret equations for lycopene production ($Pm = 1043\ mgL^{-1}$) with growth rate constant ($\alpha = 0.1491$). At last, lycopene biosynthesized from OPH was extracted and analyzed for qualitative validation. Likewise, its data on phytic acid (between 1.01 % and 0.86 %) and DPPH radical scavenging (between 38.06 % and 29.08 %) highlighted the better antioxidant capacity of lycopene. In conclusion, the OPH can be used as a fermentation feedstock which opens new possibilities of exploiting fruit crop residues for food and pharmaceutical applications.

1. Introduction

Lycopene is a kind of carotenoid (molecular formula of $C_{40}H_{56}$) belonging to lipophilic isoprenes, and it is nearly insoluble in

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methanol, ethanol, and water [1]. It gives tomatoes, apricots, and watermelons their red color and is also considered a source of functional ingredients like antioxidant agents [1]. Lycopene possesses better antioxidant activity than that of other carotenoids, including α-carotene, zeaxanthin, β-carotene, lutein, and β-cryptoxanthin [2,3]. In addition, the growth of the lycopene market is attributed to its therapeutic potential that prevents the oxidation of low-density lipoprotein and cholesterol. Agriculture Organization and United Nations Food have also identified lycopene as a class A nutrient mainly due to its myriad types of physiological characteristics, and it is widely used in agriculture, food, cosmetics, and other industry sectors, resulting in rising consumer demand [3]. Globally, lycopene production is facing an increasing trend, and its market is expected to expand at a 3.5 % growth rate annually. In 2023, it is estimated for the lycopene market size to reach the value of 133 million dollars, hence the higher demand value is supported. At industrial scale, the lycopene has been isolated by using different extraction methods from various plants, like grapefruit (0.014 g/kg), red dragon fruit (0.073 g/kg), tomato (0.371 g/kg), watermelon (0.6 g/kg), and papaya (0.5 g/kg), etc. [4–6]. However, it is found that the extraction of lycopene from plants seems to be inappropriate to fulfil the increased lycopene demand for commercial use. This might be due to a limited supply of natural bioactive compounds and high processing cost [7]. Chemical synthesis has also faced some difficulties, like high cost, low product stability, poor product quality and yield. Therefore, the microbial synthesis of lycopene has gained growing popularity because of its numerous advantages, such as controlled cultivation, cost effectiveness, and production time minimization and engineered *Escherichia coli* (E. coli) appears to be ideally suited for this purpose [8].

Interestingly, E. coli has been extensively studied due to its ability to produce a large number of isoprenoids. For instance, Xu et al. [9] found that supplementation of 20 % glycerol in culture medium led to the highest degree of lycopene production up to 925 mg/L by engineered E. coli [9]. A similar finding has been reported by Kim et al. [10] who demonstrated that the supplementation of arabinose and glucose in fermentation medium enabled the efficient production of lycopene (1350 mg/L) by engineered E. coli [10]. Even though the applicability of E. coli has been investigated for resolving the problem of low product yield, the expenditure on fermentation substrate limits the commercialization of microbial pigments. Efforts have been made to enhance the lycopene yield and, decrease process costs, by using agricultural wastes that have garnered huge attention around the world. For example, citrus as one of the most popular fruits is produced by approximately 124.73 million metric tons each year [11]. Although fruit crops are a significant part of food production, the lack of appropriate handling techniques for fruit waste leads to an increase in the volume of food losses and accumulation of waste. Meanwhile, the losses arising from fruits are estimated to be around 16 % of total food waste, which further contributes to around 6 % of emissions of greenhouse gases (GHG) both in developed and developing countries [12]. Among all fruit waste, orange peel waste (OPW) is generated in large quantities from the fruit processing industry.

To economize the process and reduce environmental pollution, orange peel waste has been valorized into value-added products like biochemicals, biopolymers, biofuels, etc. [13]. Moreover, the orange peel waste accounts for between 40 and 50 % of fruit waste, and it is often discarded [13]. OPW is a rich source of fermentable sugars and can be used in the production of several value-added bio-products [14]. A large share of about 18 % of total citrus fruit production has been utilized for commercial usage, particularly for juice production [15]. Hence, the utilization of solid wastes of orange peels into a useful product is vital. Orange peel wastes are rich in complex carbohydrates (pectin, 25 %; sugar, 23 %; cellulose, 22 %; and hemicellulose, 11 %), minerals, and numerous phytochemicals, which are suitable for making carbon-rich fermentation liquid, and used as a carbon source for lycopene [16,17]. In addition, response surface methodology (RSM) has been used to optimize the performance of bioprocess in cost-effective- and time-manner [18].

In the present study, the application of orange peel waste for lycopene production by E. coli was investigated. This study is important due to the environmental concern to minimize waste production by using it as a potential and low-cost substrate for microbial fermentation. Firstly, dilute acid pretreatment combined with enzymatic hydrolysis was performed to obtain the maximum sugar from OPW. Then, our previous E. coli strain was selected for adaptive laboratory evolution on a d-galacturonic acid minimal medium for four months. D-glucose and d-galacturonic acid are the prime carbon sources found in orange peel hydrolysate, therefore adaptive laboratory evolution of E. coli strains was performed. Thereafter, medium optimization using orange peel hydrolysate was performed via RSM methodology. Later, the kinetic study was conducted for lycopene production from OPH in 5 L batch fermentation. At last, this study also sought to determine the antioxidant activity of lycopene biosynthesized from OPH. In short, this will help to determine its therapeutic properties which may be useful in food and pharmaceutical industries.

2. Research method

2.1. Microorganism and inoculum preparation

Escherichia coli DH416 strain used in these experiments has been previously engineered in the State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, China [19]. Table S1 shows the strains and plasmids in detail. Plasmid construction was carried out by E. coli DH5α, DH1, and DMT. The company GENEWIZ Co., China manufactured all the primers. Three heterogeneous expression cassettes carrying nine genes, including mvaE, mvaS, mvaK2, mvd1, fni, mvk, idsA, crtI, and crtB were introduced into recombinant E. coli strain to produce lycopene [19]. Initially, it was first cultivated on a Luria broth agar plate having NaCl (10 g/L), tryptone (10 g/L), yeast extract (YE) (5 g/L), and agar (20 g/L) and the pH was 7.0. For the duration of 36 h, the cells were allowed to grow at 30 °C.

2.2. Reagents, feedstock, and preparation of orange peel hydrolysate

Sigma-Aldrich (USA) supplied the commercial-grade lycopene. The local market in Shanghai was the main supplier of orange peel. The orange peels were chopped into small pieces of a particle size of 2 mm. Afterward, 1 L of water was used to dissolve the 500 g

chopped peels and the addition of 1.0 % sulfuric acid was executed after mixing with water and performing the hydrolyzation for 30 min and the selected temperature was 121 °C. Later on, the enzymatic hydrolysis of this resulting OPH was carried out in 100 mL glass bottles, consisting of hydrolysate with varying concentrations of three enzymes, named as pectinase, cellulase, and β-glucosidase respectively. Then, the oven was used to heat (50 °C) the solution in a glass bottle and then stir it at 500 rpm with the aid of a magnetic stirrer.

2.3. Characterization of orange waste

The proximate analyses of experimental specimens, namely orange peel (OP) and orange peel hydrolysate (OPH) were carried out according to the method mentioned by Khedkar et al. [20], as shown in Table 1.

