SOME CHARICTERISTIC OF MICROBIAL INDOLE EXTRACTED FROM PATHOGENIC *E. COLI* IN COMPARABLE WITH STANDARD ONE

Abdulazeez H. Alqaisi **Mouruj A. Al aubydi**
Assis. Lecturer **Prof.**
Dept. Biotech., Coll. Sci., University of Baghdad, Iraq
Azeez89kc@gmail.com

ABSTRACT

Indole molecules as a signal has an important role in bacterial ecosystem, this study was aimed and conducted on the extraction and partial purification of indole from pathogenic *E. coli* and study several indole characterizations parameters compared with synthetic standard one. A total of 134 urine specimens and other stool 152 specimens were obtained from Al-Ramadi Hospitals, during the period from November / 2018 to March / 2019.The results show the percentage of isolated *E. coli* from Urinary tract infection was 100 (74.6%)/134, While 152 (100%)/ 152 are isolated from stool. Primary and secondary screening concluded, there are ten isolates are considered as the best in production of indole. The most producible one is selected with indole concentration 165.667 µM/ml. Also, indole production needs several optimal parameters to elevate its production, the study improved that the best pH is 9, temperature is 35 °C, incubation period is 18 hrs. and indole concentration is directly proportional with the amount of tryptophan added. Thin layer chromatography result reveals that no significant difference between extracted indole R_f 0.9 and 0.91 of synthetic standard one. Fourier-Transform-Infrared Spectroscopy, the results show there are some differences in the analysis in some structural positions.

Key words: Bioactive compounds, heterocyclic, TLC, FTIR, Optimization, VOCs.

بعض الخصائص التشخيصية للإندول الميكروبي المستخلص من الإشريكية القولونية المسببة للأمراض في مقارنة مع الاندول القياسي

عبد العزيز حسين القيسي **مروج عبد الستار العبيدي**
مدرس مساعد **استاذ**
قسم التقنيات الاحيائية – كلية العلوم – جامعة بغداد – العراق

المستخلص

جزيئات الاندول كإشارة لها أهمية في النظام البيئي البكتيري، تهدف هذه الدراسة إلى الاستخلاص والتنقية الجزئية للإندول من الإشريكية القولونية المرضية ودراسة العديد من خصائص الاندول مقارنةً بالاندول القياسي. إجمالي 134 عينة بول و152 عينة براز جمعت من مستشفيات الرمادي خلال الفترة من تشرين الثاني / 2018 إلى اذار / 2019، وأظهرت النتائج أن نسبة الإشريكية القولونية المعزولة من البول كانت 100 (74.6٪) / 134 بينما تم عزل 152 (100٪) / 152 من البراز. خلص الانتخاب الأولي والثانوي إلى أن هناك عشر عزلات تعتبر الأفضل في إنتاج الإندول. تم اختيار الأكثر إنتاجية بإنتاج إندول 165.667 ميكرومتر / مل. كذلك يحتاج إنتاج الإندول إلى العديد من العوامل المثلى لرفع إنتاجه، وقد اثبتت الدراسة أن أفضل درجة حموضة هي 9، ودرجة الحرارة 35 درجة مئوية، وفترة الحضانة 18 ساعة. ويتناسب تركيز الإندول بشكل مباشر مع كمية التربتوفان المضافة. كروماتوغرافيا الطبقة الرقيقة كشفت عن عدم وجود فرق معنوي بين الإندول المستخلص0,9 والصناعي 0,91. جهاز "فورييه" لتحويل طيف الأشعة تحت الحمراء، اظهرت النتائج وجود اختلافات في التحليل في بعض المواقع التركيبية.

الكلمات المفتاحية: المركبات الحلقية، المركبات العضوية المتطايرة، كروماتوغرافيا الطبقة الرقيقة، منتج بكتيري.

