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Original Research Article

Growth optimization and metabolite yield of non-pathogenic *Escherichia coli* isolated from the gut of African catfish: *Clarias gariepinus*

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Abstract

Purpose: *To investigate the optimum growth and metabolite yield of non-pathogenic Escherichia coli in the gut of African catfish for use as a substitute.*

Methods: *Optimization was statistically carried out for the growth and metabolite yield of non-pathogenic Escherichia coli. Response Surface Design was used to generate three levels of each of the following operational factors viz glucose, peptone water, potassium nitrate (KNO_3), Bambara nut, pH and temperature for the production of peptone water–glucose, KNO_3–glucose and Bambara nut culture media, respectively. The absorbance was read consecutively for 7 days using a UV-VIS double beam spectrophotometer while the metabolites were identified and analyzed using gas chromatography-MS.*

Results: *Optimum growth and metabolite yield of the non-pathogenic Escherichia coli occurred on the 4th and 7th day in Peptone water–glucose, KNO_3–glucose and Bambara nut media, with sequential p-values ranging from 0.115 (quadratic) – 0.002 (linear), 0.370 (linear) – 0.020 (quadratic), 0.470 (linear) – 0.040 (quadratic), respectively. The combination of peptone water (0.9 g), glucose (4.0 g) and pH (7.25) at 37 °C gave the optimum culture conditions for metabolite yield, with 19 classes of metabolites identified.*

Conclusion: *The quadratic model is the suitable predictive model and Peptone water–glucose has optimum metabolite yield. Therefore, it is ideal for therapeutic and nutritional purposes in aquaculture based on yield and quality of metabolites.*

Keywords: *African catfish, Metabolite yield, Non-pathogenic Escherichia coli, Optimum growth, Optimization, Quadratic model*

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INTRODUCTION

The report by Reed and Spence over a century ago serves as the foundation for studies on microbial population in fish gut. Further investigations show a dense population of microflora in the fish gut than its surrounding environment [1], perhaps due to the conducive nature of the gut. Gut bacteria florae are capable of producing wide-ranging and important bio-active molecules with extended-spectrum and biological functions suitable for medical and

nutritional purposes, where they play significant roles in nutrition, growth and immunity against pathogens in fishes [2]. However, the gut flora community can be altered by the fish's gut environmental conditions, developmental stages, stress and complexity of the digestive system [1]. Different species of fish and shellfish are seen to have a specific resident gut microbiota [3]. However, information on the gut flora of fish species is scarce concerning the specific fluctuation rate of the flora and their genetic variant.

Previous study shows that microflorae are the best sources of bio-active substances in aquaculture and have been tested for their potential in fish health and nutrient utilization [4]. An example of gut microflora of biological importance in fish is the bacteria of the genus *Escherichia*, common in endotherms [5]. Most strains of *E. coli* in the gut of fish are among the normal and harmless flora community, hence non-pathogenic and can benefit their hosts by having a mutual link that is capable of preventing pathogens from colonizing the fish gut through their antimicrobial secretion and carboxylic properties [6]. This may enhance nutrient uptake and productivity in aquaculture although there is scanty information to buttress this claim. Therefore, for optimum non-pathogenic *Escherichia coli* growth and metabolite yield, it is important to harvest the bio-active molecules that confer antimicrobial, immune and nutritional potentials to the fish [2,7], through a combination of key factors such as the state of culture and nutrient availability vis-à-vis temperature, pH, incubation period, carbon and nitrogenous sources outside the gut system. Secondary metabolites are secreted during bacteria growth stages. However, some specific secondary metabolites require the use of precursor or cofactor to stimulate the metabolic processes that produce them [7], hence the need for a controlled environment for their production.

According to Lodish *et al* [8], cultured cells have several advantages over intact organisms for studies on some fundamental aspects of biology, since culture conditions are under control. Hence, optimization techniques are the most effective and powerful approaches for maximizing microbial growth and metabolite yield. Accordingly, a more robust, economical and efficient way of growing microbial cells and metabolite yield has been through the recent innovative mathematical/statistical techniques of optimizing both microbial growth and culture media [9]. The processes involved in the growth of useful bacteria cells, according to Singh *et al* [9], are among the central components of biotechnological applications in energy production, food and pharmaceutical industries. This has the potential to maximize economic benefits and therefore, it is important to optimize the processes of producing microorganisms and their metabolites since they are proven to be biologically relevant [10]. Therefore, this study aimed to optimize culture conditions and metabolite yield of the non-pathogenic *Escherichia coli* strain encountered in the gut of sampled African catfish (*Clarias gariepinus*).

