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Synthesis of homoserine lactone from Aspartic acid and induction of quorum sensing by *E. coli* isolates:-

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Abstract:-

Homoserine L-lactone was synthesized by *E. coli* through using aspartic acid which was added to the culture media at a final concentration 1%. Homoserine lactone production was checked at different intervals of incubation (after 2, 3, 4, 5, 6 and 24 hr.) where 0.01% of KCN is added to show the time of HSL appearance in the bacterial growth. It was found that the best interval for accumulation of homoserine lactone was after 4 hours. of incubation in which the homoserine lactone reached at optimal level. When one drop of culture supernatant was added to fresh growth of *E. coli* on slide, aggregation or quorum sensing was seen as indicator of HSL production.

Introduction:-

Quorum sensing (QS) is a mechanism through which bacteria regulate gene expression by cell density. The bacteria produce hormone-like compounds called autoinducers that interact with regulatory proteins after they reach a certain threshold concentration. Several quorum-sensing systems are also extremely important to human health, as they regulate virulence determinants in bacterial pathogens (1).

Quorum-sensing molecules can be categorized into four groups (2), including: (1) N-acylhomoserine lactone (AHL; AI-1) involved in communication by gram-negative bacteria (3); (2) a furanosyl borate diester (AI-2) involved in communication by both gram-negative and gram-positive bacteria (4); (3) an unknown structure compound referred to as AI-3, involved in cross-communication with the mammalian epinephrine cells of host systems (5); and (4) oligopeptides, active only in gram-positive bacteria (6).

In the Enterobacteriaceae, however, only a few AHL-based quorum-sensing systems, such as in *Yersinia* (7) and *Serratia* (8), have been reported, whilst many other members of the family, including *Escherichia* and *Salmonella*, are generally thought to produce and utilize non-AHL signal molecules (9). All quorum-sensing systems in enterobacteria identified so far control different physiological functions related to their pathogenesis.

Quorum sensing may also play an important role in the life cycle of commensal enterobacteria, whether they exist asymptomatically in the gut environment or are provoked into causing infection elsewhere. Interestingly, many enterobacteria do not produce any AHLs under laboratory conditions, including the ATCC type strains (except for Serratia). This implies that horizontal transfer occurs during intestinal colonization and may have occurred in the clinical isolates possessing AHL synthase (10).

It is generally accepted that the *Escherichia coli* cells use quorum sensing to regulate division, based on availability of nutrients, competition from other microbes, and assessment of population density. The production of homoserine lactones in *E. coli* has been shown to be induced by the presence of glucose in the media (11).

The aim of this study is to synthesize HSL from aspartic acid by *E. coli* and then induction of quorum sensing by using HSL.

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Methods:-

1-Chemical detection of homoserine lactone:

Homoserine production was checked through the separation of supernatant from culture media, and then the supernatant was dialyzed verses LB free of KCN and then Brands test was used to detect methionine or homocystein synthesis through conversion of homoserine to homocystein. If the test is negative, the result is accepted and HSL is accumulated and homocystin or methionine was not synthesized.

2- Detection of quorum sensing in *E. coli*:-

1- LB media were prepared and supplemented with 1% aspartic acid and distributed in six flask

2- After inculation with *E. coli*, the flasks were incubated at 37°C for intervals (2, 3, 4, 5, 6, 24hr.)

3- At the end of each interval, KCN(0.01%) was added and then after 18hr. the media was filtered by Millipore filter and the supernatant was used for quorum sensing detection.

3- The quorum sensing test was done on slide through mixing one drop of supernatant and one drop of fresh bacterial growth , and then stained with gram stain and the slide was examined under microscope.

4- The positive result was scored due to the presence of aggregation bacterial cell

Results and Discussion:-

To study quorum sensing in *E. coli*, aspartic acid was used as the main focal metabolites for homoserine synthesis. It was observed that homoserine was accumulated in culture media after the addition of KCN in which the later will inhibit threonine synthesis, through its effect on threonine synthase enzyme. Homoserine lactone production was also checked by using brands test in order to ensure that quorum sensing occurs as a result of homoserine lactone synthesis.

The presence of aggregation of *E. coli* as in figure (1) was considered a positive result versus the negative results in the absence of homoserine lactone.

The best interval for accumulation of homoserine lactone was after 4 hours. of incubation in which the homoserine lactone became at maximum concentration. However, under certain condition the bacteria can form homocystein as a result of production of homocystein synthase, and the later may be used as indicator for homoserine lactone synthesis (table1).

Table (1):- effect of KCN on HSL production

Additional of KCN at interval (hr)	HSL appearance in culture media
2	(-)
3	(+) Weak
4	(+) Strong
5	(+) Weak
6	(-)

Quorum sensing in *E. coli* was first shown to occur by Surette and Bassler (12; 13). (14) proposed that quorum sensing may play an important role in stationary-phase physiology and inhibition of chromosomal replication in *E. coli.*

Escherichia coli cells use quorum sensing to regulate division, based on availability of nutrients, competition from other microbes, and assessment of population density. The production of HSL's in *E. coli* has been shown to be induced by the presence of glucose in the media (11).