Received:27/9/2021 Accepted:23/1/2022

INTRODUCTION

Indole is composed from an aromatic heterocyclic organic compound (C_8H_7N). It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered pyrrole ring with molecular weight 117.15 g/mol. Indole in different form are widely distributed in the environment and can be produced by different bacteria. Indole is a common material in the natural environment (19), More than 85 bacterial species have been found to generate substantial amounts of this volatile compound, including both Gram positive and negative bacteria of which most of them that can cause diseases. Fungal indole diterpenes (IDTs) are isolated as anti-insect ants as well as mycotoxins (21). For inducing indole-producing microorganisms such as *Escherichia coli*, the major source for indole making is an amino acid tryptophan. Tryptophanase is an enzyme coded form gene *tnaA* diminishes Tryptophan (Trp) to ammonia, pyruvate, and indole in a reversible reaction. Additionally, carbon resources like glucose can suppress *tnaA* expression (29). In addition, some ecological parameters such as temperature and pH similarly alter 1H-Indole synthesis in *E. coli*. (18) clarified that *tnaAB* gene expression can be induced at which the temperature ranging from 30 to 43°C, but bacterial cells lost indole synthesis ability at 44.5°C. They concluded the effect of 1H-Indole signaling is important at a minimize temperature (30°C) and less, rather than 37°C (18). Beside a low pH work on suppress indole production, while TnaA is one of the high active gene at pH 9.0 (19). Some worldwide researchers revealed that indole is more than just a tryptophan metabolite, and that it regulates bacteria in a variety of ways. For starters, it is linked to bacterial toxicity and drug defense. Toxin excretion by bacteria is stimulated by indole. Indole play roles as aquorum-sensing (QS) signal particle that effect on diverse characteristics of bacterial physiology begin form stop growth population to biofilm formation (29). Researchers were discovered that the poisonous of Enteropathogenic *E. coli* to *Caenorhabditis elegans* nematode is dependent on the presence of the Tryptophan amino acid and does not require direct contact, and that the nonattendance of Tryptophan in culture broth or prevent tryptophanase gene translation prohibit of its poisonous (4). Moreover, indole is a chemo-repellent and reduces motility (19), but a researcher (11) reported that acetic acid Indole-2 is a chemo-attractant in *E. coli.* Furthermore, enterohaemorrhagic *E. coli* (EHEC), a tnaA mutant, released less EspA and EspB (type III secreted proteins) and was less capable of forming attaching and effacing (A/E) lesions in HeLa cells. However, when indole was added exogenously, such abilities were restored (15). Additionally, it had proposed that indole progresses drug resistance in bacteria. Also, indole prompts the expression of multidrug active genes such as (acrD, mdtA, cusB, emrK, yceL and mdtE) that lead to defend the organism from extracellular toxic materials (24). Secondly, indole have role in sustaining genetic stability. Through contributing to the maintenance of plasmids copies, other researchers report that manifested the process is supplementary supported by the supervision that adding 4 mM indole to *E. coli* broth blocked cell division. It suggests that indole is a wide-ranging signal actively contributing in different events from metabolic feedback regulation, spores forming, to cell cycle regulation (8,9). Thirdly, indole is an important signal in metabolic regulator. It can stimulate genes related with amino acids breakdown, such *astD* (translated succinylglutamic semialdehyde dehydrogenase), as *cysK* (encoding cysteine synthase), and aforementioned *tnaB* (26). The ability of bacteria to modulate its function in response to changing dietary circumstances is one benefit of signaling via the buildup of amino acid metabolites. It was proposed that the capacity to catabolize amino acids is a key indicator of microorganism ability to stay in stationary phase, that indole excretion arranges cells for inadequate environment feeding, and that amino acid catabolism returns a vital energy source (26). Because of indole important in bacterial ecosystem, this study is aimed and conducted on the extraction and partial purification of indole from pathogenic *E. coli* and study several indole characterizations compared with synthetic standard one.

MATERIALS AND METHODS

Specimens collection and selected isolates

A total of 286 sample were collected including stool 152 specimens and 134 urine sample of patients suffering from (UTI) were obtained from Al-Ramadi Teaching Hospital for Maternity and children & Al-Ramadi Teaching Hospital, during the period from November / 2018 to March / 2019. The isolates were recognized firstly according to the general culture characteristic (color, shape, texture and size), and then characteristics parameters like Indole formation, lactose fermentation on MacConkey agar and green metallic shine on EMB agar (HiMedia, India) (1,2), this step is considered as primary screening. Secondary screening, was aimed to found indole highest produce *E. coli*. This step is summarized the optimal conditions used to induce bacterial isolate to produce indole as followings (27); culturing *E. coli* isolate using Luria Bertani broth (LB) (HiMedia, India). The pH investigated at a range from 5 –10 with addition of 0.1M (HCl or NaOH). Then, the broth autoclaved at 121℃ for 15 min at 15 pounds/square, the culture supported with different concentrations of tryptophan (Sigma, USA) included;0,1,5,10,15 and 20 µM/ml. As well as, Ampicillin (Sigma-Aldrich) was added in different concentrations which are; 0, 2, 4, 8 and16µg/ml as inducer, then incubated at 37 °C to find the more indole productive isolate. Different incubation temperatures included; 25, 30, 35, 40 and 45 ℃ were applied to find the best temperature degree for production. The broth was incubated for 10, 12, 14, 16, 18, 20, and 22 hrs to identify the incubation time that provided the highest amount of indole.

Qualitative and quantitative determination of indole: For primary qualitative of indole, Kovács reagent was used to determine the ability of each isolate to produces indole, while quantitative estimation was done through standard curve preparation using standard synthetic indole purity 98% (10) as follows; Freshly standard Indole solution was prepared by solubilized 1.8 mg in 50ml of ethanol 70% to get 300 µM stock solution, followed by serial dilution to made concentrations of 0, 25, 50, 100, 150, 200, 250 and 300 µM. Hydroxylamine based indole assay (HIA) was done as follows: 1 ml of each indole concentration was add to 0.25 ml of Sodium hydroxide (5.3M) and 0.5 ml of hydroxylamine hydrochloride (0.3M) at lab temperature. A 1.25 ml of Sulfuric acid (2.7M) was added after 15 min, thoroughly mixed and incubated at lab temperature for 30 mins a pink solution is visible. A total volume of 3 ml that was measured by spectrophotometer at 530nm wavelength including blank. The relation between absorbency (O.D.) and indole concentration was drawing to obtain the standard curve of indole (Figure 1).