EXPERIMENTAL

Preparation and screening for contamination of non-pathogenic *Escherichia coli* sample isolated from the gut of cultured *Clarias gariepinus* catfish

The study was carried out at the Enzymology Division of the Biotechnology Unit of the Federal Institute for Industrial Research Oshodi (FIIRO), Lagos State, Nigeria.

MacConkey agar was prepared following standard procedure (Oxoid Ltd, Hampshire United Kingdom). Then inoculation and incubation of isolates was monitored for 24 h to check for any impurity. Nutrient broth was prepared at 2.6 g per 200 mL (Oxoid Ltd, Hampshire United Kingdom), each in duplicate for inoculating the pure isolates. This was done by adding the respective grams of nutrients in distilled water and sterilizing. The broth was cooled and inoculated with non-pathogenic *Escherichia coli* previously isolated from the gut of cultured *Clarias gariepinus* catfish sampled from the fish farm of the Department of Aquaculture and Fisheries Management, University of Ibadan. This was then incubated in a Stuart Refrigerated Shaking Incubator (Cole-Parmer® SI-200 Series, Vernon Hills, IL 60061, USA). Cell pellets were subsequently harvested using a refrigerating centrifuge (SIGMA® 4 – 16KS, Osterode am Harz, Germany) at 10 °C for 5 min run time, 5,000 revolutions per minute (rpm) and 5087 g relative centrifugal force (RCF). The supernatant recovered from the centrifugation was discarded and cell biomass was collected and refrigerated at 4 °C for further study.

Optimization of the recovered non-pathogenic *Escherichia coli* strain

The operational factors for optimization of the recovered non-pathogenic *Escherichia coli* strain for cell growth and metabolite yield included the following: Glucose, peptone water, potassium nitrate (KNO_3), Bambara nut, pH and

temperature. The study had three (3) sets of media combinations for culture as follows: Glucose, peptone water, with pH and temperature; Glucose, potassium nitrate (KNO_3), with pH and temperature; and Bambara nut powder, with pH and temperature [11]. The total number of culture runs generated was ninety (90) for non-pathogenic *Escherichia coli* strain under investigation, and thirty (30) for each culture combination. The built information for generated culture runs in this study was as follows: Statistical software file version was Design expert v12.0.8.0, the response surface study type was used, randomized subtype, Box-Behnken design type and the built time ranging from 1 to 2 min were used.

The constituent ingredients used for the culture combination were weighed using a Digital Precision Sensitive Scale (ATOM-A 110C Platinum) for the preparation of individual culture broth. The quantity used ranged from 3 – 4 g for glucose, 0.3 – 0.9 g for peptone water and KNO_3, and 1.5 – 2.0 g for Bambara nut powder, while pH and temperature were adjusted at 7 – 7.5 and 35 – 40 °C, respectively. Each weighed ingredient was transferred into a well-labeled culture flask, sealed and autoclaved at 121 °C for 15 min and thereafter cooled below 45 °C [12].

The recovered pure non-pathogenic *Escherichia coli* biomass was inoculated by dispensing into each of the prepared sterile culture combinations using a micropipette at 2 mL per 20 mL (v/v). The inoculated sterile broths were incubated as liquid-state fermentation (LSF) at 37 °C for peptone water–glucose and KNO_3– glucose, and between 35 to 40 °C for Bambara nut powder for seven (7) days [13]. Cell growth of non-pathogenic *E. coli* strain in all culture runs at 24 h (1st day), 96 h (4th day) and 168 h (7th day) period of the liquid-state fermentation was monitored at 600 nm using the UV-6300PC VWR® double beam UV-Vis spectrophotometer (Malvern Panalytical Ltd, United Kingdom).

Quenching of culture metabolism and extraction of intracellular metabolites

The optimal non-pathogenic *E. coli* culture run from the absorbance (600 nm) readings was selected. The quenching of metabolism was done as previously [14]. Briefly, 5 mL of solvent was dispensed into each of the selected 20 mL culture runs and incubated for 20 minutes at a 1:1 Acetone / water solvent temperature of - 20 °C. This was followed by centrifugation using a refrigerating centrifuge (SIGMA® 4 – 16KS, Osterode am Harz Germany) for six (6) min run time, maximum speed of 5000 rpm and relative centrifugal force (RCF) of 5087 g at a temperature of 4 °C. After the run time, samples were removed from the centrifuge, and extracted intra and extra-cellular metabolites in the supernatant were separated from the insoluble materials.

