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Identification and Molecular Characterization of Antibiotic-Resistant Escherichia Coli Strains From Poultry Farm in Kerala

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Abstract
The steepened rise in population contributed to a heightened demand for livestock products, especially poultry products. The scenario surged the extensive use of antibiotics in poultry industry for enhanced production, and to address the growing demand. The utilization of antibiotics as growth enhancers and immunity boosters for fowls, gradually contributed to the evolution of antibiotics resistant bacterial strains including Escherichia Coli. The increasing number of antibiotic-resistant genes acquired via horizontal gene transfer contributed significantly to the increased antibiotic resistance in Escherichia Coli. Currently, this scenario is considered one of the major challenges confronting human health globally. In addition to causing grave health consequences, the bacteria serves as a reservoir of antibiotic-resistant genes that may lead to treatment failures for several diseases in both humans and animals. The current situation necessitates extensive research to identify novel strains and to explore the factors that promote antibiotic resistance in bacterial strains. The present study attempts to demonstrate the phenotypic and genotypic characterization of genetically novel Escherichia coli isolated from litter samples collected from poultry farms situated at Thiruvananthapuram district, Kerala, India. Multi Drug Resistance strains of E. coli strain were identified and the draft assembly was submitted to GenBank.
Keywords: *E. coli., Antibiotic resistance, Poultry, 16s rRNA, PCR, Gene isolation, Gel Electrophoresis.*

INTRODUCTION

The onerous journey of poultry farming is anticipated to emerge in Southeast Asia [1]. Poultry farming evolved into a livestock enterprise in the late 19th and early 20th centuries as a result of progress in nutrition and genetics. [2]. The rise in poultry product consumption has surged significantly alongside the exponential increase in the global population. The scenario necessitated the urge for a situation where poultry products must satisfy the demand while preserving their quality and safety. With an annual growth rate of about 8 to 10 percent, the poultry industry is currently one of the fastest-growing segments of the agriculture sector [3].

Although poultry farming plays a crucial role in stabilizing the global economy, the expansion of the poultry sector faces several challenges. Some of the significant factors that hamper the industry's growth include immune compromised fowls, those with reduced growth rates, and difficulties in maintaining consistent production levels [4]. The perpetual increase of various diseases further presents obstacles to the strategic future of the industry, particularly in addressing bacterial infections. Historically, attempts to the prevalent use of antimicrobials in the poultry industry had initiated in the mid-20th century, primarily for treating bacterial infections. Subsequently, these treatments were incorporated into poultry

feed and drinking water as both growth enhancers and preventive measures, thereby enhancing poultry health and productivity. Despite the significant economic advantages gained from the extensive use of antimicrobials in the poultry industry, the practice has also led to the emergence of antimicrobial drug resistance strains. Antimicrobials were initially introduced for treating diseases in human, but surprisingly 73 % of its global production was used in livestock management [5]. India and China, being the major producers of meat thus evolved as the hotspots of AMR. The Central and Southern India were predicted as emerging hotspots for AMR in poultry but lacks substantial data. Evidently, the Southern India had the highest abundance of AMR genes than the Central India [6].

Antibiotic resistance has been recognized to play a vital role in contributing to severe ailments in humans [7]. Mounting evidence highlights multitude of issues leveraged by antimicrobial resistance. It is anticipated that if the rate of evolution of multidrug resistant (MDR) bacteria continues to increase, by 2050 the mortality rate caused by resistant bacterial infections will surpass the mortality rate caused by cancer [8]. Verma et al., revealed that a single aspect of antibiotic resistance is estimated to cost humanity a $100 trillion loss in global economy and a loss of around 300 million human life [9]. Smith et al. in their study demonstrated that the decline in effective first-line antimicrobials has led to an increased dependence on second and third-line treatments, which tend to be more costly, more harmful, and necessitate longer periods of treatment [10]. Tamma et al., revealed that AMR infections likewise require more frequent visits to outpatient clinics, additional laboratory tests, and measures for isolation [11].

A study by Sebastian et al., examined four distinct types of samples (fresh fecal sample, litter from inside the shed, litter from outside the shed and agricultural soil) showed E. coli growth were tested for antibiotic sensitivity pattern and all isolates were found resistant to ampicillin, amoxicillin, meropenem and tetracycline [12]. A high prevalence of multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing Escherichia coli in intensive chicken farming in India and the prevalence of MDR avian pathogenic E. coli associated virulence genes in backyard layer chickens has been described in a recent review of antimicrobial resistance in poultry farming in low resource settings [13]. A cross-sectional survey was conducted in Assam and Karnataka interviewed 62 poultry farmers and 11 veterinarians highlighted that poultry farmers were mostly unaware of the relation between antibiotic use and antimicrobial resistance. Hence, necessary actions should be encouraged to profile the AMR genes in poultry farms in these regions. AMR emergence in them could be readily monitored as an ecological indicator of AMR gene spread [14]. Innovative technologies for detecting multi-drug resistant (MDR) strains comprise molecular techniques such as next-generation sequencing (NGS), DNA microarrays, and isothermal amplification methods (LAMP), in addition to phenotypic approaches like MALDI-TOF mass spectrometry and biosensors. These technologies provide precise and frequently more sensitive identification of drug resistance in comparison to conventional methods [15].

The present study shed insights into the domain of generation of antimicrobial resistant Escherichia Coli strains in poultry farms in Kerala where chickens are raised for meat.

METHODS

Sample Collection

Poultry litters were collected from domestic farm (Figure 1b) from Thiruvananthapuram district, Kerala, India (Figure 1a) where domestic fowls were reared for eggs. Fowls were grown at an age of 2-3 months gaining a weight of 600 to 700 grams. The fowls were grown in a standard decontaminated environment on a bed of sawdust sprayed with copper sulphate.

Isolation and identification of bacteria

Isolated samples were serially diluted to obtain the microbial population for further culture. The microbial load of the collected samples was estimated. The identification of the bacterial strains were carried out using the selective media MacConkey agar (Figure 1c and 1d) and Gram's staining (Figure 1e and 1f).

