J. Bio-Sci. 27: 101-108, 2019 ISSN 1023-8654
http://www.banglajol.info/index.php/JBS/index
DOI: https://doi.org/10.3329/jbs.v27i0.44675

THE GROWTH INFLUENCE OF *ESCHERICHIA COLI* CO-CULTURED WITH OTHER SELECTED GRAM NEGATIVE BACTERIA

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Abstract

This study examined the influence of *Escherichia coli* on the growth of other selected Gram negative bacteria (*Klebsiella pneumoniae, Shigella dysenteriae, Salmonella typhi, Pseudomonas aeruginosa* and *Proteus vulgaris*). Cultures of each bacterium at 0, 24, 48 and 72 hours of incubation were plated on MacConkey agar. Colonies that developed were counted while the optical densities were determined at 0, 24, 48 and 72 hours using spectrophotometry. Each bacterium was co-cultured with *E. coli* and their growth was determined using culturing method and spectrophotometry. The result showed an increase in growth in the cultures of each isolate co-cultured with *E. coli* when compared with single bacterium culture with the exception of *P. aeruginosa*. The result of this study revealed a positive growth influence between *E. coli* and *K. pneumoniae, S. dysenteriae, S. typhi,* and *P. vulgaris*, except for *P. aeruginosa* that showed a decrease in growth.

Key words: Culturing, *Escherichia coli,* Gram negative bacteria, Interaction, Spectrophotometry

Introduction

The interactions amongst microorganisms and with their host are key strategies in their establishment and colonization in a wide variety of environments. These interactions include: metabolite exchange, chemotaxis, signalling, physiochemical changes, genetic exchange and metabolite conversion. Microbial interactions are important in the maintenance and establishment of a microbial population (Raíssa et al. 2016). Cooperation and conflict amongst microorganisms have been recognized as a factor that is important in the organization and function of microbial communities (Karoline and Jeroen 2012).

Bacteria rarely occur as a single entity, they often exist as biofilm, thereby resulting into a large variety of types of microbial interactions (Raíssa et al. 2016). Each bacterium secretes chemical signal molecules known as autoinducers that allows cells to interact and responds to their environment in a coordinated way (Phelan et al. 2012, Oyewole et al. 2017). A cell-cell signalling process is described among bacteria which accounts for their many cooperative behaviours (Kyle and Martins 2017). A regulatory phenomenon whereby cells excrete or secrete a chemical signal into the surrounding environment is known as Quorum sensing. At sufficient concentration the signal alters the expression of specific genes. Many Gram negative and Gram positive bacteria use quorum sensing signal circuits to coordinate various array of physiological behaviours, including conjugation, symbiosis, virulence, antibiotic production, competence, motility and biofilm development. A lot of Gram negative bacteria uses LuxR/LuxL type of quorum sensing, which is based on synthesis of diffusible signal molecules that is identified as N-acyl-homoserine lactone (AHLs), to synchronize physiological behaviours (Fiorela et al. 2016).

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Many years of coevolution that occurs among different species of microorganisms has led to cohabitation such as mutualistic and endosymbiotic relationships, antagonistic, pathogenic and parasitic relationship (Faust and Raes 2012, Nicole and Michiko 2016). Secondary metabolites have been reported to be greatly involved in microbial interactions as these compounds usually are bioactive and can perform important functions in ecological interactions. Antimicrobial host defenses and environmental factors also plays an important role by the communication of microorganisms, which enables the population to collectively regulate the gene expression in response to host and environmental signals that is produced by the same or different species. This results in a coordinate response in the microbial population by achieving successful pathogenic outcomes that would not be accomplished individually by their cells (Peters et al. 2012).

It has been reported that co-cultivation with other microorganisms from the same ecosystem can induce the activation of silent biosynthetic pathways leading to the production and identification of new natural products (Netzker et al. 2015). A lot of bacteria produce numerous public goods that are released into the extracellular environment of which this diffusion allows them to be used by the neighbouring organisms despite being produced for the individual, a good example of this are siderophores. Siderophores are related to competitive and cooperative microbial interactions and can play other roles such as signalling and antibiotic activity (Neilands 1995, Kannati and Senbagam 2014, Johnstone and Nolan 2015). Hopanoids, which is a secondary metabolite plays an important role also in bacterial interaction, confer tolerance and improves the adaptation of bacteria in different environments (Schmerk et al. 2015).

