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Tryptophanase and tryptophan synthetase in *Escherichia Coli*

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Abstract
The general objective of this study was to evaluate the effects of glucose and tryptophan in the induction or repression of the enzymes tryptophanase and tryptophan synthetase in *Escherichia coli* under different incubation time.
In general, the tryptophanase activity expressed in the amount of indole produced was significantly higher in tubes with added L-tryptophan during the assay. It was also observed that tryptophanase activity has increased as the time of incubation increases. Cells B (grown in basal medium + DL-tryptophan) had the highest tryptophanse activity across time of incubation as compared with Cells A (basal medium only), Cells C (basal medium + glucose) and Cells D (basal medium + tryptophan + glucose). Cells C had the lowest tryptophanase activity followed by Cells D. The enzyme tryptophanase, which degrades tryptophan to indole, pyruvic acid and ammonia, is repressed by glucose. The inhibition by glucose and other carbohydrates of induced enzyme formation has been attributed to repression by intermediates of carbohydrate metabolism.
It appeared that tubes without DL-serine had higher tryptophan synthetase activity or lower unutilized indole as compared with tubes with DL-serine. It was expected that higher synthetase activity should be present in tubes with DL-serine because together with indole, they served as reactants in the activity of the enzyme synthetase. There was also observed increase in tryptophan synthethase activity as the time of incubation was prolonged. Cells C had the highest tryptophan synthetase activity as compared to Cells A, Cells B and Cells D.

Keywords: tryptophanase, tryptophan synthetase, *Escherichia coli*

1. Introduction
In the control of metabolism in bacteria, small molecules can regulate the physiological processes by adjusting the enzymatic constitution of the cell [1]. According to Davis [2], this control is happened via the stimulation or inhibition of enzyme activity or formation. Many enzymes concerned with degradative and synthetic pathways have been investigated in attempts to discover the mechanisms involved in these control systems. However, until present, there is still a need to elucidate the regulation of enzymes involved in both the synthesis and dissimilation of a single physiologically important compound.
Escherichia coli can use a number of mechanisms in order to regulate the expression of its tryptophan (trp) operon and control the rate of tryptophan biosynthesis. According to Yanofsky and Crawford [3], these mechanisms include repression, transcription attenuation, and feedback inhibition. The combined actions of these mechanisms permit the bacterium to vary the rate of tryptophan production over a several thousand-fold range. Since tryptophan is costly to produce, efficient shutdown of synthesis is advantageous to the bacterium whenever the amino acid is present in its environment. As compared with other bacteria, *E. coli* has the capability to transport tryptophan from its environment. Trough the action of the inducible enzyme tryptophanase, *E. coli* can use tryptophan as a sole source of carbon or nitrogen. Tryptophanase degrades tryptophan to indole, pyruvate and ammonia. This enzyme can also catalyze the synthesis of tryptophan from indole and serine or cysteine [4].
The general objective of this study was to evaluate the effects of glucose and tryptophan in the induction or repression of the enzymes tryptophanase and tryptophan synthetase in *E. coli* under different incubation time.

2. Materials and Method
2.1. Preparation of culture
E. coli was inoculated in the following media: (1) Cells A = Basal media (mineral salts-hydrolized casein); (2) Cells B = Basal medium + DL-tryptophan (0.0025 M, 51 mg/100 mL media); (3) Cells C = Basal medium + glucose (0.1 M, 1.8 g/100 mL media); and (4) Cells D = Basal medium + tryptophan + glucose. The inoculated media were incubated at room temperature for 24 hours with shaking. Around 30 to 40 mL of each growth medium was transferred in falcon tube for centrifugation (5,000 rpm, 15 minutes). The resulting pellet after two times centrifugation was resuspended in 5 mL sterile distilled water.

2.2 Induction/repression of tryptophanase
The below components were prepared in duplicate tubes (all volumes given in mL):

Component	Tube No.							
	1	2	3	4	5	6	7	8
	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	23	24
Phosphate buffer, pH 7.8 (0.2 M)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Cells A	0.1	0.1	---	---	---	---	---	---
Cells B	---	---	0.1	0.1	---	---	---	---
Cells C	---	---	---	---	0.1	0.1	---	---
Cells D	---	---	---	---	---	---	0.1	0.1
H_2O	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6
L-tryptophan (0.05 M)	---	0.1	---	0.1	---	0.1	---	0.1

The cells, buffer and water were added in the tubes. The mixture was allowed to equilibrate for about 10 minutes in a 37 °C water bath. Afterwards, the tryptophan was added in the tubes and incubated for 10, 20 and 60 minutes as follows: tubes 1 to 8 = 10 minutes incubation; tubes 9 to 16 = 20 minutes incubation; and tubes 17 to 24 = 60 minutes incubation. In order to stop the reaction, 0.2 mL of 25% trichloroacetic acid was added in each tube. The amount of indole produced was then analyzed.