2.4. Adaptive evolution

At the initial stages of adaptive evolution, the M9 minimal medium containing 2 g/L of d-galacturonic acid was used to culture the strain which was further incubated at 30 °C. The cultivation conditions were: temperature 30 °C, pH 7, and stirring at 200 rpm. The optical density was evaluated at 600 nm for adaptive evolution. Each batch culture of *E. coli* was started at a cell density of 0.4 (OD600). In the first stage of adaptation, the cells were subcultured into the minimal medium with 2 g/L of d-galacturonic acid for 10 transfers. Then, in the second, third, and fourth stages of adaptation, the cells were further subcultured into the minimal medium with 4 g/L, 5.5 g/L, and 7 g/L of d-galacturonic acid for each successive 15 transfers. After the evolution, adapted cells were streaked on minimal medium agar plates with 7 g/L d-glucose and 7 g/L of d-galacturonic acid to isolate single colonies. The specific growth rate was analyzed after cultivation. Later, the strain with the maximum specific growth rate was considered for further experiments.

2.5. Medium preparation for culturing and fermentation

OPH was used as the main carbon source with distilled water as per RSM ranges in culture media. RSM optimized the composition of the culture medium for fermentation in which the OPH was mixed with water and used as the main carbon source. To get lycopene, the cells were cultured at 200 rpm at 30 °C in a flask with 50 mL lycopene-producing medium, consisting of 15 g/L of tryptone, 12 g/L of yeast extract, 5 g/L of $NaH_2PO_4.2H_2O$, 7 g/L of K_2HPO_4. $3H_2O$, 2.5 g/L of NaCl, 5 g/L of Tween 80, 10 g/L of glycerol, 0.5 g/L of $MgSO_4$, 2 g/L of glucose, and 2 g/L of L-arabinose, and the inoculum concentration was 4.0×10^7 cells/ml (the cells were grown to OD600nm = 0.05) [19]. For plasmid maintenance, three different kinds of antibiotics, named ampicillin (50 gm/L), chloramphenicol (25 gm/L), and kanamycin (25 gm/L) were used. The initial working volume of 3 L was selected for the batch culture that took place in a 5 L stirred-type bioreactor. Meanwhile, the main objective of using optimized RSM treatment in the batch fermenter was to achieve maximum lycopene production. During fermentation, the optimum temperature and pH for culture incubation were 30 °C and 7.0 (adjusting through 2.0 M NaOH). The combination of aeration rate (1.5 vvm) with agitation speed (200 rpm) was used in a 5 L bioreactor.

2.6. Analytical methods

2.6.1. Evaluation of biomass dry cell weight (DCW)

This method was commonly used to detect the biomass. 1 mL of broth was poured in a weighted centrifuge tube and centrifuged the tube at 13,000 rpm and the time duration and temperature for centrifugation were 5 min and 4 °C, respectively. Cell pellets were separated and washed twice in phosphate buffer solution (PBS). Then, the cell pellets were incubated in the dry oven at 100 °C until a constant weight was obtained.

Table 1
Characterization of orange peel OP and OPH.

Parameters	Orange peel	Orange peel hydrolysate
Total Solids	21.08 %	10.86 %
Total Sugar	10.3 %	62.18 g/L
Reducing Sugars	–	56.23 g/L
Protein	7.4 %	2.8 %
Lipid	3.69 %	–
Cellulose	30.32 %	–
Hemicellulose	11.46 %	–
Lignin	7.3 %	–
Pectin	21.68 %	-
Limonene	3.48 %	0.14 %
Furfural	–	0.08 %
5-hydroxymethylfurfural	–	0.10 %

[a]The values of OP were calculated based on dry mass.

2.6.2. Determination of lycopene

Alper et al. [21] demonstrated the method to quantify the lycopene content produced in recombinant *E. coli* strains [21]. Centrifugation technique was used to harvest the 250 μL of recombinant *E. coli* cells and then spun it at 12000 rpm for 5 min. Afterward, the resulting cell pellets were washed with distilled water. Later, these pellets were suspended in acetone, where it incubated in the water bath at 55 °C for 15 min in the dark. After 15 min, it was subjected to centrifugation at 13000 rpm for 10 min. Then, the supernatant was collected in the tube. Then the spectrophotometer was used to check the lycopene content at 474 nm by observing the absorbance related to the resulting extract. The absorbance value was converted to lycopene concentration by standard curve and this standard curve was formed based on commercial grade lycopene.

2.6.3. Evaluation of sugar content

The dinitrosalicylic acid DNS method presented by Miller et al. [22] was exploited to measure the reducing sugar concentration of cell-free supernatant, whereas the method reported by Li et al. [23] to determine the both conversion and utilization rate of reducing sugar was followed in the present study. For the determination of total sugar concentration, phenol-sulfuric acid method was used as described in the previous study [20].

2.6.4. DPPH activity and phytic acid test

In this study, the method used by Tang et al. [24] was followed in which 2, 4-dinitrophenylhydrazone and hydroxamic acid were employed as chemical agents to determine the pyruvate and acetyl acid [24]. In this procedure, 100 mL of methanol was used to dissolve the 24 mg of DPPH to make the stock solution. After this, the 3 mL of DPPH solution was combined with different concentrations (μL) of lycopene extract. Meanwhile, the standard solution was formed, consisting of 3 mL of solution consisting of DPPH in 100 μL of methanol. Then, this tube was placed in darkness for 30 min. The absorbance was calculated at 517 nm. The formula used to determine the antioxidant percentage was:

$$\%\ \text{of antioxidant activity} = [(\text{Ac} - \text{As}) \div \text{Ac}] \times 100 \tag{1}$$

Ac = Absorbance of control reaction, and As = Absorbance of testing specimen.

On the other hand, the phytic acid content was determined by the following methods in previous studies [25].

2.7. RSM based experimental evaluation

Response surface methodology RSM was utilized as an experimental design by using software named Design Expert 8®. The mentioned approach was extremely flexible as it allowed many parameters to participate at once in a desired response to optimize the whole process in a sophisticated way. The selection of this approach was executed for this present study to get optimum lycopene production. Mainly, three variables significantly affected biomass development and lycopene production, namely A OPH (2%–8%), B YE nitrogen source (Yeast extract) (2.5%–7.5 %), and C temperature (25–35 °C). In Design Expert 8®, a three-level factorial design (central composite design) was used to design 34 experimental runs.

2.8. Statistical study

Statistical analysis was used to evaluate the RSM model and the response values after analysis had the potential to determine the model coefficients with the aid of Design Expert 8®. Reliability and accuracy of findings were determined by the analysis of variance (ANOVA).

2.9. Kinetic study

Three kinetic models were developed for the measurement of the substrate (OPH), product (lycopene), and biomass, respectively. To predict key kinetic parameters, such as microbial growth, substrate consumption, and natural products production three equations, named the Logistic equation, the Luedeking-Piretand equation, and the modified Luedeking-Piret equation were used [26–29]. All the kinetic model results were visualized, and coded by using software, known as MATLAB.

2.9.1. Kinetic evaluation of cell growth

Logistic equation is frequently used to describe the biomass concentration in the batch lycopene fermentation system. Logistic equation is given below coupled with initial conditions X = X0; t = 0

$$X(t) = \frac{X_0 e^{\mu t}}{\left[1 - \left(X_0/X_{max}\right)(1 - e^{\mu t})\right]} \tag{2}$$

In this equation, X_{max}, X_0, μt, and t represent the maximum biomass concentration (g/L), initial concentration of cells (g/L), maximum biomass growth rate (μ, h^{-1}), and time for fermentation (h). This equation provided the sigmoidal variation that may characterize the transition from exponential to stationary phase.