Gas chromatography-MS of metabolites extract

Supernatants recovered from the Acetone/water solvent after centrifugation were analyzed for metabolites using Gas Chromatography–MS. Chemical compounds were searched and identified using NIST/EPA/NIH mass spectral library search program version 2.2 (NIST14. L).

Ethical approval

The procedures complied with the U.K. Animals (Scientific Procedures) Act [15] guidelines for using fish specimens, as approved by the University of Ibadan Animal Use and Care Research Ethics Committee, and assigned no. UI-ACUREC/021-0323/29.

Statistical analysis

Optimized statistical analysis was carried out in this study using the Box-Behnken design. The response surface methodology (RSM) was used to design the experiment (DOE) to generate the levels of operational factors and the number of culture runs. In addition, fit summary models consisting of quadratic, linear and 2FI models were tested to determine the suitable model for data analysis. This was done with the aid of Design Expert statistical package version 2.

RESULTS

Optimum growth conditions and metabolite yield of non-pathogenic *E. coli*

The growth optimization and yield of metabolites of non-pathogenic *E. coli* specimens carried out on three culture media revealed that optimal growth occurred on the 4th day (mid-point) of culture in peptone water–glucose medium (1.488 nm), KNO_3–glucose medium (0.607 nm) and Bambara nut powder medium (3.05 nm). The value of the absorbance (Figures 1 – 6) declined on the 7th day (end-point) of the culture period in Peptone water–glucose (0.370), KNO_3–glucose (0.42) and Bambara nut powder (1.146).

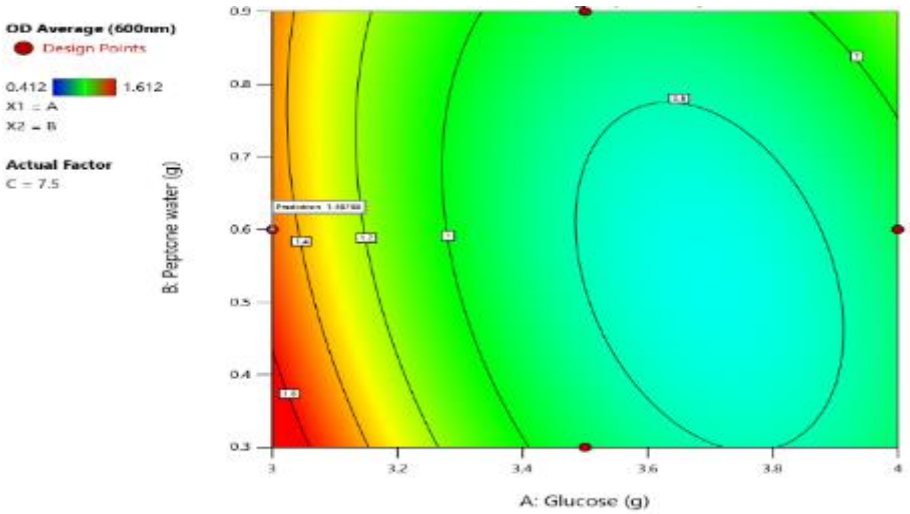

Figure 1: Contour response of non-pathogenic *E. coli* in peptone water–glucose culture media on the 4th day (mid-point) with predictive absorbance value of 1.488