The colonies emerged from the serial dilution method were inoculated into nutrient broth for subculturing the microbes for further experiments.

Determination of multi-drug resistance pattern

Standard disc diffusion method was utilized to screen the multi-drug resistance pattern of the subcultured organisms. The antibiotic discs of penicillin, ampicillin, erythromycin, tetracycline, ciprofloxacin was

aseptically transferred on the lawn culture of isolated bacterial colonies followed by an incubation at 37 ° C overnight (Clinical and Laboratory Standards Institute, 2015).

Isolation of DNA

DNA was isolated from the drug-resistant lawn cultures containing antibiotics using standard phenol chloroform method. 2mL of overnight culture was utilized for harvesting the cells by centrifugation at 10,000 rpm for 10 minutes. The supernatant was discarded and 875 µL of TE buffer was added to the cell pellets. The cells were resuspended in the buffer by gentle mixing. 100 µL of 10% SDS and 5 µL of Proteinase K were added to the cells. This mixture was incubated at 37 ^{0}C for an hour in an incubator. 1mL of Phenol-Chloroform-Isoamyl alcohol Mixture was added to the contents and mixed well by inverting and incubated at room temperature for 5 minutes. The contents are centrifuged at 10,000 rpm for 10 minutes at 4^{0}C. The highly viscous jelly-like supernatant was collected using cut tips and transferred to a fresh container. The process was repeated with Phenol-Chloroform-Isoamyl alcohol mixture and the supernatant was collected in a fresh container. 100 µL of 5 M sodium acetate was added to the contents and mixed gently. 2 mL of ice-cold isopropanol was added and mixed gently by inversion till the precipitation of DNA as white strands. The contents were centrifuged at 5000 rpm for 10 minutes. The supernatant was removed and 1mL of ice cold 70 % ethanol was added. Centrifugation was repeated at 5000 rpm for 10 minutes. Ethanol was discarded. The pellets were air dried for 1 to 2 hours till it completely dried followed by the addition of 50-100 µL of TE Buffer. The contents were stored at -20 ° C for further studies (Smith, Aldridge, & Callis., 1989).

DNA Quantification

One of the commonly used methods to estimate nucleic acid concentration is the measurement of sample absorbance at 260 nm. The 260/280, 260/230, and 260/325 absorbance ratios were used to determine DNA purity and the presence of contaminants in the biological samples during the DNA extraction process. Currently, the most useful way to estimate DNA concentration and purity is through absorbance measures of samples' microvolumes using the NanoDrop spectrophotometer. The purity and concentration of the DNA obtained from the bacterial isolate was determined through 260/280 nm absorbance measures using the MultiskanSkyHigh Microplate Spectrophotometer (THERMO SCIENTIFIC).

The Qualitative confirmation of the isolated DNA was estimated via Agarose gel electrophoresis. 400 mg of agarose was weighed and dissolved in 40mL of 1X TAE buffer by heating and constant stirring in a water bath at 95^{o}c. After cooling, 2µL of (10 mg/mL) ethidium bromide solution was added into it and the gel was cast. After solidifying, the comb was removed and transferred the gel into the electrophoretic apparatus containing 1X TAE buffer. The incubated DNA sample was mixed with DNA loading dye and loaded in the wells along with the molecular weight DNA marker and allowed to run at 50V for 1 hour. After the run, the gel was analyzed under UV transilluminator.

PCR amplification of 16s rRNA

The microbial DNA was amplified using 16s rRNA primers. PCR was carried out in SimpliAmp Thermal Cycler (Thermofischer, The Applied Biosystems). A reaction mixture (25 µL reaction volume) containing 1.5 µLof 10 µM forward primer, 1.5 µL of 10 µM reverse primer, 12 µL of Takara master mix; 5 µL of sterile autoclaved water, and 5 µL of template DNA samples. The determined conditions are mentioned in Table 1.

Agarose gel electrophoresis of PCR Product

The PCR products were electrophoresedon 2% agarose gel stained with Ethidium Bromide (1mg/mL), run at constant voltage of 50V in 1XTAE buffer. A 100bp DNA ladder was used for the comparative study. The gel documentation was carried out using the Documentation Unit. The remaining PCR product was stored at -20°C for sequencing.

Sequencing of 16srRNA

PCR products of 16S rRNA of the isolate were obtained through amplification and were purified and sequenced.

Sequence data analysis

Sequence results obtained was analyzed using applied bio systems and NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi#). A consensus sequence was generated from forward sequence data using applied bio system software. The consensus sequence was used to perform BLAST against the NCBI Genbank database. The first ten sequences were chosen based on their maximum identity score

and aligned using the multiple alignment software program. Based on the phylogenetic tree and the pair-wise distance matrix, the closest homolog of each isolate from the NCBI Genbank database was identified.

RESULTS AND DISCUSSION

Phenotypic characterization

Samples were collected using aseptic colony isolation methodologies and following serial dilution method. Two prominent colonies from the serially diluted samples, the two dilutions (10^{-3} and 10^{-5}) were used and named C1 and C2 respectively. These two colonies were used for further screening.

Visual inspection of the colonies in the plate revealed round and translucent nature. For further identification of the isolated bacterial colony, Gram's staining was used. Gram's staining is one of the preliminary steps in phenotypic characterization of bacteria. The staining differentiates the bacterial population to border classes called gram positive and gram negative. This classification is based on the structural diversity among the bacteria. Gram positive bacteria will have a thick peptidoglycan layer in their cell wall which retains crystal violet stain by complexing with iodine. Thus, the gram-positive bacteria will be observed as purple or violet in colour while gram-negative bacteria with a thinner peptidoglycan layer will be seen as red or pink after counter staining.

The isolated colonies C1 and C2 showed purple colour. Upon closer observation under a microscope the C1 was observed to be rod shaped and C2 was observed to be round shaped. To further confirm the phenotype of the bacterial colonies, Mac Conkey agar plate was used. However no prominent colonies were observed confirming that the isolated colonies were of gram positive in nature.

Determination of multidrug resistance

The criteria of clinical susceptibility breaking points such as susceptible, intermediate, or resistant depends on action of antibiotic towards a bacterial culture in in vitro condition (Munita & Arias, 2016).