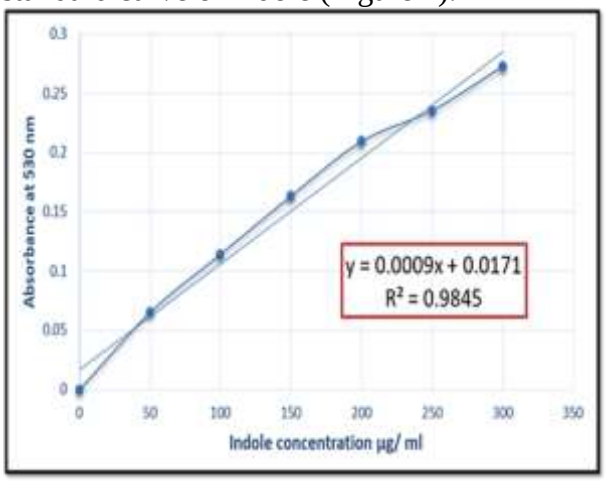


Figure 1. Standard Curve of Indole

Indole production, separation and partial purification: In a pilot scale, the selected bacterial isolate was propagated in LB broth and incubated overnight at 37 ℃. And then, bacterial growth supernatant was collected and mixed well with Ethyl acetate (Romil, UK) (1:1) in separation funnel at lab temperature, the solvent phase was collected and dehydrated using rotary evaporator (Heidolph, Germany) at 48 ℃ to evaporate all solvent and reminded yield of bacterial supernatant is considered as crude indole extract. Then, the separated yield was purified throughout dissolving the extracted product in ethanol then filtered by Centrifugal Filter Unit with 4.0-mL volume, 3,000 Nominal molecular weight limits, and allowed to passage through the ultrafiltration tubes (Merck, USA), that permit passaging molecules with molecular weight less than 3 KD. This step is considered as partial purification method.

Thin-layer Chromatography (TLC) of partial purified indole compared with synthetic : To investigate the purity of the partial purified indole, TLC technique was

carried out on silica gel (Silica Gel 60 F254, Merck), ethyl acetate: methanol: chloroform (3:1:1) that used as the mobile phase solvent. The partial purified sample was spotted at the powdered side of the silica gel plate (plastic side is the side that is spotted). A pencil line was drawn lightly nearby 1 cm from the end of a plate. A pipet was used to spot the sample, and the spot as small as possible (less than about 1 mm diameter). After applying the spot with a reasonable size. aluminum plate was kept in a TLC chamber for 46 minutes with monitoring to analyses and separate the compositions of partial purified indole depending on the Rf value which was calculated using following equation:

Rf = Distance covered by solute / Distance run by the solvent (3,6).

Characterization of extracted indole

Fourier-Transform-Infrared Spectroscopy (FT-IR) The functional groups and chemical bonds of partial purified bacterial indole extract and artificial indole were analyzed. The spectrum was limited at the range of 400-4000 cm-1 with resolution of 4 cm-1. As well as spectrophotometer was used to determine indole concentration at a wave length 530nm.

RESULTS AND DISCUSSION

From total 286 samples of urine and stool, the number and percentage of isolated *E. coli* from urine is 100 (74.6%)/134, this result is considered so high, and explained by researchers that because *E. coli* is one of gastrointestinal tract normal flora, thus is commonly considered as the main cause of urinary tract infection (UTI), which is one of the most prevalent human illnesses (7,14). Most UTIs are caused by bacteria ascending from the urethra to the bladder, and perhaps the kidneys. While 152 (100%)/ 152 were isolated from stool, this result was expected because *E. coli* is one of intestinal normal flora belongs to Enterobacteriaceae family. The gastrointestinal tract (GIT) is generally accepted as a reservoir for Uropathogenic *E. coli* (UPEC) and is supposed that healthful humans have a reservoir of UPEC strains. This bacterial strain has superior capability to persist in the gut of humans and can extent to cause extra-intestinal infections (16).

Selected *E. coli* Isolates: Dependent on primary and secondary screening, ten of *E. coli* isolates were selected {2} from urine and {8} from stool specimens' Figure 2. The most producible one is selected. Therefore, the isolate number 127 was a favorable one due to producing indole at a conc. 165.667 µM/ml.