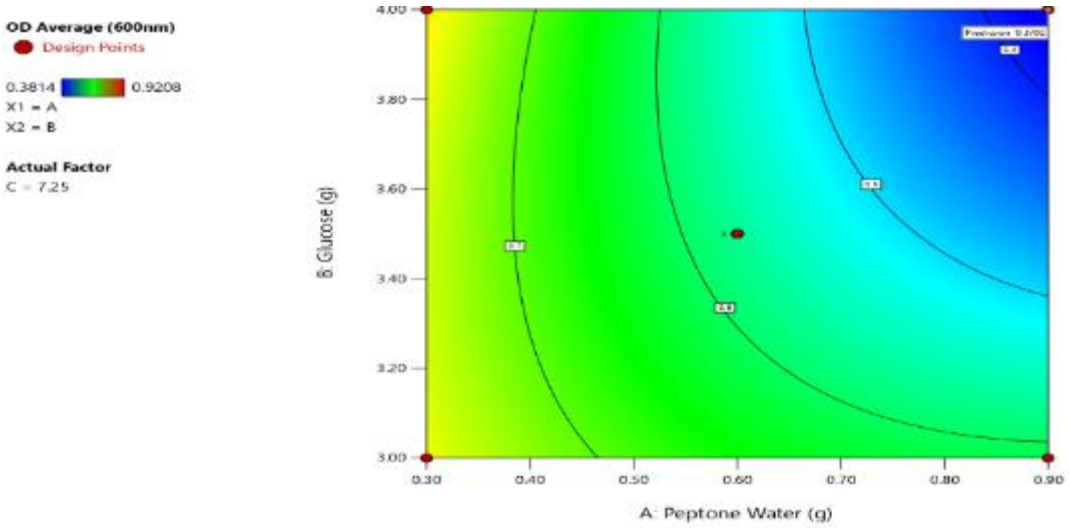

Figure 2: Contour response of non-pathogenic *E. coli* in peptone water–glucose culture media on the 7th day (mid-point) with predictive absorbance value of 0.370

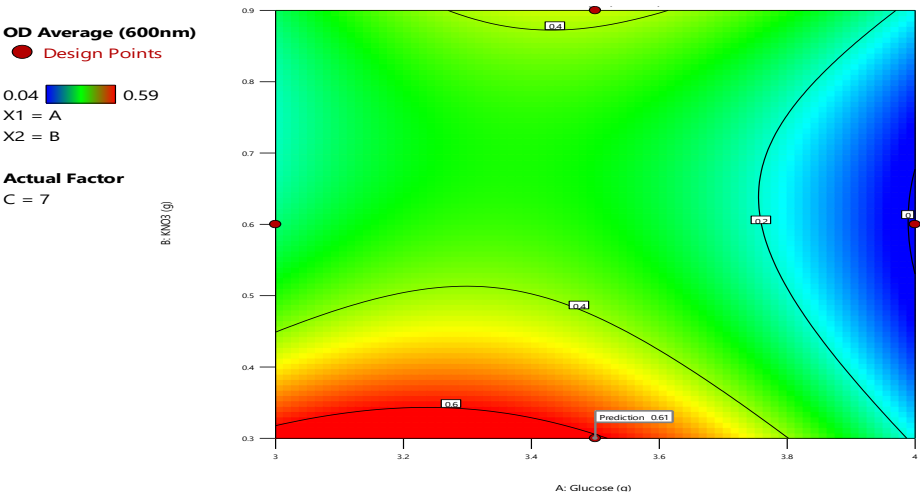

Figure 3: Contour response of non-pathogenic *E. coli* in KNO_3–glucose culture media on the 4th day (mid-point) with predictive absorbance value of 0.607

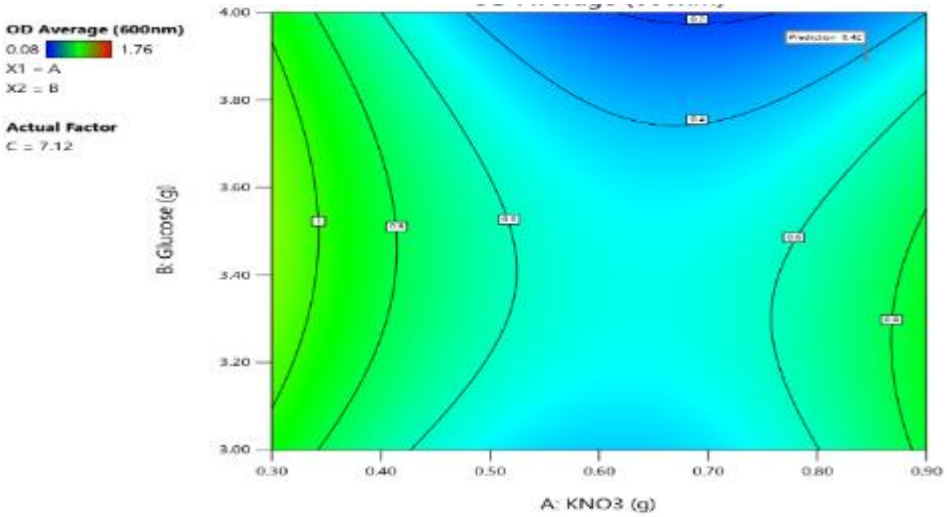

Figure 4: Contour response of non-pathogenic *E. coli* in KNO_3–glucose culture media on the 7th day (mid-point) with predictive absorbance value of 0.42