Disc diffusion test was employed to determine the antibiotic resistance of the isolated bacteria. For this purpose, antibiotic discs of ampicillin, penicillin, Erythromycin, Tetracycline, Ciprofloxacin.

Both C1 and C2 showed complete resistance to Penicillin, Ampicillin, and tetracycline by showing no zone of inhibition (Figure 2). C1 showed a zone of inhibition of 24 mm in Erythromycin and 17 mm in Ciprofloxacin. C2 showed a 26 mm zone of inhibition in Erythromycin and 17mm in Ciprofloxacin. However, according to antibiotic resistance sensitivity charts C1 and C2 show a partial resistance towards Erythromycin while completely resistant to ampicillin, penicillin, Tetracycline, Ciprofloxacin.

Genotypic characterization

Microbial samples from dairy farms were collected aseptically and the antimicrobial resistant microbial population was isolated. Among the antibiotics tested the colony showed resistance towards all the antimicrobial agents. From preliminary phenotypic analysis of the microbial population two colonies C1 and C2 were obtained, among which the C1 showed a rod-shaped morphology and C2 showed a round morphology. The gram staining of the two colonies revealed that both were gram negative in nature. To further characterize and classify the obtained antimicrobial resistant strains, Ribotyping experiments were employed. Subculture colonies were subjected to DNA isolation by standard phenol- chloroform method, precipitated by ethyl alcohol and dissolved in deionised water. For further genetic analysis the quality of the obtained DNA was analysed by means of Agarose gel electrophoresis and spectrophotometer. The Gel doc image under UV light of ETBR stained DNA gel showed two intact DNA bands of 10 kb in range when compared to the DNA ladder. To further evaluate the DNA quality the nanodrop spectrophotometric analysis was employed. The concentration of DNA obtained were 269 µg/mL and 416 µg/mL in concentration for samples C1 and C2 respectively (Table 2). The purity of the samples was observed to be 1.81 and 1.78 samples C1 and C2 respectively.

PCR amplification of 16srRNA

For ribotyping the DNA samples were amplified in a PCR reaction with 16srRNA specific primers in a reverse transcriptase PCR. Agarose gel electrophoresis was done for the qualitative analysis of PCR products. The horizontal gel electrophoresis unit was used to run the sample on the gel to determine the size of amplicons. In both samples, C1 and C2, a bright band of DNA was observed at 600 kbps.

Sequence analysis.

Sequence data analysis using BLAST was done to verify the nearest species of isolated colonies. The strains' resemblance to E. coli species was more pronounced. Using BLAST analysis, a novel antibiotic-

resistant strain of E. coli was discovered. The colony C1 was found to have 98% homology with Escherichia coli strain whereas C2 with 97.17% similarity with Escherichia coli strain. An assembly draft has been placed in GenBank (accession number OP727565; OP727566).

CONCLUSION

Antimicrobial resistance has now emerged as a global concern. The resistance shown by bacteria to numerous active antibiotics poses a significant threat to the curability of diseases. Various sources have been identified as contributors to the rise of AMR, with poultry being one notable example. It has been observed that antibiotics are frequently utilized in poultry farming to enhance growth, prevent disease, and treat infections. While these practices have resulted in increased productivity, they also play a role in the emergence of antibiotic-resistant bacteria. Microorganisms adapting to their surrounding environment to survive is a strategy that has proven successful for their biological existence as living organisms. However the acquired resistance to antimicrobials by means of mutation or acquiring genetic determinants of intrinsically resistant organisms, along with overuse of antimicrobials contributes majorly to a global health threat.

Here in this study, we isolated AMR strains of microbes from a poultry farm at Trivandrum and identified the organism by phenotypic and genotypic methods to be Escherichia coli. We describe here for the first time the discovery and description of this unique sequence type, C1 and C2. The isolated strains exhibited moderate resistance to erythromycin and high resistance to ampicillin, penicillin, tetracycline, and ciprofloxacin. Utilizing 16s rRNA for ribotyping DNA samples revealed the bacteria' resemblance to E. coli species. Future research on poultry farming and the prevention and management of antibiotic resistance on E.coli is encouraged.

REFERENCES

1.J. Peters, O. Lebrasseur, E.K. Irving-Pease, P.D. Paxinos, J. Best, R. Smallman, C. Callou, A. Gardeisen, S. Trixl, L. Frantz, N. Sykes, D.Q. Fuller, G. Larson, The biocultural origins and dispersal of domestic chickens, Proc. Natl. Acad. Sci. U.S.A. 119 (24) e2121978119. (2022).

2.https://www.hipra.com/en/animal-health/knowledge/1-origins-poultry-farming. Accessed on August 8 2025.

3.Toor Singh Jasdeep, Goel Rudhita (2024). Poultry Farming in India with Special Reference to Punjab: An Overview . Agricultural Science Digest. 44(6): 1000-1004.

4.Hafez HM, Attia YA. Challenges to the Poultry Industry: Current Perspectives and Strategic Future After the COVID-19 Outbreak. Front Vet Sci. 2020 Aug 26;7:516.

5.Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A. 2015 May 5;112(18):5649-54.

6.Ajmal Aseem, Prarthi Sagar, Nerellapally Samyukthakumar Reddy, Shobi Veleri. The antimicrobial resistance profile in poultry of Central and Southern India is evolving with distinct features. Comparative Immunology, Microbiology and Infectious Diseases.2024;114: 102255.

7.Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. Virulence Mechanisms of Bacterial Pathogens, (6), 481–511.

8.EIP AGRI Focus Group Reducing Antimicrobial Use in Poultry Farming. 2021. Accessed on August 8 2025.

9.Verma V, Valsan C, Mishra P, Mund K, Dutta S, Anke G, Sasi H, Shah D. Antimicrobial Resistance Profile in ICU Patients Across India: A Multicenter, Retrospective, Observational Study. Cureus. 2024 Apr 2;16(4):e57489.