2.9.2. Kinetic evaluation of product formation

This Luedeking-Piret equation adequately describes the product formation. This model explained the product formation relating to cell growth with a focus on both growth-associated constant (α) and non-growth-associated constant (β) in the batch fermentation.

$$P(t) = P_0 + \alpha X_0 \left\{ \frac{e^{\mu t}}{\left[1 - \left(X_0/X_{max}\right)(1 - e^{\mu t})\right]} - 1 \right\} + \beta \frac{X_{max}}{\mu} ln\left[1 - \left(X_0/X_{max}\right)(1 - e^{\mu t})\right] \quad (3)$$

Whereas, the concentration of the product and the initial P value are denotated as P and P_0, respectively. And α is growth-associated product formation coefficient (mg/g), whereas β is non-growth-associated product formation coefficient (mg/g·h) in the Luedeking–Piret model.

2.9.3. Kinetic evaluation of sugar uptake

The total sugar uptake is evaluated by the well-known modified Luedeking-Piret equation. This equation considers the substrate conversion to product and biomass. Meanwhile, the relation of product development with respect to total sugar concentration was explained by two constants, γ (g/g) and δ (g/g/h) both of these are substrate kinetic constants.

$$S(t) = S_0 - \gamma X_0 \left\{ \frac{e^{\mu t}}{\left[1 - \left(X_0/X_{max}\right)(1.0 - e^{\mu t})\right]} - 1 \right\} - \delta \frac{X_{max}}{\mu} \ln\left[1 - \left(X_0/X_{max}\right)(1.0 - e^{\mu t})\right] \quad (4)$$

In this equation, S, S0 and μ represent the concentration of substrate (g of substrate/kg solution), initial concentration of substrate, specific growth rate ($\mathbf{h^{-1}}$) of considered microbe.

3. Results

3.1. Saccharification of orange peel waste

Orange peel waste primarily consists of pectin, cellulose, hemicellulose, and lignin. Therefore, saccharification is considered a viable option to get a higher concentration of fermentable sugar from orange peel wastes. Saccharification was executed by various physical, chemical, and biological means. However, biological (enzymatic hydrolysis) and chemical (dilute acid treatment) pretreatment have gained growing popularity due to their efficient bioconversion of lignocellulosic biomass to achieve a higher level of total soluble sugar [20]. On considering this, the orange peel waste was subjected to dilute sulfuric acid treatment (pretreatment temperature of 121 °C, 1 % sulfuric acid for 30 min) which resulted in achieving 42.21 g/L of total sugar from orange peel. During enzymatic hydrolysis (3 mg cellulase, 1 mg of pectinase, and 1 mg of β-glucosidase), the maximum total sugar of 62.18 g/L (mainly galacturonic acid and glucose) was obtained from the sample that was already pretreated by dilute acid. The concentration of total sugar and reducing sugar is presented in Table 1.

3.2. Shake flask studies for assessing lycopene production by Escherichia coli strain DH416

The DH416 strain was assessed for its ability to consume d-galacturonic acid and further fermented into lycopene. In fermentation, 0.2 % of arabinose serves as an inducer during the induction phase. Highly pure d-galacturonic acid was used as a substrate for the growth of strain DH416, the rapid consumption of d-galacturonic acid was noticed and nearly the entire d-galacturonic acid (14 g/L) was depleted in 28 h with a significant rise in cell growth ((Fig. 1(A)). Moreover, an OD600 of 22 was recorded in 14 h until an

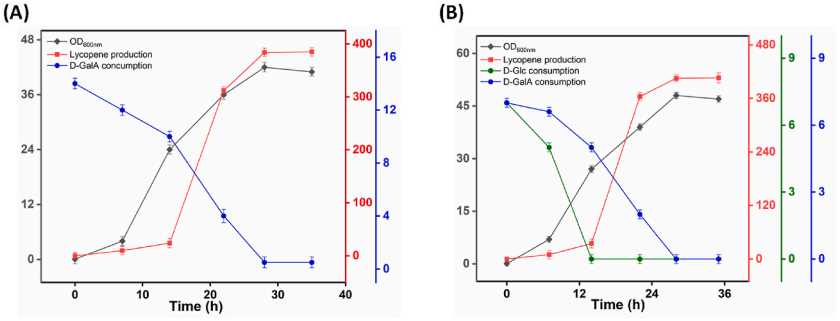

Fig. 1. Production of lycopene by DH416 strain in shake flask. (A) lycopene level (mg/L), growth rate of DH416 strain on pure d-galacturonic acid and d-galacturonic acid concentration (g/L); B) lycopene level (mg/L), growth rate of DH416 strain on the mixture of pure d-galacturonic acid and glucose and concentration of d-glucose (g/L) and d-galacturonic acid (g/L). Induction of L-arabinose was carried out at 14 h.

absorbance at OD600nm reached to 40. The starting pH was 7.2 and dropped below 5.5 after 36 h. It was observed that 312 mg/L of lycopene was accumulated at 24 h and, after 36 h of fermentation, the highest lycopene concentration of 385 mg/L was achieved ((Fig. 1(A)). According to these results, the isolated strain showed a substantial ability to grow on d-galacturonic acid and fermented it to lycopene. The co-fermentation of glucose and d-galacturonic acid by strain DH416 was carried out and the glucose exhibited a significant impact on the growth pattern, and lycopene formation with time ((Fig. 1(B)).

Glucose was preferred over d-galacturonic acid, and the glucose (7 g/L) was rapidly consumed during the initial 14 h of fermentation. Then, the d-galacturonic acid consumption escalated after glucose depletion in the next 16 h. The cell growth was boosted in the presence of glucose and a higher OD600 was achieved. Therefore, it is indicated that the co-fermentation seems to be ideally suited as it diverted more d-galacturonic acid toward lycopene formation when compared to fermentation with only-galacturonic acid. In total, ~90 % of the d-galacturonic acid was consumed within 30 h, and the lycopene of 406 mg/L was accumulated ((Fig. 1(B)). In several bacteria, glucose is the preferred carbon source that represses the utilization or uptake of other carbon substrates. This carbon catabolite repression was attributed either to competition between different protein transporters that exhibit different affinity towards distinct types of hexose and pentose sugars or carbon repression enzymes that suppress the uptake of d-galacturonic acid. Moreover, it was reported by Anat et al. [30] that the E. coli consumed glucose first in the sugar mixture during fermentation. Herein, the rapid consumption of glucose led to the highest production of lycopene [30].

3.3. Adaptive evolution of DH416 strain

The recombinant DH416 strain was grown in a d-galacturonic acid-minimal medium under aerobic conditions. D-galacturonic acid was used as a primary carbon source rather than glucose. In the present study, the growth-coupled adaptive evolution contributed to enhanced galacturonic acid consumption rate, improved lycopene production, and optimized co-utilization of glucose and d-galacturonic acid. Starting in a minimal medium (consisting of 7 g/L glucose), the concentration of d-galacturonic acid was successively increased. In four adaptation stages over a total of 18 weeks, the d-galacturonic acid concentration was increased from 2 g/L to 7 g/L, and the culture was successfully adapted to intensifying conditions. After reaching the concentration of 7 g/L d-glucose and 7 g/L d-galacturonic acid in a minimal medium, the minimal medium agar plate was used to isolate the best-growing single colonies.