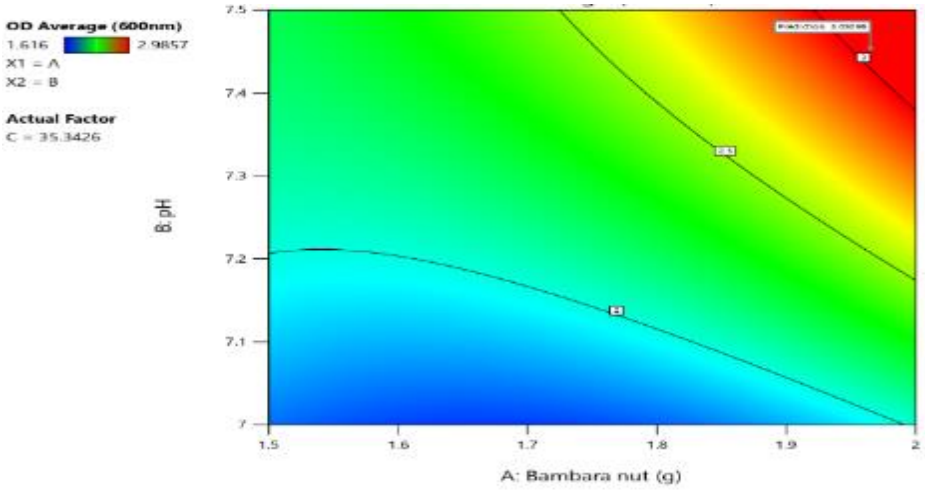

Figure 5: Contour response of non-pathogenic *Escherichia coli* in Bambara nut powder culture media on the 4th day (mid-point) with predictive absorbance value of 3.05

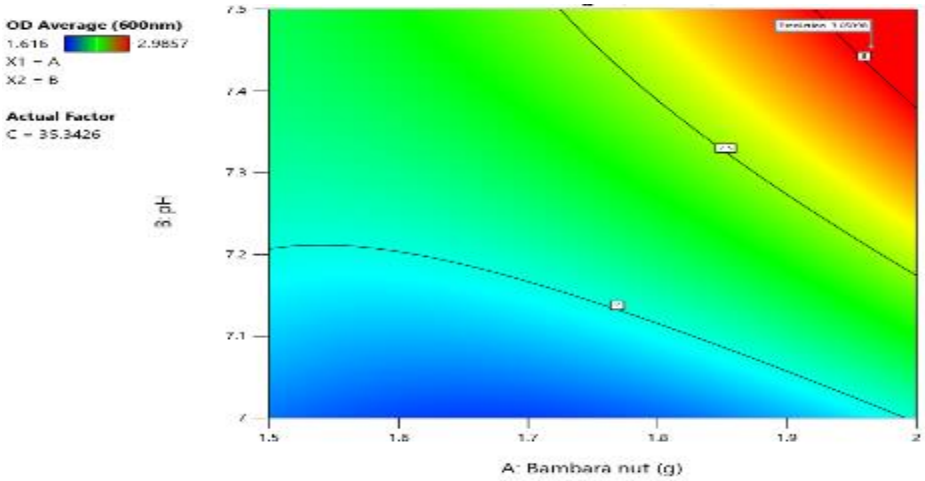

Figure 6: Contour response of non-pathogenic *E. coli* in Bambara nut powder culture media on the 7th day (mid-point) with predictive absorbance value of 1.146

The results of the response surface design confirming the location of factors for optimal growth revealed that the peak values occurred at the following culture conditions namely 0.6 g peptone water, 3.0 g glucose, 7.5 pH and 37 °C temperature on the 4th day and 0.9 g peptone water, 4.0 g glucose, 7.25 pH and 37 °C temperature on the 7th day for peptone water–glucose culture; 0.3 g KNO_3, 3.5 g glucose, 7.00 pH, 37 °C temperature on the 4th day and 0.84 g KNO_3, 3.89 g glucose, 7.12 pH and 37 °C temperature on the 7th day for KNO_3–glucose culture; and 1.96 g Bambara nut powder, 7.45 pH, 35.34 °C temperature (4th day) and 1.5 g Bambara nut powder, 7.50 pH, 37.5 °C temperature (7th day) for Bambara nut powder.