10. Smith R, Coast J. The true cost of antimicrobial resistance. Bmj. 2013 Mar 11;346.

11. Tamma PD, Cosgrove SE. Antimicrobial stewardship. Infectious Disease Clinics. 2011 Mar 1;25(1):245-60.

12. Stelvin Sebastian, Antriya Annie Tom, Joyal Anna Babu, Merin Joshy. Antibiotic resistance in Escherichia coli isolates from poultry environment and UTI patients in Kerala, India: A comparison study. Comparative Immunology, Microbiology and Infectious Diseases.2021;75:101614.

13. Brower CH, Mandal S, Hayer S, Sran M, Zehra A, Patel SJ, Kaur R, Chatterjee L, Mishra S, Das BR, Singh P, Singh R, Gill JPS, Laxminarayan R. The Prevalence of Extended-Spectrum Beta-Lactamase-Producing Multidrug-Resistant Escherichia Coli in Poultry Chickens and Variation According to Farming Practices in Punjab, India. Environ Health Perspect. 2017 Jul 20;125(7):077015.

14. Sharma G, Dey TK, Hazarika RA, Shome BR, Shome R, Singh VP, Deka RP, Grace D, Lindahl JF. Knowledge and practices related to antibiotics among poultry producers and veterinarians in two Indian states. One Health. 2024 Mar 5;18:100700.

15. Shubhi Singh, Sahithya Selvakumar, Priya Swaminathan. Harnessing advanced molecular diagnostics and bioinformatics to ascertain antimicrobial resistance in ESKAPE organisms. The Microbe. 2025;7:100316.

Table 1. PCR primer sequence for 16s rRNA

Primer	Sequence
27F	CGGCCCAGACTCCTACGGGAGGCAGCA
1492R	GCGTGGACTACCAGGGTATCTAATTC

Table 2. Genotypic characterization of the isolated strains C1 and C2

Sample code	Concentration	Purity
C1	269 µg/mL	1.81
C2	416 µg/mL	1.78

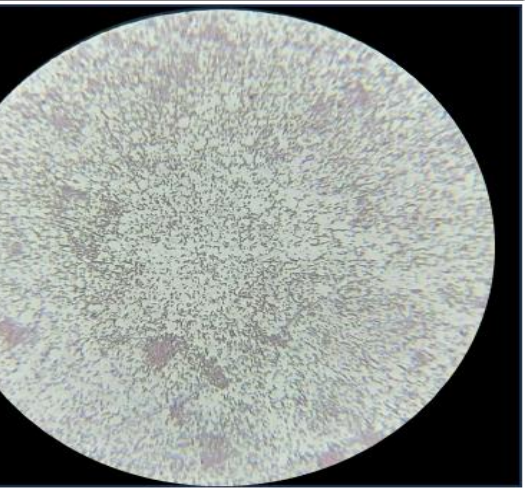

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Fig.1. Collection of samples and phenotypic identification of microbial population from poultry farm. (a) Poultry farm: Ambady Egger Nursery in Poovathoor, Thiruvananthapuram district, Kerala, India; (b) Sample collection of poultry litters; (c) Streak plate culture of bacterial population isolated from sample (serial dilution 10^{-3}); (d) Streak plate culture of bacterial population isolated from sample (serial dilution 10^{-5});(e) Gram staining- rod shaped bacterial cells in 10X magnification (serial dilution 10^{-3}); (f) Gram staining- rod shaped bacterial cells in 10X magnification (serial dilution 10^{-5}).

Fig.2. Plates showing resistance pattern of C1 against Ampicillin, Penicillin and Erythromycin

Fig.3. Plates showing resistance pattern of C1 against Tetracycline and Ciprofloxacin

Fig.4. Plates showing resistance pattern of C2 against Ampicillin, Penicillin and Erythromycin

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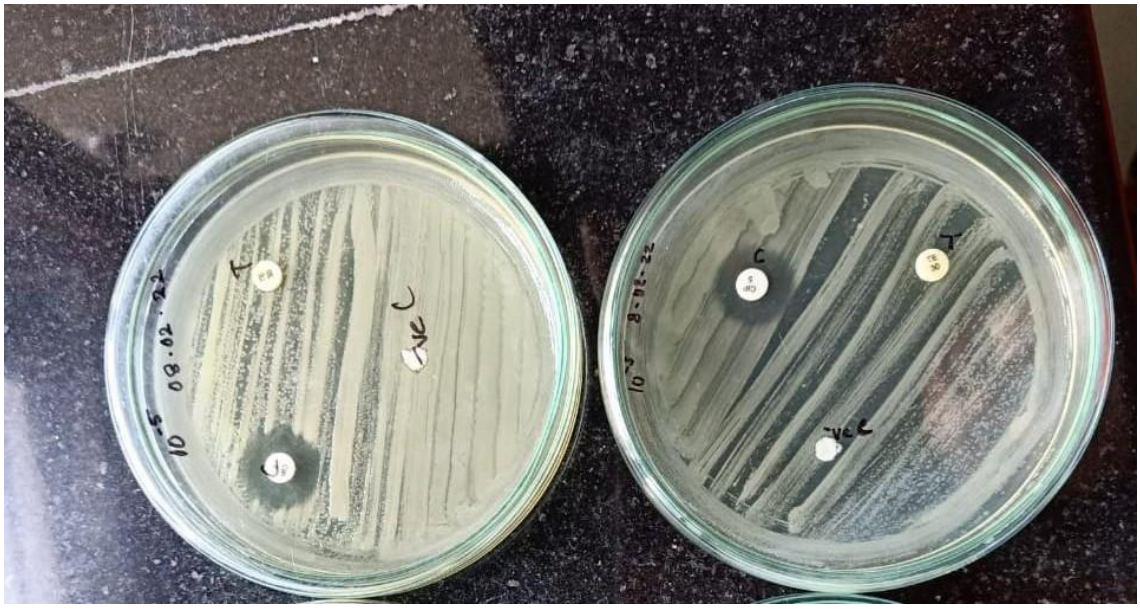

Fig.5. Plates showing resistance pattern of C2 against Tetracycline and Ciprofloxacin