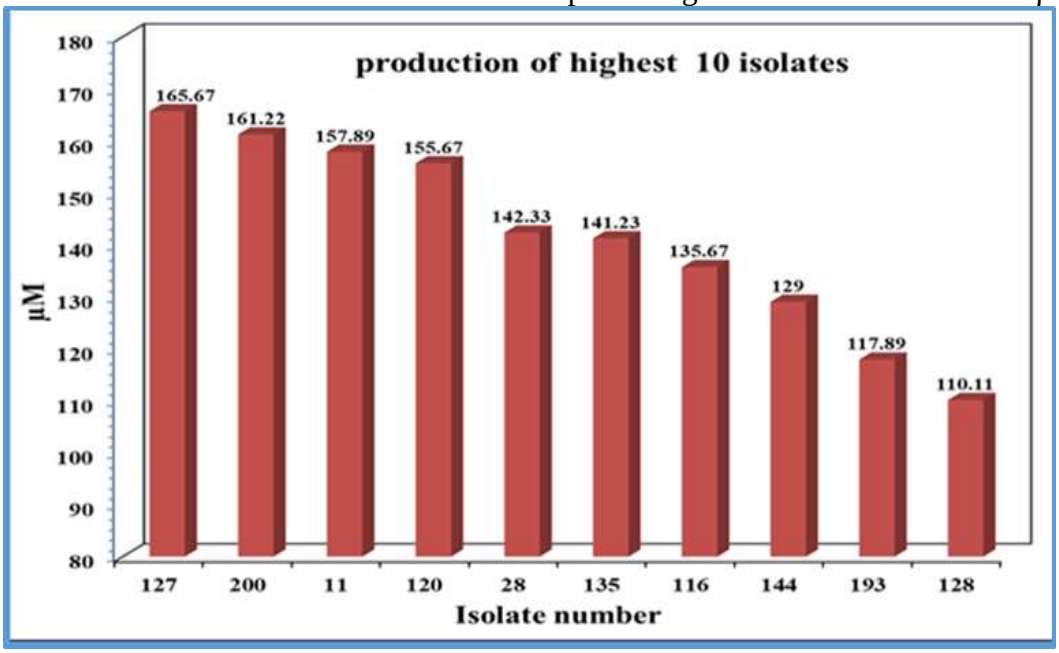


Figure 2. Indole concentration for highest produce 10 *E. coli* isolates

This amount of producible indole in recent study is represent as primarily production after secondary screening. Dependent on (13) they reveal that the extracellular pH is a significant feature for indole formation that moreover effects biofilm formation of *E. coli*. Additionally, *E. coli* produced high level of extracellular indole when antimicrobials such

as ampicillin is found, and then increased indole improved cell survival during antimicrobial stress as show in Figure3. Moreover, they found that indole is a constant volatile organic product, and *E. coli* may use indole to shelter itself against another microorganisms (13). While, the recent study when implementing the optimal condition, it suggesting the following; the best pH is 9 as show in Figure 4.