In the first stage of adaptation, the cells were sub-cultured in M9 minimal medium consisting of 2 g/L d-galacturonic acid for successive 10 transfers. Then, in the second adaptation stage, the cells were sub-cultured in M9 minimal medium with a higher concentration of d-galacturonic acid concentration (4 g/L) for 15 transfers. Afterward, the cells were again sub-cultured into the M9 minimal medium with 5.5 g/L of d-galacturonic acid for an additional 15 transfers (third stage of adaptation), and lastly, in the fourth stage of adaptation, the concentration of d-galacturonic acid was enhanced to 7 g/L for 15 transfers (total roughly 200 generations). These four stages of adaptation maintain the strong selection pressure to evolve the E. coli strain. Specific growth rates of evolved populations were determined after 10, 25, 40, and 55 transfers. The results indicated that the adapted cells exhibited no improvement in specific growth rates after 55 transfers. Thus, post 55 transfers, the isolation and selection of the best single colonies on minimal medium agar plate (7 g/L d-glucose and 7 g/L d-galacturonic acid) depended on their growth performance and colony morphology. After 3 days of incubation, small and large single isolated colonies appeared on the agar plate.

Later, these cells with different morphologies were categorized into two different groups based on size, one group, named DH416-S showed small colonies, and the second group, DH416-L consisted of large colonies on M9 minimal agar plate. These two colonies

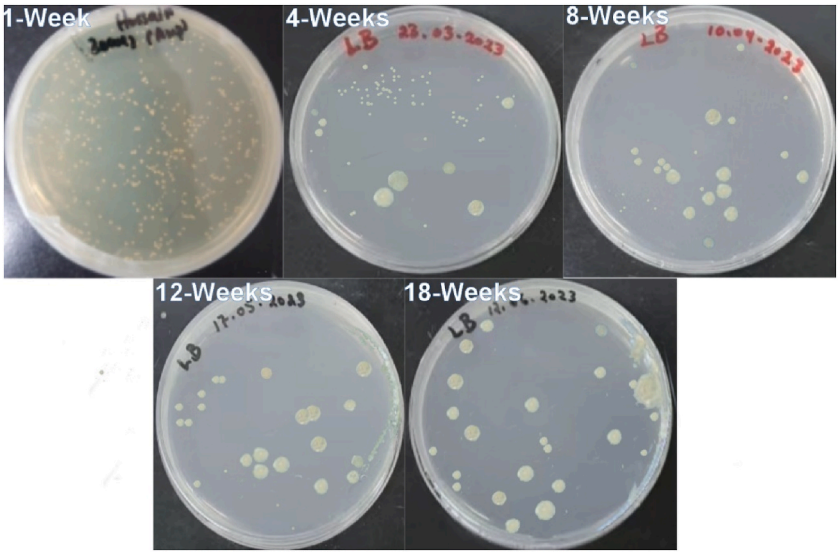

Fig. 2. Relative proportion of different colonies (wild-type, large and small colonies) that grow on d-galacturonic acid minimal medium agar plates with respect to time.

having different morphologies as compared to wild-type DH416 were identified after 4 weeks. Moreover, their relative proportion was continuously increasing for the rest of the 18 weeks (Fig. 2). At week 1, the proportion of wild-type colonies was about 90 % on the agar plate. Over time, the decrease in the proportion of wild-type colonies was observed and the proportion of large colonies was enhanced. Larger colonies might be carrying beneficial mutations. Similar findings have also been reported by Stanek et al. [31], who stated that evolved E. coli populations were characterized by enhanced growth rates which further resulted in increased cell size. It was due to the beneficial mutation that occurred after serial propagation for 2000 generations [31]. Various single colonies were isolated from agar plates and continued further to shake flask culture on M9 minimal medium with d-galacturonic acid. Interestingly, the DH416-S exhibited weaker growth than that of DH416-L in liquid culture. The results are in line with Godara and Kao [32], who found that the large colonies are associated with enhanced terpene production. This improvement in the coupling of growth/survival and production is thought to be a result of a successful evolution strategy [32]. Therefore, we selected eight large colonies A, B, C, D, E, F, G, and H for further testing. Of those, colony A exhibited the highest specific growth rate (0.374 g/L h) in liquid culture. Subsequently, we selected colony A as the best glucose and d-galacturonic co-fermenting strain and named it DH416-LA. It was selected for further experiments. M9 minimal medium was utilized with one of two different carbon sources, named d-galacturonic acid and glucose. The maximum growth rate was denoted as μmax(h^{-1}) whereas the biomass yield was calculated during the exponential growth phase (Table 2) (see Fig. 3).

3.4. Assessing lycopene production from d-galacturonic acid-rich orange peel hydrolysate

Fruit wastes such as orange peels were used as an excellent and cheaper feedstock in comparison to pure d-galacturonic acid to produce biofuels and biochemicals by microbial hosts. Therefore, it was imperative to assess the ability of E. coli to manufacture biochemical such as lycopene by using this lignocellulosic feedstock. At the shake flask level, the capability of E. coli to produce lycopene on pure substrate and orange peel hydrolysate was examined. Meanwhile, the pH of pre-hydrolysate was adjusted to 7.2 to extenuate the toxic effect of inhibitory compounds [28]. The mutant (DH416-LA) E. coli strains were grown on the mixture of glucose and d-galacturonic acid and also on d-galacturonic acid-rich hydrolysate (Fig. 3). The lycopene production obtained with mutant DH416-LA strain on the mixture of glucose and d-galacturonic acid was 668 mg/L, while the similar mutant strain was able to accumulate 497 mg/L lycopene on orange peel hydrolysate. More importantly, the mutant outperformed the wild-type strain again for lycopene production, and, therefore, it was further used in experiments. It is in accordance with the study conducted by Prabhu et al. [33] in which the mutant strain (15 min exposure time to ethyl methanesulfonate) produced more target product than that of the wild-type strain in a shake flask [33]. It was noted that the decrease in lycopene production on hydrolysate was accompanied due to a substantial amount of inhibitory compounds like furfural, acetic acid, and hydroxymethylfurfural (HMF), but the mutant strain grew well and accumulated an optimal amount of lycopene. In terms of d-galacturonic acid metabolism, lycopene production, and biomass development, the result indicates the robustness of E. coli in valorizing lignocellulosic waste.

3.5. Optimization of media components for maximizing lycopene production statistics for analysis of experimental data

3.5.1. Production of lycopene (Response Y1)

The design matrix and the corresponding experimental data are reported in Table S2. This model was predicted to use the analysis of variance (ANOVA), evaluating the influence and interaction of experimental parameters on the response lycopene production (Table S3).

The overall model test determines that the model is significant as it has a smaller P-Value <0.0001 and a higher F-value. Meanwhile, the model F-value of 259.64 and P-value less than 0.0001 have confirmed the significance of the model. Some studies reported that the F-value demonstrates the effect of distinct operation parameters on the response, while the idea about the statistical significance of the model was provided by the P-value [34]. Furthermore, its R^2 value is about 0.9898 which is approximately equal to the model value 1. Thus, a higher level of precision was found in experimental trials. The adj-R^2 is the modified version and a close relationship existed between both adj-R^2 and R^2. It is also reflected that the model consists of variables that are statistically related. In the present case, it is indicated that the model fits well and its lack of fit is not significant with a P-value of 0.3626.

The quadratic regression equation in terms of the coded factor for lycopene production is:

$$\text{Lycopene production} = +822.84 + (10.10A) + (17.82B) - (5.66C) + (1.95A \times B) - (0.72A \times C) - (4.42B \times C) - (18.63A^2) - (33.22B^2) - (91.72C^2) \quad (5)$$

The influence of different operation parameters with their interactions on lycopene production was represented by three-

Table 2
Growth characteristics of DH416, DH416-LA, & DH416-S in shake flask.