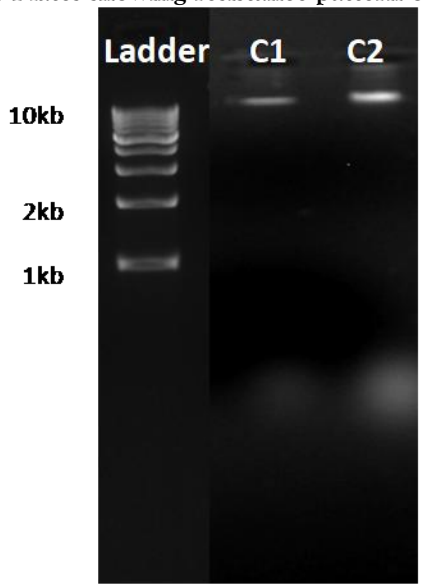

Fig.6. Gel Image of isolated DNA from C1 and C2

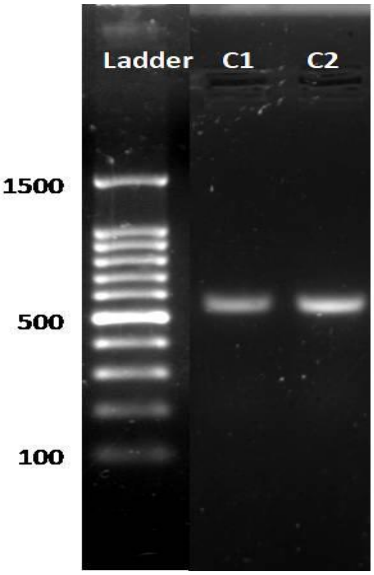

Fig.7. Gel Image of PCR product of C1 and C2

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GCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACG
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TTCCCCACTGCTGCCTCCCGTAAGAATCTGGGCCGG

Fig. 8. Sequence Data C1

>0622_792_015_PCR_C2_Bacteria_16S_F_C06.ab1
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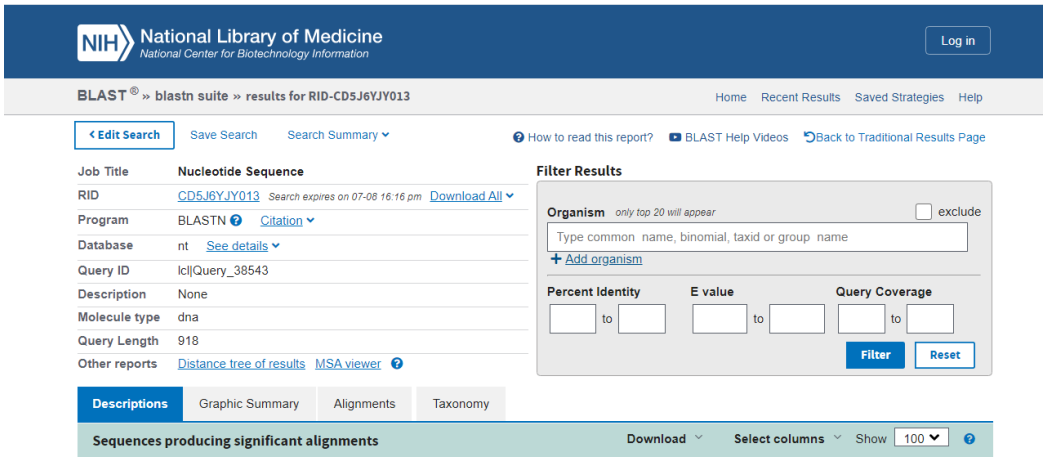
Fig. 9. Sequence Data C2