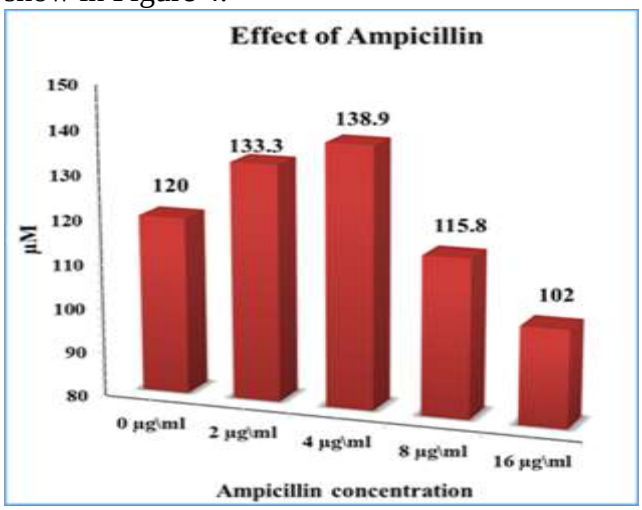


Figure 3. Effect of Ampicillin concentration on extracellular indole production

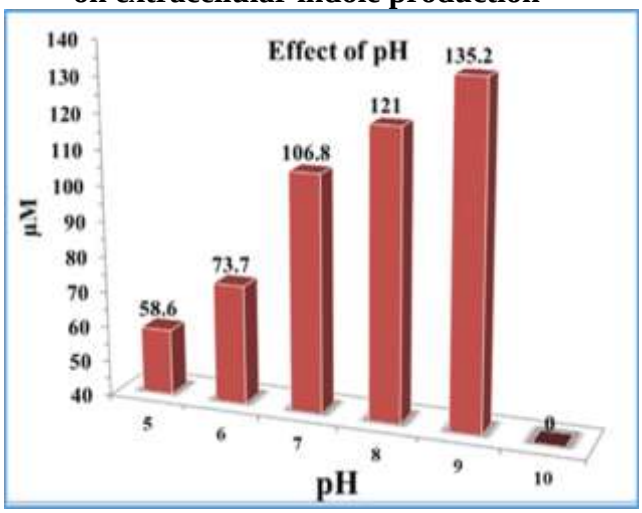


Figure 4. Effect of pH on extracellular indole production

Bacterial cells are unfavorably reliant on pH regulation. The present result was compatible with **(**17) they proved that, *E. coli* is a neutrophilic bacterium that can propagate at external pH from 5.0 to 9.0 but commonly conserves its cytoplasmic pH in the range 7.2-7.8 and that explained by research demonstrated that indole works as a significant role in the instruction of the cytoplasmic pH of *E. coli* (5). Cells maintain their cytoplasmic pH at 7.2 under specific circumstances permitting indole synthesis. Under adverse circumstances, where no indole is generated, the cytoplasmic pH is close to 7.8. They demonstrate that pH control is caused by pulse (28). Furthermore, the capacity of indole to transmit protons across the cytoplasmic membrane is important. Furthermore, the action of the indole pulse, which typically occurs during a stationary phase pass in rich medium, acts as a memory to maintain the cytoplasmic pH until entrance into the subsequent stationary phase. The indole-mediated reduction in cytoplasmic pH may provide a response, why indole be responsible for *E. coli* protection against external stresses, including some bactericidal antimicrobials. As well as, temperature is another important factor, the recent result revealed that, 35°C is the best for indole production Figure 6. This result was incompatible with that confirmed by researcher who improved that, 50°C is the best to produces microbial indole (13). Whereas second research, shown that the one of quorum-sensing signals is indole which influence the biofilm formation of *E. coli*. temperature, affects indole signaling in *E. coli*, it may result in additional general variance gene expression at 30°C, that include (186 genes) than at 37 °C (59 genes), that indole decreases biofilm formation (without impact growing) more dramatically at 25 and 30 °C than at 37 °C, and that the action is linked to the QS SdiA proteins of *E. coli* and *Salmonella enterica* (13). American society for microbiology advised in indole test protocol, to incubate bacterial isolate at 35 °C for producing indole.

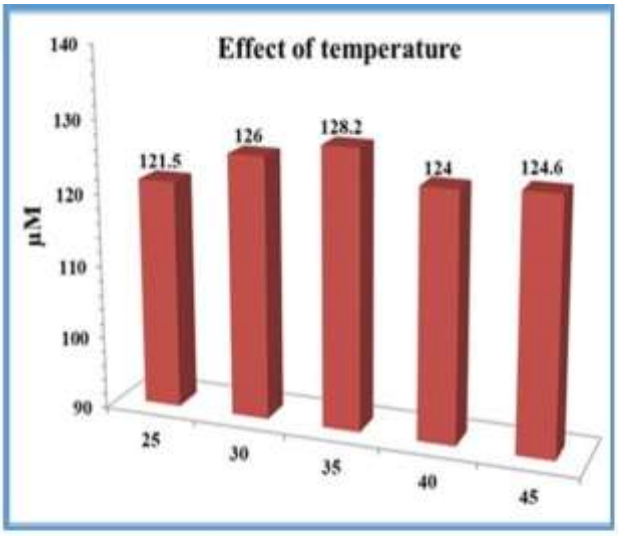


Figure 5. Effect of temperature on extracellular indole production.

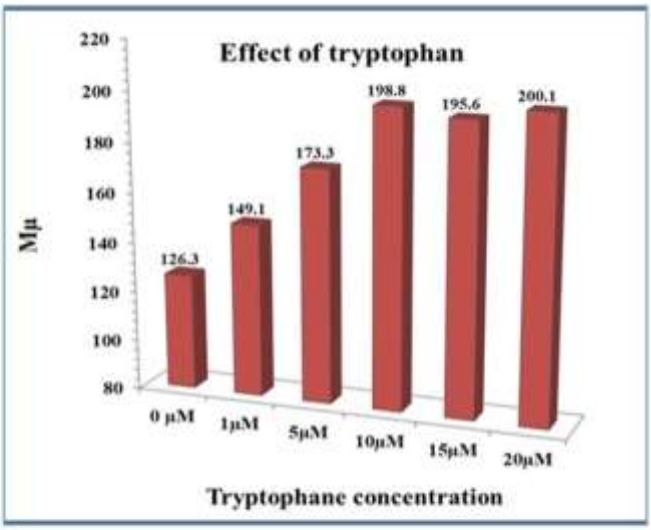


Figure 6. Effect of tryptophan concentration on extracellular indole production.

Whereas, the increase in indole production is proportional to the increase in the concentration of tryptophan, Figure 6. The finale yield product of indole depends directly, and perhaps solely, on the amount of exogenous tryptophan. When added with a range of Trp. Conc., *E. coli* changed this amino acid into an identical quantity of indole (20). Finally, the results show that the best incubation period is 18hrs. As shown in Figure 7. It was documented that, the concentration of indole in *E. coli* cultures ordinarily increases during the lag and early log phases of bacterial growth, thereby permitting early recognition (29). Whilst, in a stationary phase culture at high temperatures, *E. coli* cells have been shown to create abundant amounts of indole, which has been hypothesized to promote survival (22). The amount of indole production reach to 386 µM/ml. and the final product of pilot scale was 2.17g/ 40 L of LB broth.

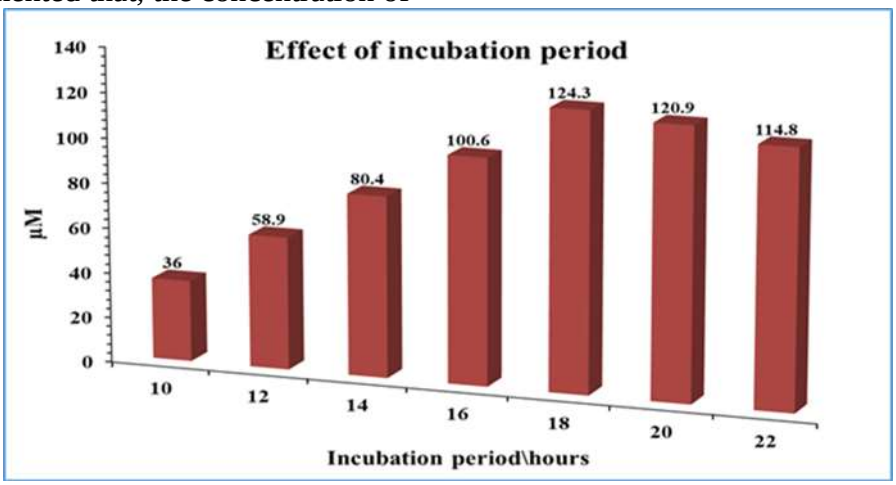


Figure 7. Effect of incubation period on concentration of extracellular indole production