Carbon source	Growth parameters	Strain		
		DH416	DH416-LA	DH416-S
D-galacturonic acid	μmax(h^{-1})	0.281 ± 0.03	0.374 ± 0.03	0.316 ± 0.03
Glucose	μmax(h^{-1})	0.512 ± 0.04	0.698 ± 0.03	0.637 ± 0.03

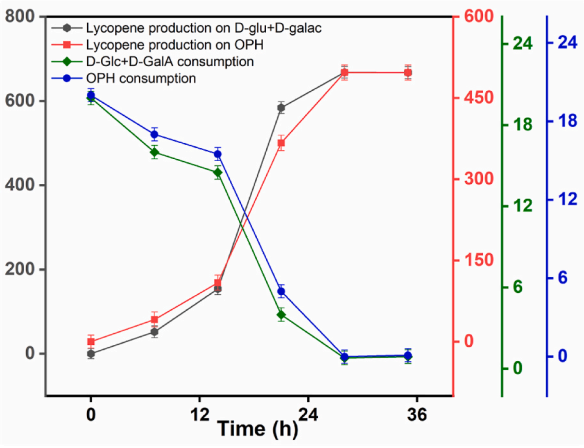

Fig. 3. Production of lycopene by DH416-LA strain in shake flask. Black line indicates the lycopene production by DH416-LA on the mixture of glucose and d-galacturonic acid; Red line indicates the lycopene production by DH416-LA on orange peel hydrolysate. Green line indicates the consumption of d-glucose and d-galacturonic acid by DH416-LA and Blue line indicates the consumption of orange peel hydrolysate (rich in d-galacturonic acid) by DH416-LA. Induction of L-arabinose was carried out at 14 h. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dimensional 3D surface plots. The plot was so built against any two independent variables that the response (lycopene titer) was plotted on the z-axis while maintaining other variables at their mid-levels, as shown in Fig. 4(A-C). It is evidenced from the graph that there is a direct correlation between OPH concentration and lycopene production. There is a steep rise in lycopene production with an increasing initial concentration of OPH (2%–8%), along with combined effects of other operation parameters (temperature and nitrogen source), as displayed in Fig. 4(A–C). Its F- and P-value were 26.54 and <0.0001.

This type of response regarding lycopene biosynthesis elucidates food waste as a potential source of carbon which is not only essential for microbial-derived biochemical production but also for cellular growth. These results are quite similar to other past studies where food waste was used to cultivate E. coli for industrial production [35]. Likewise, the same pattern is shown by yeast extract, a nitrogen source ((Fig. 4(A and B)), where the increase in yeast extract concentration from 2.5 % to 7.5 % is positively correlated with the lycopene production. These results are in line with the previous study conducted by Wang et al. [36] in which lycopene titer was uplifted significantly up to 1.21 g/L in *Saccharomyces cerevisiae* by increasing the yeast concentration between the range of 3–12 g/L [36]. From these graphs, it can be noted that maximum lycopene production was achieved with a mid-level concentration of OPH (5 %) and a yeast extract (5 %). Furthermore, the mid-range of temperature (30 °C) ((Fig. 4(B and C)) was considered to be ideal in increasing the lycopene production. This result is in line with the finding of Jinendiran et al. [37], who stated that the development of biomass and production of β-carotene in strain S01 was better at 30 °C than that at either 40 or 20 °C [37].

Design Expert 8® software was used for optimization as it evaluated the optimum values of temperature (30 °C), carbon source of OPH (5.53 %), and nitrogen source of yeast extract (6.57 %). These optimum values were used to evaluate the theoretical value of lycopene yield (825.58 mg/L). Subsequently, the experiments were performed to get an actual lycopene yield of 825.30 mg/L under the selected optimal conditions. Interestingly, the actual yield was close to the predicted yield value (825.58 mg/L).

Previously, Zuorro et al. [38] found that the central composite design was used to optimize the lycopene production obtained from fruit peel waste [38]. Similar findings have also been reported by Zhang et al. [39], who adapted the central composite design of RSM design to increase lycopene production by PTS01 strain E. coli [39]. Later, the two different sets of optimal batch experiments were performed in a shake flask: with pure d-galacturonic acid Fig. 5(A) and d-galacturonic acid rich hydrolysate ((Fig. 5(B)). The high initial d-galacturonic acid concentration was 7.5 % causing a lag phase of 12 h. D-galacturonic acid uptake was significantly improved

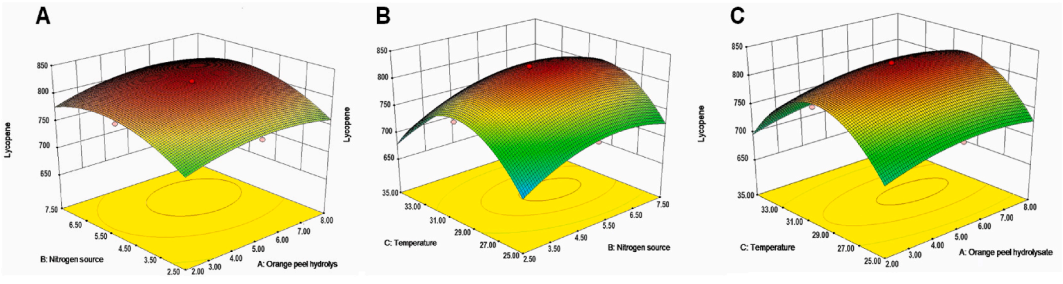

Fig. 4. Evaluation of combined effect exerted by carbon source of OPH A (2–8%), nitrogen source of yeast extract B (2.5–7.5 %), and temperature range C (25–35 °C) by response surface methodology on the production of lycopene.

after 18 h, the growth was dramatically increased, and cells entered the log phase. The cell growth was increased linearly and steady-state growth was achieved after 26 h and reached ~60 (OD600) at the end of the fermentation. It could be assumed from the results that the inhibitors of OPH had little influence on cell growth. With pure d-galacturonic acid as a carbon source, the DH416-LA was able to obtain lycopene with a titer of 926.72 mg/L. In the case of hydrolysate, the strain was able to accumulate 825.30 mg/L of lycopene.

3.6. Optimization of media components for biomass development

3.6.1. Biomass development (Response Y2)

Table S2 shows the design matrix and the corresponding experimental data. This model was predicted to use the analysis of variance (ANOVA) to evaluate the influence and interaction of several experimental parameters on the response biomass development (Table S4).

This model exhibits a higher F-value (8.00) and a smaller P-Value (<0.0001). Some studies reported that the F-value demonstrates the influence of distinct process parameters on the response, while the idea about the statistical significance of the model was provided by P-value [34]. Furthermore, its R^2 value is about 0.7500 which is approximately equal to the model value 1. Thus, a higher level of precision was found in experimental trials. The adj-R^2 is the modified version and a close relationship existed between both adj-R^2 and R^2. It is also reflected that the model consists of variables that are statistically related. Presently, it is clearly indicated that the model fits well and its lack of fit in RSM is 0.6532 (not significant).