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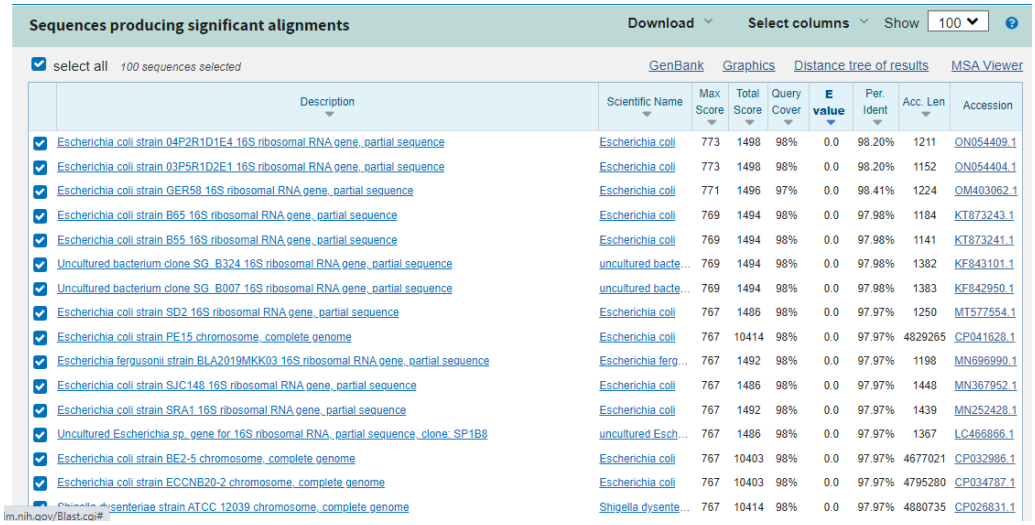
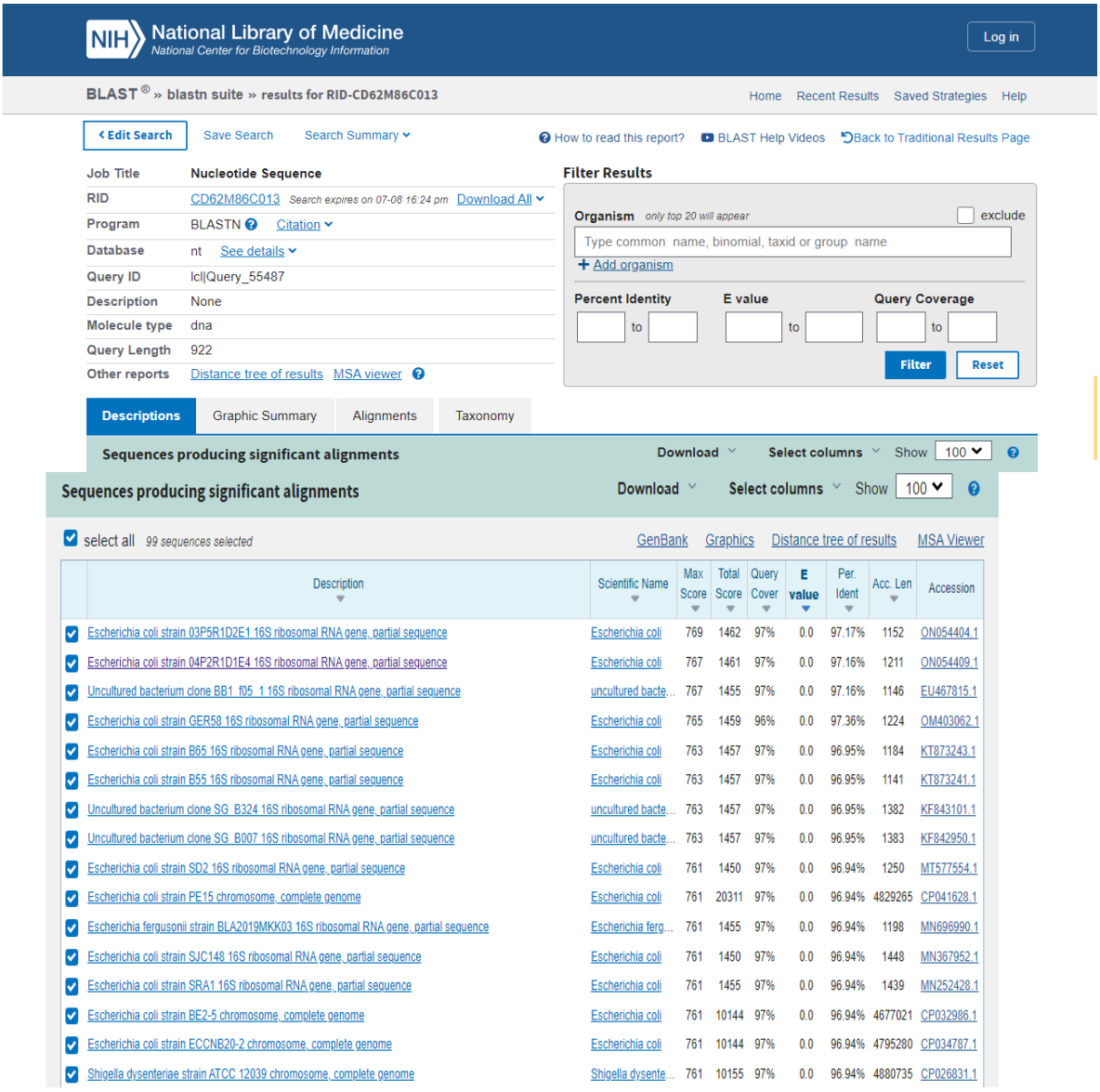


Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Escherichia coli strain 04P2R1D1E4 16S ribosomal RNA gene, partial sequence	Escherichia coli	773	1498	98%	0.0	98.20%	1211	ON054409.1
Escherichia coli strain 03P5R1D2E1 16S ribosomal RNA gene, partial sequence	Escherichia coli	773	1498	98%	0.0	98.20%	1152	ON054404.1
Escherichia coli strain GER58 16S ribosomal RNA gene, partial sequence	Escherichia coli	771	1496	97%	0.0	98.41%	1224	OM403062.1
Escherichia coli strain B65 16S ribosomal RNA gene, partial sequence	Escherichia coli	769	1494	98%	0.0	97.98%	1184	KT873243.1
Escherichia coli strain B55 16S ribosomal RNA gene, partial sequence	Escherichia coli	769	1494	98%	0.0	97.98%	1141	KT873241.1
Uncultured bacterium clone SG_B324 16S ribosomal RNA gene, partial sequence	uncultured bacte...	769	1494	98%	0.0	97.98%	1382	KF843101.1
Uncultured bacterium clone SG_B007 16S ribosomal RNA gene, partial sequence	uncultured bacte...	769	1494	98%	0.0	97.98%	1383	KF842950.1
Escherichia coli strain SD2 16S ribosomal RNA gene, partial sequence	Escherichia coli	767	1486	98%	0.0	97.97%	1250	MT577554.1
Escherichia coli strain PE15 chromosome, complete genome	Escherichia coli	767	10414	98%	0.0	97.97%	4829265	CP041628.1
Escherichia fergusonii strain BLA2019MKK03 16S ribosomal RNA gene, partial sequence	Escherichia ferg...	767	1492	98%	0.0	97.97%	1198	MN696990.1
Escherichia coli strain SJC148 16S ribosomal RNA gene, partial sequence	Escherichia coli	767	1486	98%	0.0	97.97%	1448	MN367952.1
Escherichia coli strain SRA1 16S ribosomal RNA gene, partial sequence	Escherichia coli	767	1492	98%	0.0	97.97%	1439	MN252428.1
Uncultured Escherichia sp. gene for 16S ribosomal RNA, partial sequence, clone: SP1B8	uncultured Esch...	767	1486	98%	0.0	97.97%	1367	LC466866.1
Escherichia coli strain BE2-5 chromosome, complete genome	Escherichia coli	767	10403	98%	0.0	97.97%	4677021	CP032986.1
Escherichia coli strain ECCNB20-2 chromosome, complete genome	Escherichia coli	767	10403	98%	0.0	97.97%	4795280	CP034787.1
Shigella dysenteriae strain ATCC 12039 chromosome, complete genome	Shigella dysente...	767	10414	98%	0.0	97.97%	4880735	CP026831.1

Fig. 10. Sequence Data Analysis of C1



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BLAST® » blastn suite » results for RID-CD62M86C013 — Home — Recent Results — Saved Strategies — Help

Edit Search — Save Search — Search Summary — How to read this report? — BLAST Help Videos — Back to Traditional Results Page