Predictive models for optimal growth and metabolite yield of *E. coli* isolate in culture media

Peptone water–glucose culture media

The fit summary models for the growth and metabolite yield of non-pathogenic *E. coli* isolate in peptone water-glucose culture media (Table 1) showed sequential and lack of fit *p*-values of 0.26 and 0.34 at predicted R^2 = - 0.13 (linear), 0.86 and 0.26 at predicted R^2= - 0.85 (2FI), 0.03 and 0.58 at predicted R^2= - 0.36 (quadratic), respectively on the 4th day. On the 7th day, the sequential and lack of fit *p*-values were 0.0028 and 0.0307 at predicted R^2 = 0.4383 (linear), 0.1126 and 0.0414 at predicted R^2 = 0.5017 (2FI), 0.1155 and 0.8649 at predicted R^2 = 0.2558 (quadratic), respectively. The 4th-day and 7th-day predictive responses both suggested the quadratic model as being suitable and are represented in terms of coded factors in Eq 1 and 2.

On the 4th day:

$$\text{Absorbance } (Average) = +0.5608 - 0.2359\,(A) + 0.0314\,(B) - 0.0100\,(C) + 0.1350\,(AB) - 0.0568\,(AC) - 0.034\,(BC) + 0.0405\,(A^2) + 0.027\,(B^2) + 0.0761\,(C^2) \quad \text{..... (1)}$$

On the 7th day:

$$\text{Absorbance } (Average) = +0.5722 - 0.1485\,(A) - 0.055\,(B) + 0.0015\,(C) - 0.0658\,(AB) + 0.0387\,(AC) + 0.0684\,(BC) + 0.0405\,(A^2) + 0.0272\,(B^2) + 0.0761\,(C^2) \quad \text{..... (2)}$$

Where: Absorbance (Average) is the average absorbance (600 nm), A is glucose (in grams), B is Peptone water (in grams) and C is pH.

KNO_3–glucose culture media

The fit summary models for the growth and metabolite yield of non-pathogenic *E. coli* isolate in KNO_3 –glucose culture media (Table 2) showed sequential and lack of fit *p*-values of 0.66 and 0.53 at predicted R^2 = - 0.7216 (linear), 0.79 and 0.43 at predicted R^2 = - 2.51 (2FI), 0.04 and 0.87 at predicted R^2 = - 0.01 (quadratic), respectively, on the 4th day. The sequential and lack of fit *p*-values on the 7th day were 0.37 and 0.29 at predicted R^2 = - 0.45 (linear), 0.68 and 0.24 at predicted R^2 = - 1.67 (2FI), 0.02 and 0.66 at predicted R^2 = 0.01 (quadratic), respectively. The 4th-day and 7th-day predictive responses both suggested the quadratic model as being suitable and are represented in terms of coded factors by Eqs 3 and 4.

On the 4th day:

$$\text{Absorbance } (Average) = +0.2512 - 0.0886\,(A) - 0.0324\,(B) + 0.0160\,(C) + 0.0889\,(AB) + 0.0470\,(AC) + 0.0615\,(BC) - 0.2004\,(A^2) + 0.1904\,(B^2) + 0.0894\,(C^2) \quad \text{..... (3)}$$

On the 7th day:

$$\text{Absorbance } (Average) = +0.3982 - 0.2737(A) - 0.0307\,(B) - 0.0626\,(C) - 0.1153(AB) - 0.2209(AC) + 0.1427(BC) + 0.4607\,(A^2) - 0.2068\,(B^2) - 0.3525\,(C^2) \quad \text{..... (4)}$$

Where: Absorbance (Average) is the average absorbance (600 nm), A is KNO_3 (in grams), B is glucose (in grams) and C is pH.

Bambara nut powder culture media

The fit summary models for growth and metabolite yield of non-pathogenic *E. coli* isolate in KNO_3 - glucose culture media (Table 3) showed sequential and lack of fit *p*-values of 0.05 and 0.06 at predicted R^2 = - 0.10 (linear), 0.01 and 0.16 at predicted R^2 = 0.31 (2FI), 0.04 and 0.35 at predicted R^2 = 0.60 (quadratic), respectively on the 4th day. On the 7th day, the sequential and lack of fit *p*-values were 0.47 and 0.04 at predicted R^2 = - 0.61 (linear), 0.14 and 0.05 at predicted R^2 = - 0.82 (2FI), 0.04 and 0.72 at predicted R^2 = - 0.47 (quadratic), respectively. The 4th-day and 7th-day predictive responses both suggested the quadratic model as being suitable and are presented in terms of coded factors in Eqs 5 and 6 below.