The quadratic regression equation in terms of the coded factor for biomass production is:

$$\text{Biomass production} = +3.91 + (0.19A) + (0.24B) + (0.77C) - (0.043A \times B) + (0.080A \times C) - (0.014B \times C) - (0.30A^2) - (0.22B^2) - (0.23C^2) \quad (6)$$

The effect of different operation parameters with their interactions on biomass production was represented by three-dimensional 3D surface plot. The plot was so built against any two independent variables that the response (biomass development) was plotted on z-axis while maintaining other variables at their mid-levels, as shown in Fig. 6(A-C). There is a steep rise in biomass production from 2.16 g/L to 4.47 g/L has been achieved as a result of an increase in temperature between 25 and 35 °C along with combined effects of other operation parameters (OPH and nitrogen source), as displayed in Fig. 6(B and C). This result is quite adjacent to the previous study, where the temperature of 35 °C was selected as the optimal temperature to attain the maximum growth rate of Bacillus subtilis T9-05 participated in the production or degradation of biogenic amines (BA) during fermentation [40]. Likewise, the same pattern is shown by yeast extract concentration ((Fig. 6(A–C)), where the optimum yeast extract concentration (7.5 %) showed improved biomass production. From these graphs, it can be noted that maximum biomass development was achieved with a high-level concentration of yeast extract (7.5 %) and a higher value of temperature (35 °C). On the contrary, at a mid-level concentration of OPH (5 %) as a carbon source Fig. 6(A and B) seemed to be more appropriate in enhancing biomass production. However, biomass production was not the target of our fermentation process, our main aim was to enhance the lycopene yield. Therefore, we further carried the optimized values of the fermentation process for higher lycopene production in a 5 L bioreactor.

3.7. Kinetic modelling with optimal conditions in 5 L batch fermentation

The data was scaled up from a shake flask to the bioreactor, and the batch experiments were performed in a 5 L bioreactor with a 3 L working volume using orange peel hydrolysate. The optimized treatment of the shake flask experiment that gave the best lycopene production was used here. Similar process parameters from shake flask validation studies were selected for scale-up cultivation. The agitation speed, aeration rate, and pH were set at 1.5 vvm, 200 rpm, and 7.2, respectively. Lycopene titer and biomass were significantly improved from shake flask to bioreactor cultivation. According to fermentation optimization results, the temperature was fixed

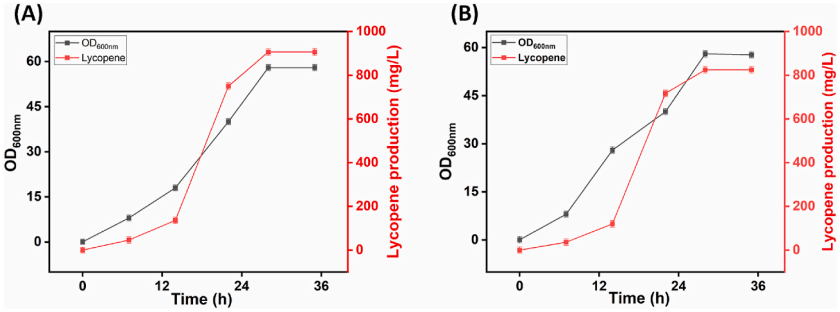

Fig. 5. Production of lycopene by DH416-LA strain under optimized parameters in shake flask (A) lycopene level (mg/L) and growth rate of DH416-LA strain on pure d-galacturonic acid; B) lycopene level (mg/L) and growth rate of DH416-LA strain on the hydrolysate. Induction of L-arabinose was carried out at 14 h.

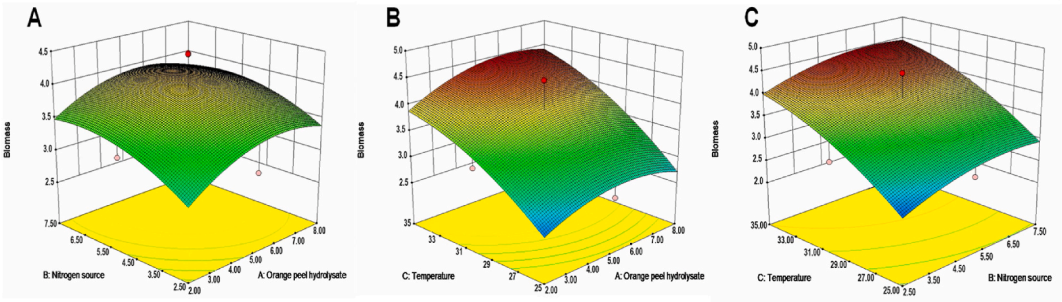

Fig. 6. Evaluation of combined effect exerted by carbon source of OPH A (2–8%), nitrogen source of yeast extract B (2.5–7.5 %), and temperature C (25–35 °C) by response surface methodology on the production of biomass.

at 30 °C during the whole cultivation process [40]. After the inoculation in bio-fermenter, the biomass concentration started growing quickly and entered into an exponential phase of growth which was then followed by a stationary phase of growth that usually arrived after a few hours. At that point, the biomass concentration was about 6.69 g/L DCW. The findings of this result are in correspondence with Xu et al. [41], achieving the highest biomass (E. coli) of 7.67 g/L DCW [41]. Eventually, after 36 h of fermentation, the highest lycopene concentration of 1043 mg/L was achieved in a 5-L fermenter, whereas the biomass titer was 7.02 g/L ((Fig. 7(A)).

The metabolic processes involve multiple interconnected biochemical processes that take place within the microbial cells and unfortunately, the data gathered from experiments are incapable of explaining the various facts regarding metabolic production and cell growth [28,29,42]. Therefore, the increased selection has been noticed for kinetic modeling that compresses large and complex data sets obtained from experimental statistics into simpler ones. Hence, it becomes easy to comprehend the underlying mechanisms for microbial metabolism. The unknown model parameter values and distinct error functions are displayed in Table 3.

3.7.1. Kinetic analysis of biomass concentration of E. coli

Model predictions and experimental data for biomass development of *E. coli* by using a Logistic equation are presented in Fig. 7(A). During batch fermentation, the cell growth was determined by Eq. (2) consisting of Xmax (7.00 g/L) and X0 (0.55 g/L), respectively. The accuracy of the Logistic model in predicting the growth rate of E. coli was verified by its correlation value ($R^2 = 0.9914$), and it is found to be very significant. The different error functions confirmed the accuracy and validity of the Logistic growth model (Table 3). After assessing the values of error functions, it is concluded that the existing model exhibited good agreement with the experimental data. After inoculation, the biomass concentration initially increased sharply in the 5-L fermenter, and then the maximum growth rate of cells ($\mu m = 0.441\ h^{-1}$) was noticed at the stationary growth phase that immediately arrived after 12 h of growth. A few hours later, the growth rate of the cell was gradually accelerated until it attained the maximum biomass concentration of 7 g/L DCW in the log phase. At 18 h, the growth rate of the cell was 6.85 g/L DCW, and a little change in growth rate was observed after 18 h. This may be due to the shortage of essential nutrients for the sustainable growth of the bacterial population.