Job Title	Nucleotide Sequence
RID	CD62M86C013 Search expires on 07-08 16:24 pm Download All
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_55487
Description	None
Molecule type	dna
Query Length	922
Other reports	Distance tree of results MSA viewer

Filter Results

Organism only top 20 will appear — exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity — E value — Query Coverage — to

Filter — Reset

Descriptions — Graphic Summary — Alignments — Taxonomy

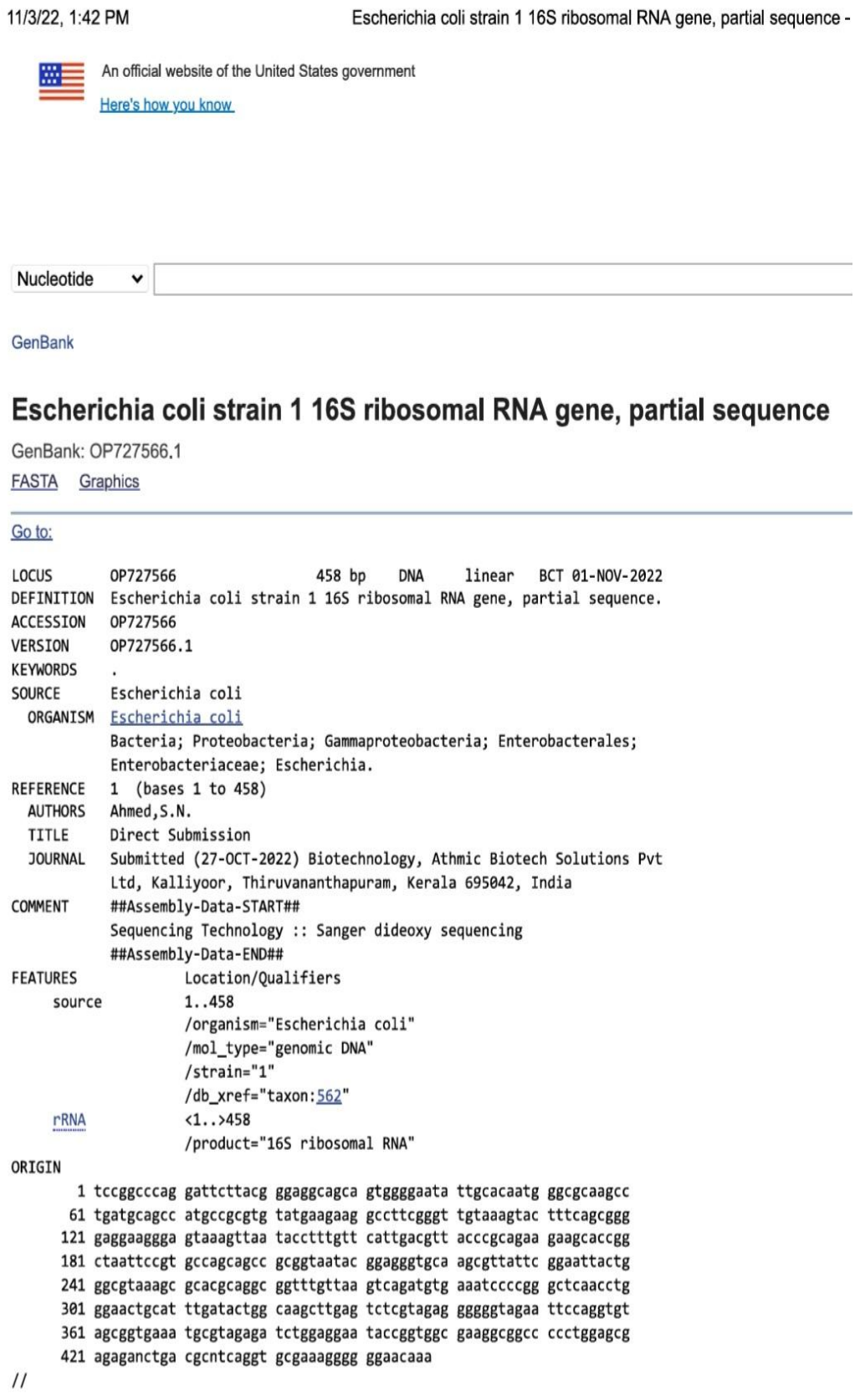
Sequences producing significant alignments — Download — Select columns — Show 100

Sequences producing significant alignments — Download — Select columns — Show 100

select all 99 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Escherichia coli strain 03P5R1D2E1 16S ribosomal RNA gene, partial sequence	Escherichia coli	769	1462	97%	0.0	97.17%	1152	ON054404.1
Escherichia coli strain 04P2R1D1E4 16S ribosomal RNA gene, partial sequence	Escherichia coli	767	1461	97%	0.0	97.16%	1211	ON054409.1
Uncultured bacterium clone BB1_f05_1 16S ribosomal RNA gene, partial sequence	uncultured bacte...	767	1455	97%	0.0	97.16%	1146	EU467815.1
Escherichia coli strain GER58 16S ribosomal RNA gene, partial sequence	Escherichia coli	765	1459	96%	0.0	97.36%	1224	OM403062.1
Escherichia coli strain B65 16S ribosomal RNA gene, partial sequence	Escherichia coli	763	1457	97%	0.0	96.95%	1184	KT873243.1
Escherichia coli strain B55 16S ribosomal RNA gene, partial sequence	Escherichia coli	763	1457	97%	0.0	96.95%	1141	KT873241.1
Uncultured bacterium clone SG_B324 16S ribosomal RNA gene, partial sequence	uncultured bacte...	763	1457	97%	0.0	96.95%	1382	KF843101.1
Uncultured bacterium clone SG_B007 16S ribosomal RNA gene, partial sequence	uncultured bacte...	763	1457	97%	0.0	96.95%	1383	KF842950.1
Escherichia coli strain SD2 16S ribosomal RNA gene, partial sequence	Escherichia coli	761	1450	97%	0.0	96.94%	1250	MT577554.1
Escherichia coli strain PE15 chromosome, complete genome	Escherichia coli	761	20311	97%	0.0	96.94%	4829265	CP041628.1
Escherichia fergusonii strain BLA2019MKK03 16S ribosomal RNA gene, partial sequence	Escherichia ferg...	761	1455	97%	0.0	96.94%	1198	MN696990.1
Escherichia coli strain SJC148 16S ribosomal RNA gene, partial sequence	Escherichia coli	761	1450	97%	0.0	96.94%	1448	MN367952.1
Escherichia coli strain SRA1 16S ribosomal RNA gene, partial sequence	Escherichia coli	761	1455	97%	0.0	96.94%	1439	MN252428.1
Escherichia coli strain BE2-5 chromosome, complete genome	Escherichia coli	761	10144	97%	0.0	96.94%	4677021	CP032986.1
Escherichia coli strain ECCNB20-2 chromosome, complete genome	Escherichia coli	761	10144	97%	0.0	96.94%	4795280	CP034787.1
Shigella dysenteriae strain ATCC 12039 chromosome, complete genome	Shigella dysente...	761	10155	97%	0.0	96.94%	4880735	CP026831.1