Table 1: Fit summary model on the 4th and 7th day for non-pathogenic *E. coli* in Peptone water–glucose culture media

Parameter	4th day mid-point			7th-day end-point		
Source	***Linear***	***2FI***	***Quadratic***	***Linear***	***2FI***	***Quadratic***
Sequential *p*-value	0.2621	0.8647	0.0398	0.0028	0.1126	0.1155
Lack of Fit *p*-value	0.339	0.2610	0.5896	0.0307	0.0414	0.8649
Adjusted R^2	0.1016	-0.1325	0.6119	0.6283	0.7482	0.8649
Predicted R^2	-0.1322	-0.8507	-0.3643	0.4383	0.5017	0.2558
Suggestion			Suggested	Suggested		Suggested

Table 2: Fit summary model on the 4th and 7th day for non-pathogenic *E. coli* in KNO_3-glucose culture media

Parameter	4th day (mid-point)			7th day (end-point)		
Source	***Linear***	***2FI***	***Quadratic***	***Linear***	***2FI***	***Quadratic***
Sequential *p*-value	0.6614	0.7982	0.0481	0.3745	0.6851	0.0239
Lack of Fit *p*-value	0.5292	0.4359	0.8749	0.2966	0.245	0.6644
Adjusted R^2	-0.1079	-0.3518	0.4989	0.0298	-0.1191	0.6895
Predicted R^2	-0.7216	-2.5134	-0.0183	-0.4594	-1.6777	0.0143
Suggestion			Suggested			Suggested

Table 3: Fit summary model on the 4th and 7th day for non-pathogenic *E. coli* in Bambara nut culture media

Parameter	4th day (mid-point)			7th day (end-point)		
Source	***Linear***	***2FI***	***Quadratic***	***Linear***	***2FI***	***Quadratic***
Sequential *p*-value	0.0578	0.0098	0.0492	0.4714	0.1482	0.0482
Lack of Fit *p*-value	0.0669	0.1671	0.3541	0.0441	0.0553	0.7230
Adjusted R^2	0.3381	0.7649	0.9120	-0.0216	0.2538	0.7230
Predicted R^2	-0.1059	0.3178	0.6064	-0.6122	-0.8219	-0.4729
Suggestion			Suggested			Suggested

On the 4th day:

Absorbance

$$(Average) = +2.30 - 0.3286(A) + 0.0890(B) + 0.0188(C) + 0.2055(AB) + 0.0008(AC) - 0.3804(BC) + 0.1764(A^2) - 0.0553(B^2) - 0.1253(C^2 \quad \text{.... (5)}$$

On the 7th day:

$$\text{Absorbance } (Average) = +2.73 + 0.3285(A) - 0.0878(B) + 0.143(C) + 0.3750(AB) + 0.0044(AC) + 0.6145(BC) - 0.0964(A^2) - 0.6960(B^2) - 0.0087(C^2) \quad \text{..... (6)}$$

Where: Absorbance (Average) is the average absorbance (600 nm), A is Bambara nut (in grams), B is pH and C is the temperature (°C).

Profile of the active metabolome extracted from non-pathogenic *E. coli*

The profile of extracted metabolites of non-pathogenic *E. coli* as presented in Table 4 showed the presence of nineteen bio-active chemical groups from Gas chromatography-MS. The abundance of metabolites ranged in percentage from 5-Hydroxymethylfurfural (33.34 %), followed by Imidazole-4-carboxylic acid, 1-met hyl- (16.86 %), then 1,3-Propylene glycol, O, O-di(pivaloyl), Cycloserine, and DL-Asparagine (11.62 %) and DL-3-Aminoisobutyric acid, N-dimethylamino methylene-, ethyl ester (11.45 %) to 2-Formyl-9-(beta-D-ribofuranosyl) hypoxanthine (0.17 %). The six most abundant constituents of the metabolome made up 61.41 % of the metabolite composition of non-pathogenic *E. coli.*

DISCUSSION

The growth of *E. coli* strain in the three (3) culture media indicated the suitability of either of the culture media for its growth. The optimal growth occurred on the 4th day (mid-point) of culture in peptone water–glucose, KNO_3–glucose and Bambara nut media. This implies that the 4th day of culture is important for peak harvest of bacteria, irrespective of the culture media. It was observed that the highest production or yield of non-pathogenic *E. coli* strain was obtained using the Bambara nut medium, while the least yield was obtained in the KNO_3–glucose medium. It thus follows that Bambara nut medium is most suitable for optimal growth of non-pathogenic *E. coli* strain which might be due to its organic nitrogenous source.