3.7.2. Kinetic analysis of lycopene production

The predicted model and experimental data for lycopene biosynthesis by using the Luedeking-piret equation in batch fermentation system are shown in Fig. 7(B). It was estimated by analyzing the values of error functions that the predicted model fitted well with the data (Table 3). It is also found that the coefficient (R^2) is 0.96 which indicates the excellent fit of the model with the experimental data. During batch culture fermentation, the Luedeking Piret model (Eq. (3)) was effectively employed to measure the production of lycopene. Meanwhile, its values of growth-associated constant (α), and biomass-associated constant (β) were 0.149 g lycopene/g cell, and 0.012, respectively. It was observed that the production of lycopene was associated with biomass development in batch fermentation. Notably, the α value is higher, and the β value was lower. It means that the production during the stationary phase was non-growth associated. The growth associatedness of carotenoid production was in line with previous findings like Roadjanakamolson et al. [43], who investigated the production kinetics of carotenoid pigment in Rhodotorularubra [43]. For further increase in production, there is a need for agreement with the continuous process in which fresh nutrients are maintained in the batch fermentor. In the present study, orange peel waste and yeast extract are one of the main governing factors to achieve optimal lycopene production. At the start of fermentation, the progressive increase in lycopene production (650 mg/L) was seen until 6 h whereas, the maximum lycopene production of 1043 mg/L was achieved at the end of 36 h of fermentation. The obtained production in the bioreactor was 20.90 % higher than that of the production rate achieved in the shake flask as displayed in Fig. 7(B). After 36 h, both lycopene content and cell growth are not increased. Thus, the present results suggest that the optimum fermentation time for the strain DH416-LA using OPH is 36 h. The findings of this work are in line with previous studies using the kinetic model to determine the carotenoid and curdlan production on raw feedstock materials like dairy wastes and orange peel wastes [27–29].

3.7.3. Kinetic analysis of substrate consumption by E. coli

The experimental data and model predicted values of substrate consumption by the Modified Luedeking-Piret model are presented in Fig. 7(C). In the 5 L fermenter, the experimental data related to the concentration of residual sugar was described by the Modified Luedeking Piret model (Eq. (4)). On analyzing the values of error functions, it is stated that the Modified Luedeking-Piret model fits

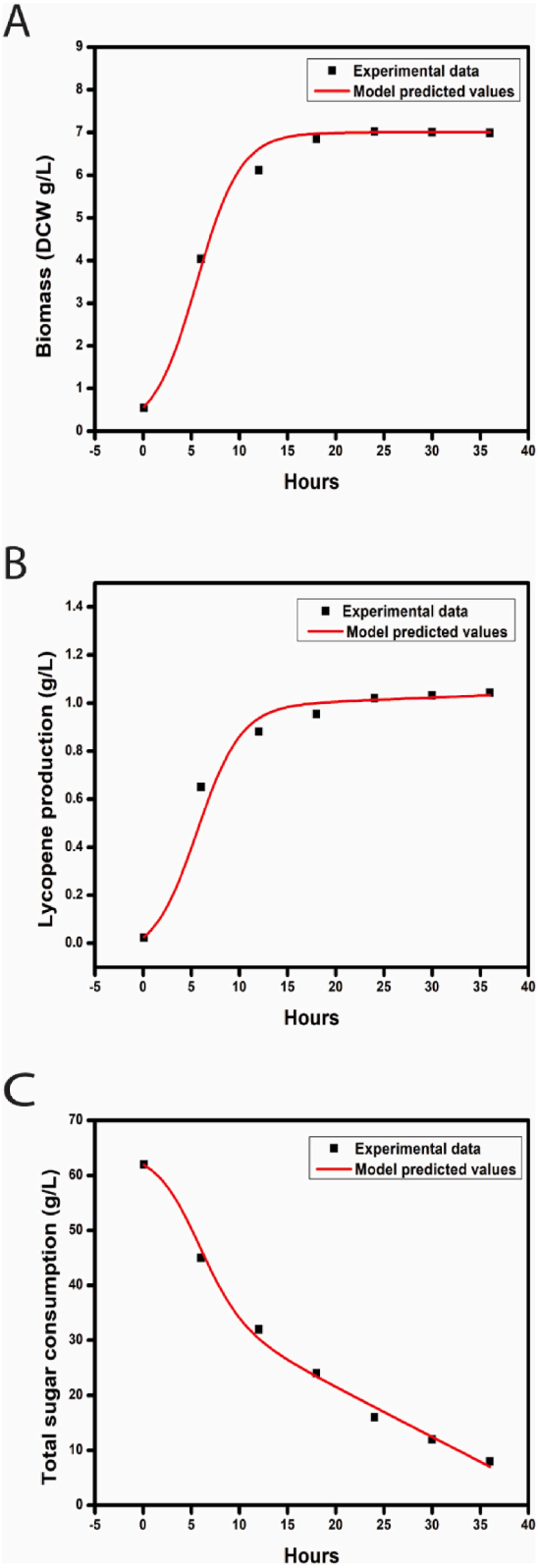

Fig. 7. Relationship between the time of fermentation and (A) biomass concentration (g/L), (B) lycopene production (g/L), and (C) total sugar consumption (g/L) along with model prediction values. Dots represent experimented data, whereas solid lines represent calculated data.

Table 3
Parameters estimated by Logistic, Luedeking-Piret, and Modified Luedeking-Piret equation.

Model	Parameters values			Values of different error functions		
Logistic	$\mu(h^{-1})$	Xo (g/L)	Xm (g/L)	R^2	SSE	MSE
	0.441	0.55	7.00	0.9914	0.0056	0.065
Luedeking-Piret	–	A	B	R^2	SSE	MSE
		0.1491	0.012	0.9640	0.0049	0.086
Modified Luedeking-Piret	–	Γ	Δ	R^2	SSE	MSE
		4.280	0.1294	0.9945	0.2845	0.013

Mean squared error (MSE), and sum of squares error (SSE) are used to determine the model performance based on residuals between simulated and measured values.

well with the experimental data **(**Table 3**).** Meanwhile, its values of correlation coefficient (R^2), and growth-associated constant (γ) were 0.994, and 4.280, respectively. It was predicted that the residual sugar consumption was tightly correlated with the concentration of cell growth. This prediction was made based on higher growth-associated constant value. And the lycopene production and development of biomass were inversely proportional to total sugar. At the start of fermentation, the concentration of total sugar content was approximately 62.18 g/L which was later reduced to 8.16 g/L after 36 h of fermentation shown in Fig. 7(C). It is concluded that the adopted models remain successful while describing the lycopene production, E. coli growth rate, and OPH consumption rate. It also indicates that the adaptive evolutionary approach applied to *Escherichia coli* is successful, as it creates a stable strain with improved growth rate, OPH utilization, and lycopene yield.

3.8. Antioxidant characteristics of lycopene biosynthesized from OPH

Lycopene is recognized as a potent antioxidant that can destroy free radicals in the body which is often associated with poor diet and environmental pollution. Additionally, lycopene prevents cell damage that can lead to cancer of the prostate, colon, cervix, and pancreas as well as heart disease and macular degeneration [44]. Meanwhile, the role of antioxidants in combatting microbial inflammation has gained acknowledgment in recent years. Optimally, they exhibit synergistic effects with existing antimicrobial agents conferring a high level of antimicrobial resistance in the host bacteria. More importantly, lycopene has unique physicochemical properties besides potential functional characteristics, such as reducing lipid peroxidation, preventing DNA damage, and acting as a scavenger for reactive oxygen species (ROS) [45]. These findings of literature are validated with our present work in which lycopene exhibits better antioxidant properties confirmed by DPPH assay **(Eq. (1))**, as shown in Fig. 8(A). Antioxidant activity of different concentrations of lycopene extract was measured by DPPH free radical scavenging methods. The percentage of DPPH inhibition of different concentrations of lycopene extract was found in the range of 38.06 %–29.08 % ((Fig. 8(A)). These results are in agreement with an opinion established by Tambunan et al. [46], reporting that the antioxidant activity of pasteurized tomato juice increased from 34.2746 % to 44.7245 % [46].