Fig. 11. Sequence Data Analysis of C2


11/3/22, 1:42 PM Escherichia coli strain 1 16S ribosomal RNA gene, partial sequence -

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Nucleotide

GenBank

Escherichia coli strain 1 16S ribosomal RNA gene, partial sequence

GenBank: OP727566.1

FASTA Graphics

Go to:

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  AUTHORS   Ahmed,S.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-OCT-2022) Biotechnology, Athmic Biotech Solutions Pvt
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Fig. 12. Gene Bank submission record of E.Coli C1 strain

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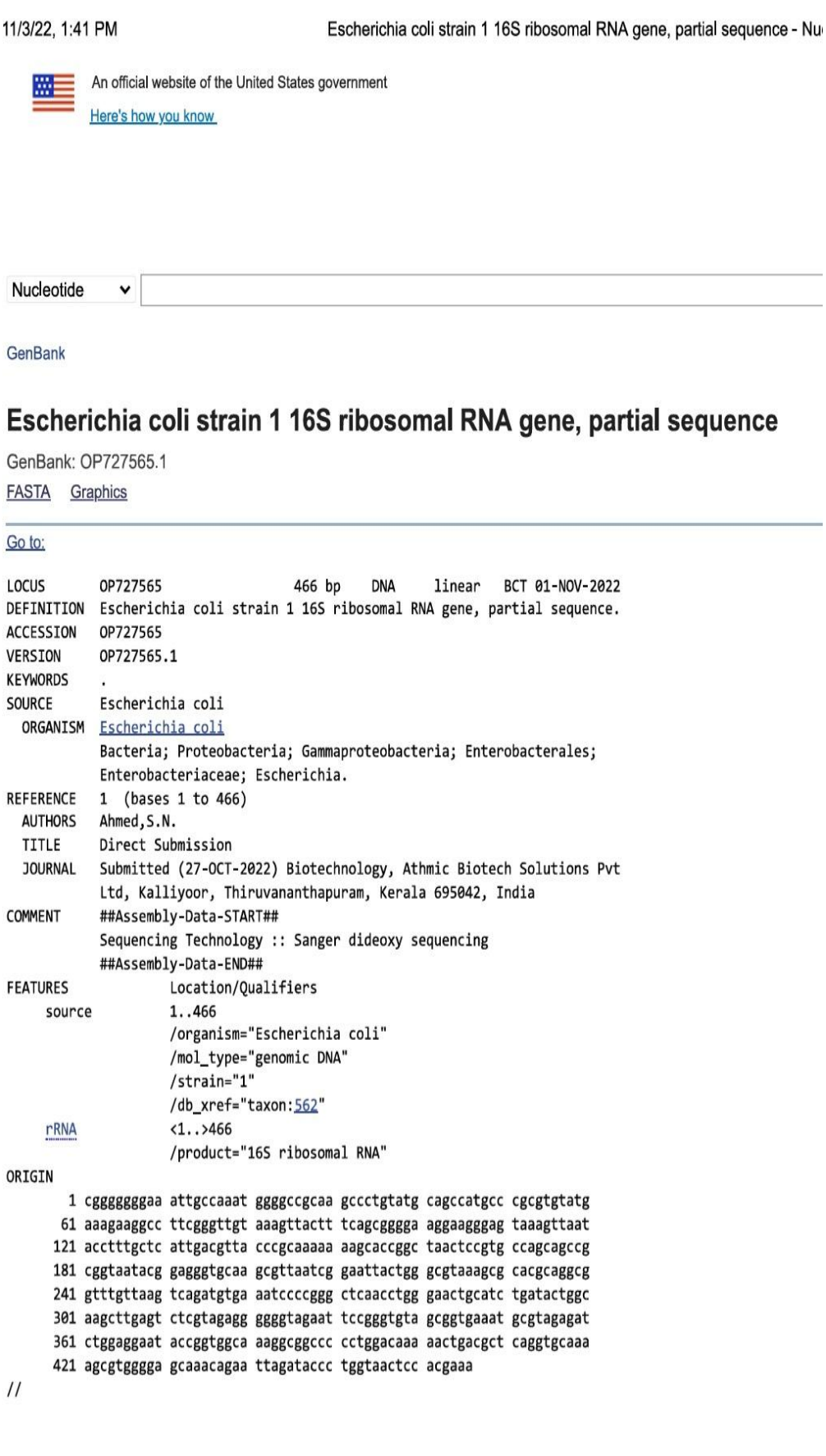
https://theaspd.com/index.php



11/3/22, 1:41 PM Escherichia coli strain 1 16S ribosomal RNA gene, partial sequence - Nu

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Nucleotide

GenBank

Escherichia coli strain 1 16S ribosomal RNA gene, partial sequence

GenBank: OP727565.1

FASTA Graphics

Go to:

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REFERENCE   1  (bases 1 to 466)
  AUTHORS   Ahmed,S.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-OCT-2022) Biotechnology, Athmic Biotech Solutions Pvt
            Ltd, Kalliyoor, Thiruvananthapuram, Kerala 695042, India
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Fig. 13. Gene Bank submission record of E.Coli C2 strain