The interactions between Gram negative and other groups of bacteria could either be positive, negative or neutral. Many of Quorum sensing -regulated phenotypes exhibits a good sign of cooperation that results in the secretion of products that are produced by individuals with benefits that are available to all cells in a population. Examples are exoenzymes for the degradation of biopolymers, exopolysaccharide (EPS) for the formation of biofilms and antibiotics for microbial warfare (Kyle and Martin 2017). In an environment with individual microbes, mutualism is most often performed in order to increase individual fitness benefits while in a community; microorganisms interact on a large scale to allow for the persistence of the population, which will thereby increase their own fitness (Guimarães et al. 2016).

As bacteria are mostly found in colonies, neighbouring bacteria are likely to express genetic commonality thereby, increasing the chances for a nearby bacterium to grow and divide. In the case of siderophores production, positive correlation exists among bacterial lineages and siderophore production (Miethke and Marachiel 2007). Group selection is an important organizing principle of cooperation (James and Jeff 2012). In a microbial community composed of species that compete using different strategies, each of the individual cells possess a fitness that depends on the strategy of the individuals with whom it interacts. Individuals that use more successful strategies have higher chances to propagate and their frequency in the community will increase. Enzymes produced by bacteria that are responsible for the digestion of macromolecules such as extracellular lipases and proteases are good examples of public goods and their production in a complex microbial community is influenced by the interactions between its members (Matteo et al. 2017).

The interaction between one bacteria and other can lead to the depletion of the other bacteria population (Micheal et al. 2010). During the cooperative process, certain bacteria can switch to selfish behaviour in which they do not contribute to the production of public goods but benefits from the ones produced by others. If the ratio of the cheaters increases to a critical level, this may lead to the collapse of the community (Bige et

al. 2016). Examples of antagonistic behaviour displayed by Gram negative bacteria include the secretion of toxins such as colicins or antibiotics that inhibit the growth of other bacteria (Glen et al. 2018). Cooperating bacterial populations are more likely to survive in changing habitats. Bacteria neutral interactions in its environment have impact on how best to cultivate bacteria strains and how to treat bacterial infections (Mauro and Erwin 2017). In nature, microorganisms interact with other microorganisms in the environment and the products from the microorganisms (Wikieł et al. 2014). Knowing how microorganisms interact would help in enhancing their products for biotechnological use and disease prevention. Therefore, the aim of this study was to assess the interaction between *E. coli* and other selected Gram negative bacteria.

Materials and Methods

Microbial isolates

The test organisms used for the study were *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa,* and *Proteus vulgaris.* The organisms were collected from culture collection centre of the Centre for Genetic Engineering Federal University of Technology, Minna, Nigeria. The organisms were stored in slant bottles in the refrigerator at temperature of 4°C.

Confirmatory tests on the organisms

The organisms were confirmed using cultural and biochemical characterization. The media used for this work were nutrient broth and MacConkey agar. The tests carried out were Gram staining, indole test, citrate utilization test, oxidase test using test tube method, catalase test, urease test, methyl red test, Voges Proskaeur test and sugar fermentation test (Cheesbrough 2006).

Assessment of the interaction between *E. coli* and other selected Gram negative bacteria

The 9 ml of nutrient broth was dispensed into 6 test tubes and sterilized at 121°C for 15 minutes. The 0.5 ml pure culture of each test organism was inoculated into each of the test tube and incubated for 24 hours at 37°C. The 0.1 ml culture was plated on MacConkey agar to confirm the purity of the culture. The 9 ml of nutrient broth was also dispensed into 12 test tubes and 0.5 ml overnight culture of each test organism was inoculated into each of the test tubes and incubated for 24 hours at 37°C. For cultures co-cultured with *E. coli,* 0.5 ml of both organisms was inoculated into the test tube. Serial dilution was carried out on each culture (singly and combined) at 0, 24, 48 and 72 hours of incubation and fifth diluent was plated using 0.1 ml of the organism on MacConkey media plate using pour plate method. Colonies were counted while the optical densities were also determined using UV-VIS spectrophotometry (model 752, China).