3.9. Benefits of phytate (antioxidant potential)

Naturally, the phytic acid exists in various biological systems. It plays a vital role in nutrition. Interestingly, information regarding its antinutritional properties has been available in several kinds of literature. This antinutritional property may be due to its capability of binding with proteins, carbohydrates, minerals, and lipids [47]. Furthermore, the phosphate group is found to be an effective site for non-covalent interactions and coordination bonds. The phytic acid as a dietary agent has gained growing popularity in the food industry because of its therapeutic potential [48]. The phytic acid percentage of different concentrations of lycopene extract was measured by spectrophotometric method. Phytic acid concentration was obtained in the range of 1.01 %–0.86 % ((Fig. 8(B)). According to the current observation, the concentration of phytic acid in 1 ml exhibited a higher concentration of phytic acid of 1.01 %

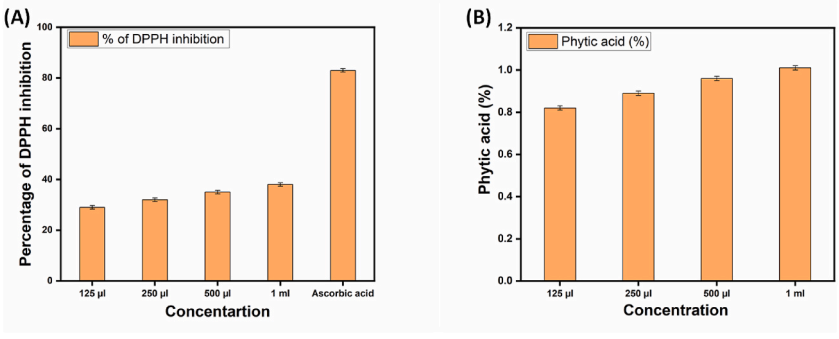

Fig. 8. Antioxidant activity of lycopene extract in fermentation broth measured by (A) DPPH free radical scavenging method; B) and phytic acid test.

while 125 μl treatment exhibited the lowest concentration of phytic acid. These findings proved that phytic acid can bind with different minerals like iron, zinc, and calcium, which can increase the concentrations of phytic acid in lycopene extract. According to Lott et al. [49], phytic acid acts as an antioxidant in lycopene extract [50].

4. Discussion

The astonishing increase in agricultural waste is due to the growing demand for food and the continuous increase in world population. The amount of agriculture waste generated in China was around 0.9 billion t. The discarding of this agricultural waste can contaminate the soil, air, and water supplies. Therefore, agro-industrial waste, such as orange peel can be exploited for the production of various value-added products like biofuels, and biochemicals, particularly lycopene through microbial fermentation [13]. In this regard, it allows the efficient recovery of lignocellulosic waste which will further promote the circular economy and environmental protection.

The saccharification methods are known to affect the amount of fermentable, sugar derived from orange peel wastes [7,8]. In the present study, the dilute acid treatment with enzymatic hydrolysis was recognized as a better option to liberate the optimum level of fermentable sugar in the fermentation medium which was fermented to lycopene by strain DH416 [19]. However, the level of lycopene and growth of strain wasn't optimal from orange peel hydrolysate. Sevgili and Erkmen reported low production of lycopene (4.9 mg/L) by *Blakeslea trispora* from orange powder in a fermenter [7]. This low yield is thought to be a result of increased inhibitory compounds present in orange peel waste. Therefore, in the present study, the strain was subjected to adaptive laboratory evolution for four months on the d-galacturonic acid minimal medium which provided improvement to strain DH416 for its lycopene production at a large scale. The results of this study are similar to the research conducted by Zhou et al. [49], who adopted both atmospheric and room-temperature plasma (ARTP) mutagenesis and adaptive laboratory evolution to improve lycopene yield in *Saccharomyces cerevisiae* [49]. Similar findings have also been reported by Phosriran et al. [51], who overcame the problem of poor growth with no succinate production in *Klebsiella oxytoca* KIS004-91T strain through an adaptive laboratory evolution approach [51].

Three key operating parameters, such as temperature, orange peel hydrolysate, and yeast extract were optimized by response surface methodology (RSM)-central composite design (CCD). Thus, RSM modelling led to the significantly higher value of lycopene and cell growth in resulting mutant DH416-LA using both orange peel hydrolysate and pure d-galacturonic acid. This model fitted well as its lack of fit was non-significant. Furthermore, the optimized value was further continued from the shake flask to the 5-L fermenter.

Distinguished from the shake flask system, various physical parameters, such as temperature, pH, agitation speed, and aeration rate are controlled in the bioreactor to attain maximum yield of the target product at industrial scale-up [52]. Notably, the dissolved oxygen is considered one of the key factors in a bioreactor system and its level can be improved with the increase of aeration rate and agitation speed [53]. In aerobic fermentation, these two above-mentioned factors are critical and have a significant impact on the production of several biochemicals and biopolymers. The agitation is attributed to the important shearing and mixing role which not only induces the proper mixing of nutrients, oxygen, and heat but also facilitates their better transfer in the fermentation broth. Aeration, on the other hand, ensures the optimum supply of oxygen to the microbial cells. Moreover, the pH inside the bioreactor is continuously monitored and maintained at optimal levels to improve the coupling between biomass development and energy production [53].

More importantly, the higher agitation speeds $<$300 rpm are accompanied by an unbearable shear force which can lead to cell damage. Moreover, the low aeration rate was unable to fulfill the dissolved oxygen (DO) requirement, as well as under the low aeration rate, the conversion of β-carotene to torularhodin did not occur efficiently due to the poor mass transfer of the substrate [54]. Therefore, in the present study, the moderate aeration rate (1.5 vvm) and agitation speed (200 rpm) were optimally used for maximum lycopene production by *Escherichia coli* DH416-LA. These findings align with Nasrabadi et al. [55], who stated that the aeration rate (1.5 vvm) gave rise to optimal lycopene production by *Dietzia natronolimnaea* [55]. Moreover, the high aeration rate and agitation speed negatively influence terpene production as these factors cause oxidation of the pigment.

To better understand the fermentation process, the kinetic modelling based on Logistic and Luedeking-Piret equations was applied that evaluated the cell growth, lycopene production, and substrate consumption at the same time. In the end, the antioxidant evaluation was performed by using a phytic acid test and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Phytic acid and DPPH tests have shown that the DH416-LA-derived lycopene exhibited better antioxidant properties. In short, this current study presents an economical and sustainable approach to producing highly value-added lycopene by using evolutionary-adapted recombinant *Escherichia coli* from orange peel waste.

5. Conclusion

Herein, the RSM strategy is adopted to develop the systematic and scalable approach that ensures optimum lycopene production by using orange peel waste as a feedstock material. To further increase the lycopene production, the optimized treatment is continued further in a 5 L bio-fermentor under the surveillance of a kinetic model to achieve maximum lycopene production of 1043 mg/L from pretreated orange peel hydrolysate. This work generates the best results and aims to motivate the potential of utilizing myriad types of wastes as carbon sources to produce different useful metabolites as part of fermentation as well as reduce the environmental burden and cost of disposal too.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Muhammad Hammad Hussain: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Subra Sajid:** Writing – review & editing, Formal analysis, Data curation. **Maria Martuscelli:** Writing – review & editing, Funding acquisition, Data curation. **Waleed Aldahmash:** Software, Methodology, Data curation. **Muhammad Zubair Mohsin:** Writing – review & editing. **Kamran Ashraf:** Writing – review & editing, Data curation. **Meijin Guo:** Writing – review & editing, Funding acquisition. **Ali Mohsin:** Writing – review & editing, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34366.

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