Characterization of microbial indole by thin layer chromatography

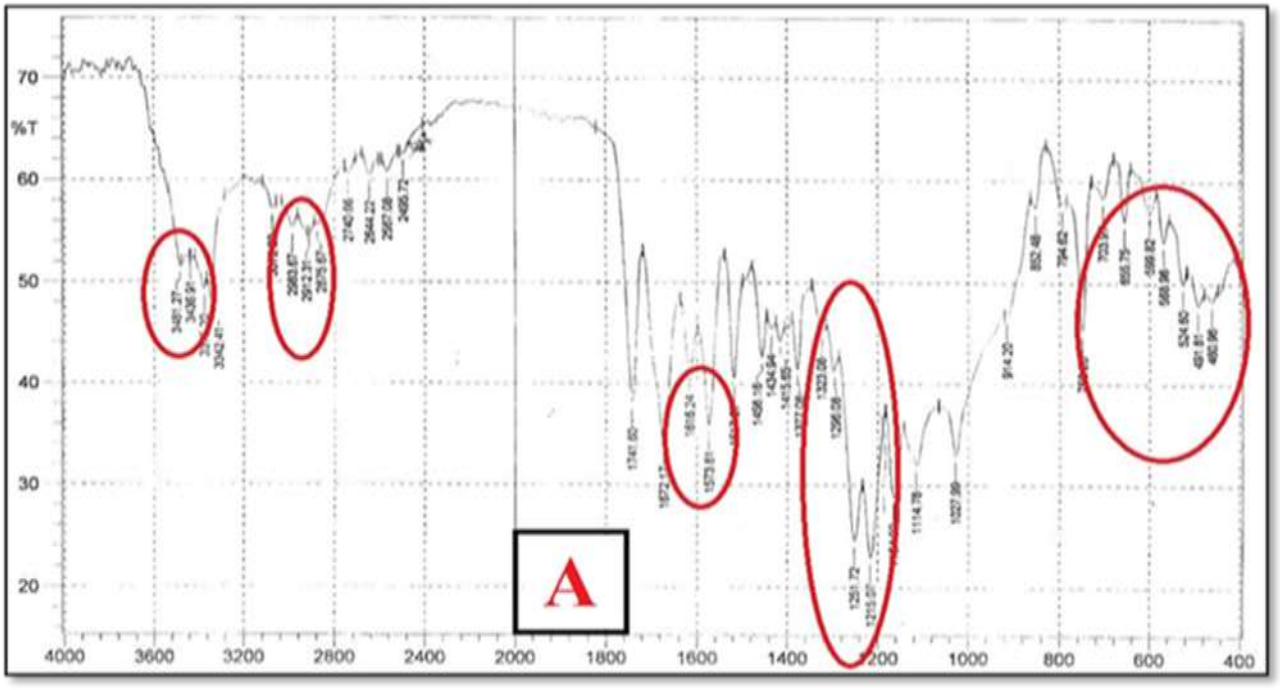
The following figures established the values of RF for both standard indoles which is 0.91, and for extracted indole which is 0.9. These values revealed no significant difference between them, as well as reflecting a little difference in the structure of microbial indole than standard once Figure 8, Table 1.

Table 1. R_F calculation results for extracted and standard indole

	Distance travelled (cm)	R_F
Standard indole	**13.2**	**13.2/14.4 = 0.91**
Extracted indole	**15.3**	**15.3/17 = 0.9**

Fourier-Transform-Infrared Spectroscopy (FTIR) analysis: The functional groups and chemical bonds of purified bacterial indole extract and synthetic indole were analyzed by using FTIR spectrometry. The spectrum was limited at the range of 400-4000 cm-1 with resolution of 4 cm-1. FT-IR spectrum of both standard and bacterial indoles are presented in Figure 9 The FT-IR of sample showed stretching vibration band at 3481.27, 3436.91 and 3444, 3417.86 cm-1 which was attributed to the N-H band. The band at 1616.24 and 1635.84 cm-1 as a result of C=C-C from aromatic ring. The peak at 2875.67 and 2839.22 cm-1 attributed to C-H stretching C-N strong stretching were appeared at 1573.81 and C=C-N banding at 1550.77 cm-1 in the sample. Vibrations peaks at 1323.08, 1251.07 cm-1, and 1330.88, 1253.73 cm-1 were due to C-N stretching in the sample. The FTIR region 1226–949 cm-1 (several) exhibits Aromatic C-H in-plan, while regions 900–670 cm-1 (several) exhibits Aromatic C-H out of plan bend. Other vibration peaks were observed at the rang 2000-1660 were represent aromatic combination bands. The observed FT-IR data is depended on reported literature (12,23).