Results

Optical densities of *E. coli* and other selected Gram negative bacteria

Each growth of the test organism in the medium appears turbid and the growth was determined using spectrophotometry. The result is indicated in Fig. 1. There was generally an increase in the optical densities as the incubation period increases from 0 to 72 hours. Thus, highest optical densities (turbidity) were observed on the last day of incubation (72 hours). There was an increase in the optical densities of *S. dysenteriae, S. typhi, K. pneumonia* and *Proteus vulgaris* co-cultured with *E. coli* compared to the single cultures. However, there was a decrease in the optical density of *P. aeruginosa* co-cultured with *E. coli* compared with single cultures of *P. aeruginosa.*

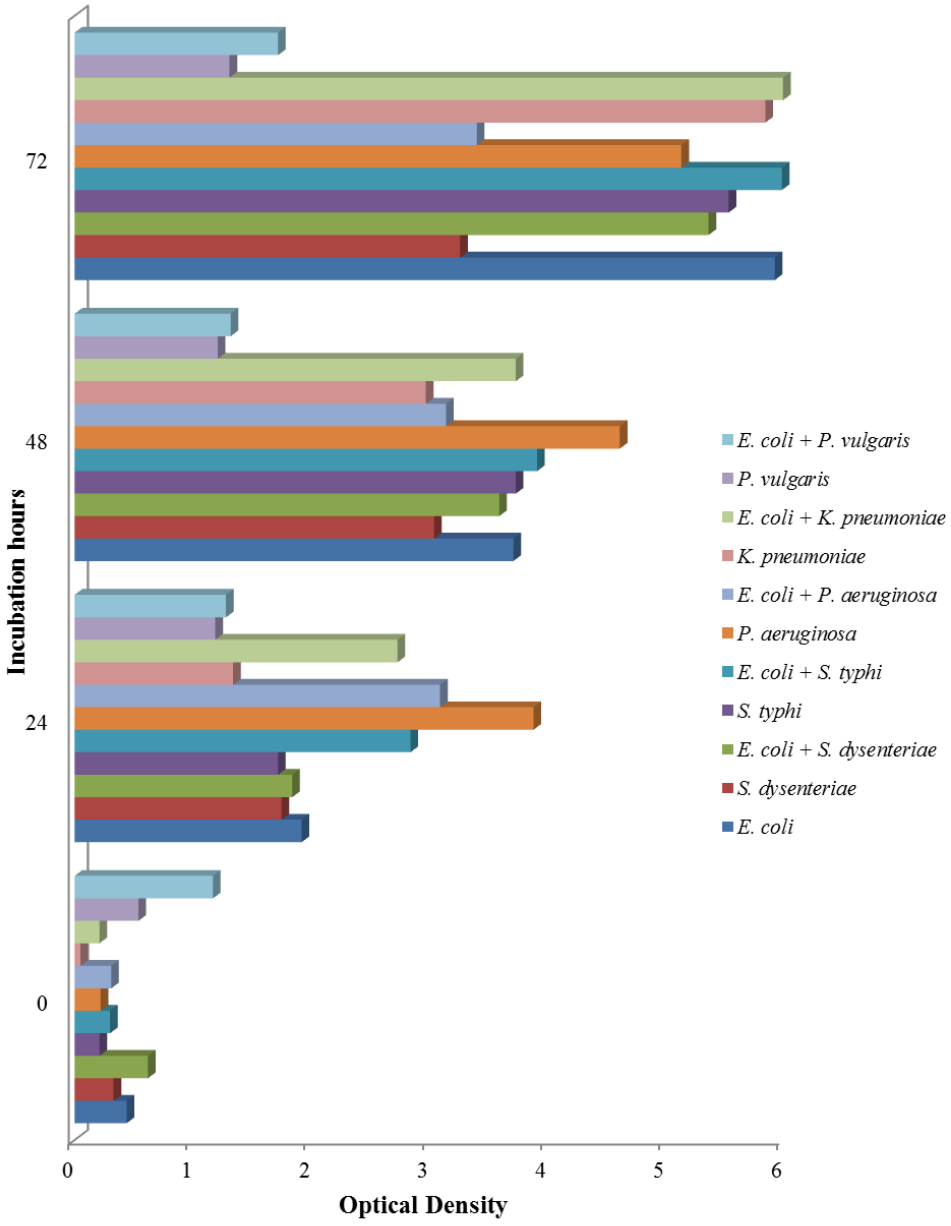

Fig. 1. Optical densities of *E. coli* and other selected Gram negative bacteria.