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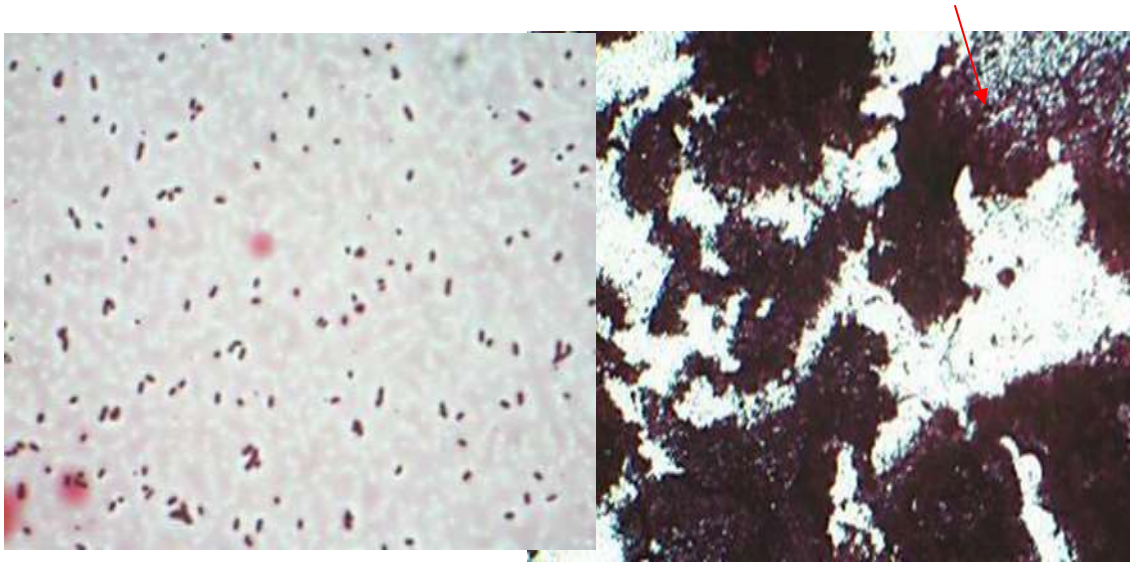
AHL-mediated cell-to-cell signaling has been observed in many natural environments (3) and has been shown to play a role in diverse functions such as pathogenesis, biofilm development and stress resistance (3). Furthermore, AHL cross-talk can result in differential gene expression due to the presence of exogenous AHLs produced by other species in mixed populations (15). Although Escherichia coli is not capable of synthesizing AHL molecules because they lack an AHL synthase encoding gene, they do produce a receptor-like protein, which shows an amino acid sequence similar to that of the LuxR-type transcriptional activators in AHL-dependent quorum sensing (16). It is known that the compound homoserine lactone (HSL), a metabolite synthesized from intermediates in threonine biosynthesis, can induce the expression of rpoS, a stationary phase-specific sigma factor of RNA polymerase and the master regulator of the general stress response in *E. coli* (17; 18). (19) have shown that temperature affects the AHL responsiveness and could have an impact on the survival of *E. coli* between hosts. Survival of *E. coli* outside the host in competitive environments requires the ability to overcome low nutrient availability, wide temperature fluctuations and other stresses (like pH). Also, it found mixed communities such as biofilms containing AHL producing bacteria.

AHLs produced by bacteria could serve as potential biomarkers in the management of bacterial diseases and, thus, monitoring them in biological samples may be a significant analytical tool for the investigation of such diseases (20). Specifically, altered bacteria-host interaction has been implicated in several health imbalances and disorders. For instance, there are several reports role of bacteria in GI disorders such as inflammatory bowel disease and irritable bowel syndrome (21). Using quorum sensing, bacteria can amass a high cell density before virulence determinants are expressed, and is so doing, the bacteria are able to make a concerted attack, produce ample virulence factors, and be present in sufficient numbers to overwhelm the host defenses (22). It has been shown that many pathogens use quorum sensing to regulate virulence (6).



A B

Figure (1): Detection of quorum sensing in *E. coli* (100x)
A: control (absence of homoserine); B: positive result ( ) →

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الخلاصة:-
في هذه الدراسة تم تصنيع Homoserine- L- lactone بواسطة بكتريا القولون المعوية من خلال اضافة حامض الأسبارتيك اسد الى الوسط الزرعي بتركيز نهائي 1 ٪. وقد تم فحص انتاجL- lactone - Homoserine في فترات مختلفة من الحضانة (بعد 2، 3 ، 4، 5 ، 6 و 24 ساعة.) حيث تم إضافة 0.01 ٪ من KCN لمعرفة الوقت الذي يلاحظ فيه HSL في النمو البكتيري. وقد وجد أن أفضل فترة لتراكم homoserine- L- lactone هو بعد 4 ساعات من الحضانة التي وصل فيها homoserine lactone الى المستوى الأمثل. وعندما تمت إضافة قطرة واحدة من النمو البكتيري من بكتريا القولون المعوية على الشريحة قد لوحظ تجمع للبكتريا دلالة على انتاجها ل HSL