Table 4: Gas chromatography profile of Amino acid in non-pathogenic *E. coli* metabolites extract

Library ID	Area (%)	Quality	Retention time (min)
DL-3-Aminoisobutyric acid, N-dimethylamino methylene-, ethyl ester	11.45	64	3.663
Methacrylamide	1.36	59	3.735
1,3-Butadiene, 1-{(1-methyl ethyl)t hio}-, (E)-	1.53	43	3.754
Methacrylamide	2.67	59	3.773
1,3-Propylene glycol, O,O-di(pivaloyl)-	11.62	47	3.939
Butanal, 3-methyl-	5.1	32	3.996
1,3-Propanediamine, N-methyl-	5.23	35	4.044
Cyclohexanamine, N-3-butenyl-N-methyl-	1.79	42	4.301
Imidazole-4-carboxylic acid, 1-met hyl-	6.92	37	4.606
4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	7.63	53	4.639
1,6-Diaminohexane-N,N,N',N'-tetraa cetic acid	0.85	50	5.601
Acetic acid, {(aminocarbonyl)amino} oxo-	0.72	50	5.616
1-Propanol, 2-amino-, (+/-)-	0.99	50	5.654
2(3H)-Furanone, dihydro-4-hydroxy-	1.42	50	5.758
2-Butene ozonide	1.12	53	5.773
5-Hydroxymethylfurfural	9.88	53	6.330
Alloxan	0.48	27	7.916
2R,3S-9-{1,3,4-Trihydroxy-2-butoxy methyl}guanine	0.33	43	14.259
2-Formyl-9-{beta.-d-ribofuranosyl}hypoxanthine	0.17	50	14.559

Peptone water–glucose culture medium was next to Bambara nut medium in growth yield. The growth of *E. coli* in peptone water–glucose culture medium, as an organic nitrogenous source, supported higher growth than KNO_3–glucose, which is an inorganic source of nitrogenous nutrients. This agrees with the reports of Hepburn *et al* [16], where buffered peptone water broth gave a significant increase in recovered *E. coli* compared to other tested media, and Shu [17], where the type of nitrogenous source was shown to have a key effect on microbial cell growth. On the expiration of the 7-day culture period, bacteria yield from the entire medium declined from optimal values recorded on the 4th day, indicating evidence of system collapse with time, as the *E. coli* under culture failed to grow and was dying out. The *E. coli* growth in all culture settings drastically declined after the late exponential phase. The system collapse stimulated the secretion of extra-cellular secondary metabolites by bacteria in the face of starvation due to the depletion of nutrients from both nitrogenous and sugar catabolism in the set cultures and also ammonia accumulation [18].

Generally, the design of this study was to create an environment where the non-pathogenic *E. coli* strain can optimally mass produce and suddenly be upset through starvation for them to secret extra-cellular secondary metabolites. Specifically, the secretion of extra-cellular secondary metabolites by non-pathogenic *E. coli* under liquid-state fermentation was optimal on the 7th day of culture. The peptone water–glucose culture showed a decline from an absorbance of 1.48 to 0.37 indicating a 75.1 % reduction while KNO_3–glucose culture reduced from 0.607 to 0.417, indicating a 31.3 % reduction. Lastly, Bambara nut powder reduced from 3.05 to 1.146 indicating a 62.4 % reduction. The peptone water–glucose, having had the highest percentage of bacteria reduction, was therefore expected to also have the optimum extra-cellular metabolite yield.

CONCLUSION

The combination of peptone water (0.9 g), glucose (4.0 g), and pH (7.25) at 37 °C gives the optimum culture conditions for non-pathogenic *E. coli* metabolite yield with an absorbance value of 0.3702 on the 7th day of culture. The culture media shows a more rapid utilization of nutrients as it depletes faster and the primary goal of accessing a comprehensive print of small metabolite molecules in the non-pathogenic *E. coli* strain is achieved.

DECLARATIONS

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None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Samuel B. Umma conceived, designed the study, and wrote the manuscript. Yetunde E. Agbeja and Olusegun O. Oyebola wrote the manuscript. George D. Ametefe collated and analyzed the data.

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