The interaction between each organism and *E. coli* was positive and the growth of colony increases with increase in incubation period as shown in Fig. 2, still with the exception of *P. aeruginosa.*

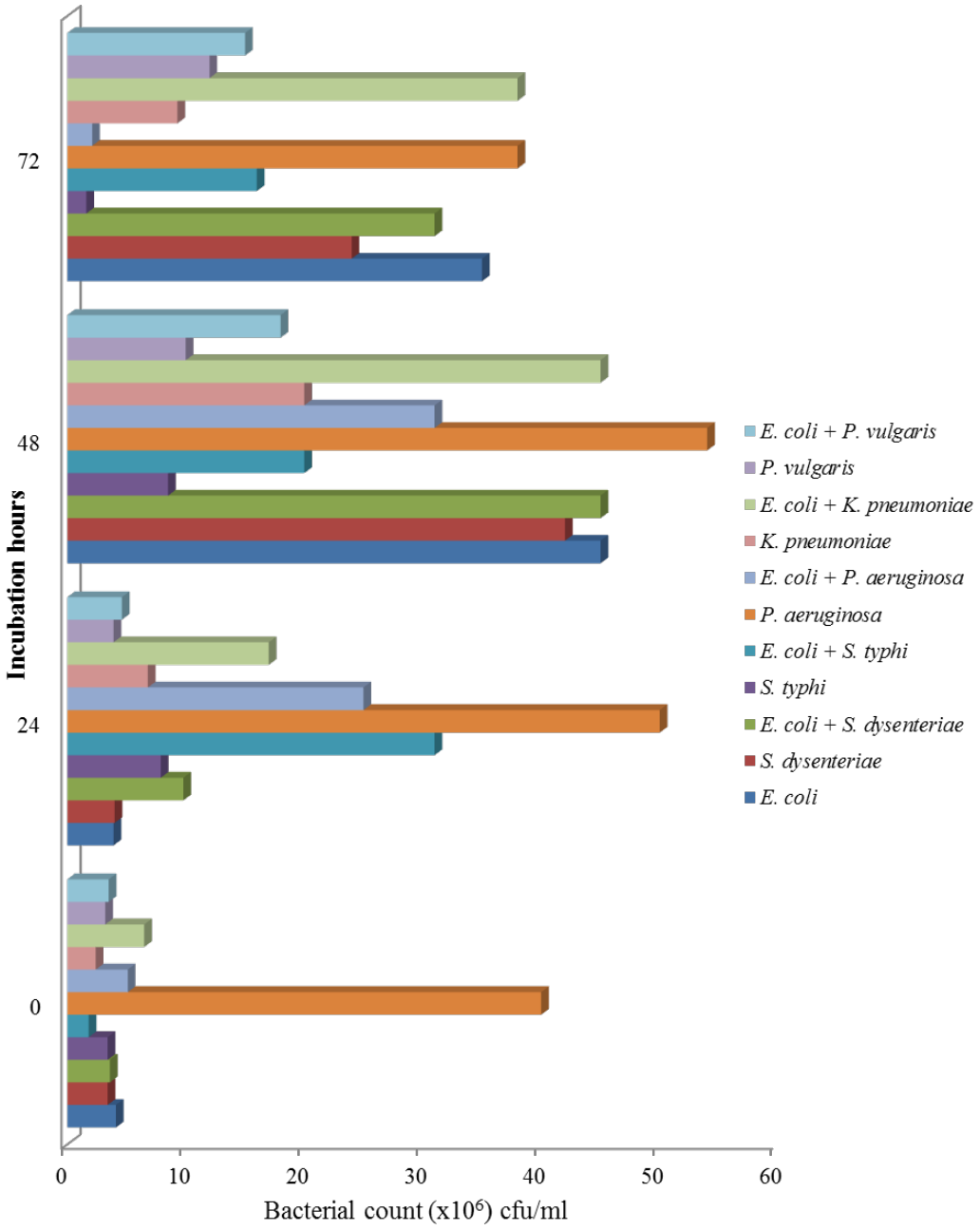

Fig. 2. Colony count of *E. coli* and other selected Gram negative bacteria.