Figure 8. TLC experiment of A) extracted indole B) standard indole, imaged under visible light and UV illumination



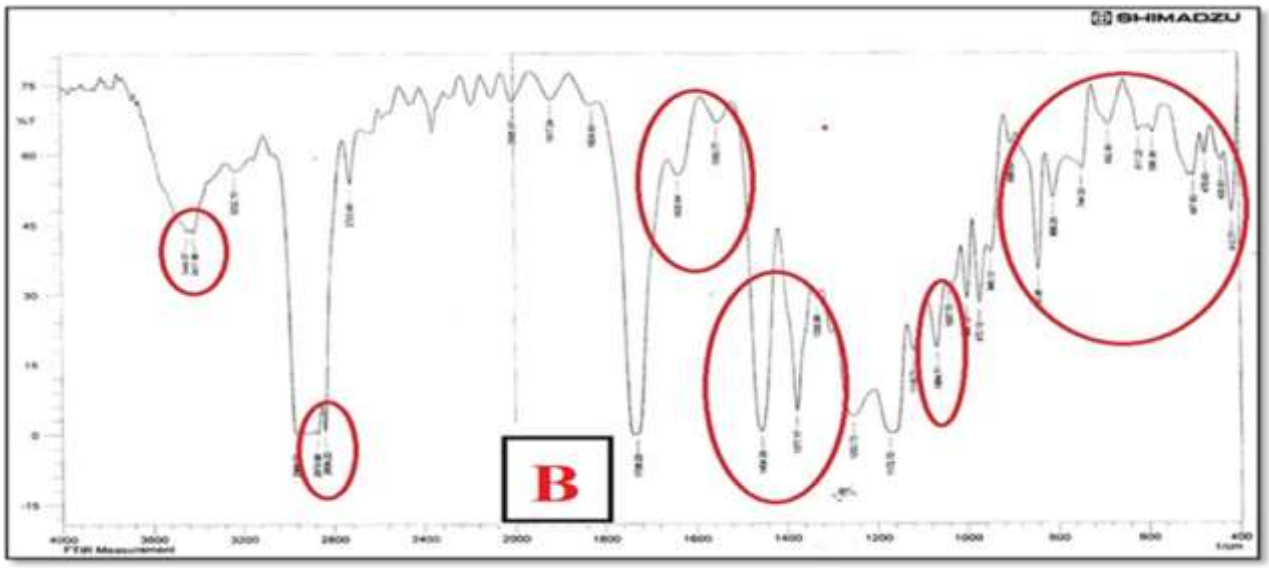


Figure 9. FTIR analysis for A) Standard indole, B) Partial purified bacterial indole

CONCLUSION

E. coli has different capability to produce indole dependent on different parameters such as pH, temperature, incubation period, inducer type and precursor.

REFERENCES

1. Abdulridha, R. N., and O. M. Ibrahim. 2018. Activity of bacterial antibiotics against some pathogenic bacteria isolated from calves diarrhoea in Baghdad (part I). The Iraqi Journal of Agricultural Science, 49(5): 847. https://doi.org/10.36103/ijas.v49i5.45

2. Ahmed, N. A., Mahmood, S. S., and Abbas, A. H. 2019. A comparative study of some virulence factors and phylogenetic charactrization of *Escherichia. coli* isolates causing urinary tract infection and the commensal gut. The Iraqi Journal of Agricultural Science, 50(4), 1193-1198. https://doi.org/10.36103/ijas.v50i4.763

3. Alshaikh Faqri, A. M., N. H. Hayder, , and A. J. Hashim. 2019. Lab-scale production of rhamnolipid by *Pseudomonas aeruginosa* A3 and study its synergistic effect with certain antibiotics against some pathogenic bacteria. Iraqi Journal of Agricultural Sciences, 50(5) :1290-1301. https://doi.org/10.36103/ijas.v50i5.794

4. Anyanful, A., J. M. Dolan-Livengood, T. Lewis, S. Sheth, M. N. DeZalia, M. A. Sherman, and D. Kalman. 2005. Paralysis and killing of Caenorhabditis elegans by enteropathogenic *Escherichia coli* requires the bacterial tryptophanase gene. Molecular Microbiology, *57*(4): 988-1007

5. Ashraf Zarkan, Marta Matuszewska, Stephen B. Trigg, Meng Zhang, Daaniyah Belgami, Cameron Croft, Junyan Liu, Sawssen El-Ouisi, Jack Greenhalgh, James S. Duboff, Taufiq Rahman & David K. Summers. (2020) Inhibition of indole production increases the activity of quinolone antibiotics against E. coli persisters. Scientific Reports volume 10, Article number: 11742

6. Atwan, Q. S., and N. H. Hayder .2020. Eco-friendly synthesis of Silver nanoparticles by using green method: Improved interaction and application in vitro and in vivo. Iraqi Journal of Agricultural Science, 51(Special Issue):201-216. https://doi.org/10.36103/ijas.v51iSpecial.898

7. Behzadi, P., E. Urbán, M. Matuz, R. Benkő and M. Gajdács 2020. The role of gram-negative bacteria in urinary tract infections: Current concepts and therapeutic options

8. Chant, E. L., and D. K. Summers. 2007. Indole signalling contributes to the stable maintenance of *Escherichia coli* multicopy plasmids. Molecular Microbiology, 63(1): 35-43

9. Chattoraj, D. K. 2007. Tryptophanase in sRNA control of the *Escherichia coli* cell cycle. Molecular Microbiology, 63(1): 1-3

10. Darkoh, C., C. Chappell, C. Gonzales, and P. Okhuysen. 2015. A rapid and specific method for the detection of indole in complex biological samples. Applied and Environmental Microbiology, 81(23): 8093-8097

11. Englert, D. L., M. D. Manson, and A. Jayaraman. 2009. Flow-based microfluidic device for quantifying bacterial chemotaxis in

stable, competing gradients. Applied and Environmental microbiology, 75(13): 4557-4564

12. Faqri, A. A., N.H. Hayder, and A. J. Hashim .2019. Induction of rhamnolipid production by pseudomonas aeruginosa A3 using chemical and physical mutagenic factors. Iraqi Journal of Agricultural Sciences, 50(4):1174-1185 https://doi.org/10.36103/ijas.v50i4.766

13. Han, T. H., J. H. Lee, M. H. Cho, T. K. Wood, and J. Lee. 2011. Environmental factors affecting indole production in *Escherichia coli*. Research in Microbiology, 162(2): 108-116.

14. Haugan, M. S., F. B. Hertz, G. Charbon, B. Sahin, A. Løbner-Olesen, and N. Frimodt-Møller. 2019. Growth rate of *Escherichia coli* during human urinary tract infection: Implications for antibiotic effect. Antibiotics, 8(3): 92.

15. Hirakawa, H., T. Kodama, A. Takumi-Kobayashi, T. Honda, and A. Yamaguchi. 2009. Secreted indole serves as a signal for expression of type III secretion system translocators in enterohaemorrhagic *Escherichia coli* O157:H7. Microbiology, 155(2): 541-550

16. Katouli, M. 2010. Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. Iranian Journal of Microbiology, 2(2): 59

17. Krulwich, T. A., G. Sachs, and E. Padan. 2011. Molecular aspects of bacterial pH sensing and homeostasis. Nature Reviews Microbiology, 9(5): 330-343

18. Lee, J. H., and J. Lee. 2010. Indole as an intercellular signal in microbial communities. FEMS Microbiology Reviews, 34(4): 426-444

19. Lee, J., X. S. Zhang, M. Hegde, W. E. Bentley, A. Jayaraman, and T. K. Wood. 2008. Indole cell signaling occurs primarily at low temperatures in *Escherichia coli*. The ISME Journal, 2(10): 1007-1023

20. Li, G., and K. D. Young. 2013. Indole production by the tryptophanase TnaA in *Escherichia coli* is determined by the amount of exogenous tryptophan. Microbiology, 159(Pt_2): 402-410

21. Liu, C., A. Minami, T. Ozaki, and H. Oikawa. 2020. Biosynthesis of Indole Diterpenes. Comprehensive Natural Products III (Third Edition). Chemistry and Biology, 2: 446-466

22. Liu, J., and D. Summers. 2017. Indole at low concentration helps exponentially growing *Escherichia coli* survive at high temperature. Plos One, 12(12): e0188853

23. Nandiyanto, A. B. D., R. Oktiani, and R. Ragadhita. 2019. How to read and interpret FTIR spectroscope of organic material. Indonesian Journal of Science and Technology, 4(1): 97-118

24. Nishino, K., T. Honda, and A. Yamaguchi. 2005. Genome-wide analyses of *Escherichia coli* gene expression responsive to the BaeSR two-component regulatory system. Journal of Bacteriology, 187(5): 1763-1772.

25. Sharma, S., P. P. Verma and M. Kaur 2014. Isolation, Purification and Estimation of IAA from Pseudomonas sp. using High-performance liquid Chromatography. Journal of pure and applied microbiology, 8(4), 1-6

26. Wang, D., X. Ding, and P. N. Rather. 2001. Indole can act as an extracellular signal in *Escherichia coli*. Journal of Bacteriology, 183(14): 4210-4216.

27. Younis, R. W. 2020. Production, purification and inhibition of alginate ly-ase from local isolate of pseudomonas aeruginosa na11. Iraqi Journal of Agricultural Sciences, 51(6):1726-1739. https://doi.org/10.36103/ijas.v51i6.1201

28. Zarkan, A., S. Cano-Muniz, J. Zhu, K. Al Nahas, J. Cama, U. F. Keyser, and D. K. Summers. 2019. Indole pulse signalling regulates the cytoplasmic pH of *E. coli* in a memory-like manner. Scientific Reports, 9 (1): 1-10

29. Zhang, S., W. Zhang, N. Liu, T. Song, H. Liu, X. Zhao, and C. Li. 2017. Indole reduces the expression of virulence related genes in Vibrio splendidus pathogenic to sea cucumber Apostichopus japonicus. Microbial Pathogenesis, 111: 168-173

30. Zhong, Q., F. Cheng, J. Liang, X. Wang, Y. Chen, X. Fang, and Y. Hang. 2019. Profiles of volatile indole emitted by *Escherichia coli* based on CDI-MS. Scientific Reports, 9(1): 1-6.