Discussion

The influence of *E. coli* on the growth of *P. aeruginosa, S. typhi, S. dysenteriae, K. pneumoniae* and *P. vulgaris* was carried out in this study. The growth that exists between *E. coli* and the other four Gram negative bacteria was determined by optical densities using spectrophotometry (Fig. 1) and by colonial count (Fig. 2). The increased optical densities and bacterial counts observed in *E. coli* in test tubes co-cultured with other bacteria compared to single colonies may be due to the secretion of products through cell-to-cell signal systems (Høiby et al. 2010) or exhibition of quorum sensing-regulated phenotypes by *E. coli* that has benefits to the growth of the other Gram negative bacteria in the medium. Kyle and Martin, (2017) argued that *E coli* produces a lot of public goods that are used by other Gram negative bacteria such as extracellular lipases and proteases, siderophores, exoenzymes use for the degradation of biopolymers, exopolysaccharide (EPS) for the formation of biofilms and antibiotics for microbial warfare. In quorum sensing, each bacterium secretes chemical signal molecules known as auto-inducers (AI). As bacterial density increases, the level of the auto-inducers in the immediate environment also increases and this increases their interaction with cell signal receptors on surrounding bacteria (Fuqua and Greenberg 1998).

The optical densities of *E. coli, P. aeruginosa, S. typhi, S. dysenteriae, K. pneumoniae* increase with increase in hours of incubation which implies multiplication of cells in the medium. This implies that the interaction between *E. coli* and the other Gram negative is favourable to them and it is not detrimental to *E. coli* itself. It has been established that bacteria interact with other organisms *in vitro* and *in vivo*. Neilands (1995) which explains that, a lot of bacteria produce numerous public goods that are released into the extracellular environment of which this diffusion allows them to be used by the neighbouring organisms despite being produced for the individual. This is as a result of *E. coli* expressing genetic commonality thereby, increasing the chances for the nearby bacterium to grow and also *E. coli* produces siderophore that enhances the growth of the other Gram negative bacteria in agreement to Miethke and Marachiel (2007) which explains that bacteria are mostly found in colonies, neighbouring bacteria are likely to express genetic commonality, thereby increases the chances for a nearby bacterium to grow and divide.

The interaction between *E. coli* and *K. pneumoniae* in humans is an example. This interaction leads to respiratory disease in mule foals (Carneiro et al. 2017). *E. coli* and *K. pneumoniae* interaction leads to the resistant of carbapenem-resistance of which the carbapenemase are OXA-48 and NDM producers (Hosam et al. 2014). Similarly, *Salmonella* evolved to aid *E. coli* by excreting amino acids. In lactose media, *Salmonella* consumes metabolic waste from *E. coli* creating a mechanism of reciprocation for cooperation (Harcombe 2010). The interactions between *E. coli* and *S. typhi* may lead to infections, which may also lead to transfer of resistance to antimicrobial drugs (Moini et al. 2015). The opportunistic pathogens use hierarchical quorum-sensing network to regulate virulence factor production that cooperatively benefit the population at a cost to the individual (Ron et al. 2018). During the interaction between these two organisms, Freya et al. (2006) asserts that *P. aeruginosa* produces siderophores that results in more rapid host death thereby, causing mixed infections.

Mohandass (2004) also reported that siderophores are mostly novel compounds and contain many modified amino acids that are naturally not found elsewhere. Also, Martinez et al. (2003), Ali and Vidhale (2013) and Ahmed and Holmström (2014) added that siderophores have several biotechnological, agricultural, environmental and medicinal applications. According to Martinez et al. (2003), most bacteria synthesize one or more types of siderophores, which are often secreted into the growth medium.

There was an increased growth of all the other Gram negative bacteria co-cultured with *E. coli* with the exception of *P. aeruginosa.* This may be because *P. aeruginosa* does not utilize the metabolic products produced by *E. coli* for its growth instead makes use of the nutrient in the medium. It may also be as a result

of secretion of toxins by *E. coli* that is harmful to *P. aeruginosa* in the medium. Zuo (2007) reported the production of compounds by *Paenibacillus polymyxa* and *Bacillus licheniformis* that inhibit the growth of sulphate reducing bacteria. The result of this study revealed a positive effect of *E. coli* on the growth of all other selected Gram negative bacteria with the exception of *P. aeruginosa* that showed a decrease in growth when co-cultured with *E. coli*. Further studies need to be carried out on the identity of substances that are secreted by *E. coli* that confer growth induction or growth inhibition to these bacteria.

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(Manuscript received on July 23, 2018 and revised on December 29, 2018)