2.3. Induction/repression of synthetase

The below components were prepared in duplicate tubes (all volumes given in mL):

Component	Tube No.							
	1	2	3	4	5	6	7	8
	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	23	24
Phosphate buffer, pH 7.8 (0.2 M)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Indole (5 mg/10 mL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Cells A	0.5	0.5	---	---	---	---	---	---
Cells B	---	---	0.5	0.5	---	---	---	---
Cells C	---	---	---	---	0.5	0.5	---	---
Cells D	---	---	---	---	---	---	0.5	0.5
H_2O	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6
DL-serine (0.1 M)	---	0.2	---	0.2	---	0.2	---	0.2

The cells, buffer, indole and water were added in the tubes. The mixture was allowed to equilibrate for about 10 minutes in a 37 °C water bath. Afterwards, the serine was added in the tubes and incubated for 10, 20 and 60 minutes as follows: tubes 1 to 8 = 10 minutes incubation; tubes 9 to 16 = 20 minutes incubation; and tubes 17 to 24 = 60 minutes incubation. To establish the initial level of indole, a set of four tubes were prepared; the components were the same as tubes 2, 4, 6 and 8 except that 0.2 mL of 25% trichloroacetic acid was added before the addition of other components. In order to stop the reaction, 0.2 mL of 25% trichloroacetic acid was added in each tube. The amount of indole produced was then analyzed.

2.4. Determination of indole

Two millilitre of toluene was added in each tube in order to extract the indole. The contents were thoroughly mixed and made it stand for 15 minutes. Upon the separation of layers, 0.5 mL of the toluene layer (upper layer) was transferred to another tube. One millilitre of Ehrlich's reagent and 8.5 mL of acid alcohol were added in the new tube. The mixture was set aside for 15 minutes to allow color development. The absorbance was read at 540 nm. The amount of indole produced was calculated by using a standard curve prepared by toluene extraction of indole standard.

2.5. Preparation of indole standard curve

The below components were prepared in test tubes (all volumes given in mL):

Component	Tube No.						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Indole solution (50 ug/mL)	---	0.2	0.4	0.8	1.2	1.6	2.0
H_2O	2.0	1.8	1.6	1.2	0.8	0.4	---

The indole was extracted as described above. The standard indole curve was plotted and the linear portion of the curve was used to determine the indole produced (trytophanase) or utilized (synthetase)

3. Results and Discussion

3.1. Induction/repression of tryptophanase

The tryptophanase activity (amount of indole produced, µg/ml) in general was higher in tubes with added L-tryptophan during the assay (Tables 1a to 1d). In Cells A, tubes with L-trytophan (2, 10, 18) had 10 to 13 times higher amount of indole produced than tubes without L-tryptophan (1, 9, 17); pairwise comparison showed significant difference ($p<0.05$) (Table 1a). The same result was obtained in Table 1b, wherein tubes with added L-tryptophan (4, 12, 20) had 15 to 27 times higher indole than tubes without L-tryptophan (3, 11, 19) and statistical analyses showed significant difference ($p<0.05$). In Tables 1c and 1d, no significant difference resulted when indole production was compared between tubes with L-tryptophan and without L-tryptophan. In tubes with added L-tryptophan, the highest amount of indole produced was in Cells B (grown in basal medium + DL-tryptophan) (Table 1b) and followed by in Cells A (grown in basal medium only) (Table 1a). It appeared that the addition of L-tryptophan had a positive effect in the induction of tryptophanase activity. The enzyme tryptophanase is responsible for the degradation of tryptophan to indole, pyruvic acid and ammonia, thus, higher availability of tryptophan in the tubes means more indole to be produced in the degradation process.

Table 1a: Pairwise comparison of indole produced in Cells A (basal medium only) with (tubes 2, 10, 18) or without (tubes 1, 9, 17) addition of tryptophan

Without Tryp	Indole Produced (µg/mL)	With Tryp	Indole Produced (µg/mL)
1	1.00±0.00	2	13.31±0.62*****
9	1.44±0.44	10	13.69±1.15*****
17	1.25±0.18	18	15.38±1.40*****

Asterisk shows significance at $p<0.05$

Table 1b: Pairwise comparison of indole produced in Cells B (basal medium + tryptophan) with (tubes 4, 12, 20) or without (tubes 3, 11, 19) addition of tryptophan

Without Tryp	Indole Produced (µg/mL)	With Tryp	Indole Produced (µg/mL)
3	2.25±0.35	4	33.81±3.27*
11	1.5±0.35	12	40.75±2.47*
19	2.31±0.62	20	56.88±3.71*

Asterisk shows significance at $p<0.05$

Table 1c: Pairwise comparison of indole produced in Cells C (basal medium + glucose) with (tubes 6, 14, 22) or without (tubes 5, 13, 21) addition of tryptophan

Without Tryp	Indole Produced (µg/mL)	With Tryp	Indole Produced (µg/mL)
5	1.88±0.17	6	1.81±0.44
13	1.31±0.27	14	0.88±0.00
21	1.19±0.27	22	1.00±0.35

Table 1d: Pairwise comparison of indole produced in Cells D (basal medium + tryptophan + Glucose) with (tubes 6, 14, 22) or without (tubes 5, 13, 21) addition of tryptophan

Without Tryp	Indole Produced (µg/mL)	With Tryp	Indole Produced (µg/mL)
7	1.00±0.35	8	0.75±0.18
15	1.38±0.35	16	1.38±0.18
23	0.75±0.18	24	1.63±0.88

In Cells A, Cells B and Cells D (Table 2), it was observed that tryptophanase activity has increased as the time of incubation increases. This will give more time for the enzyme tryptophanase to degrade tryptophan and produce more indole in the process. The comparison was significant in Cells B, wherein amount of indole produced in 60 minutes incubation (56.88±3.71 µg/mL) was significantly higher to the amount of indole produced during 10 (33.81±3.27 µg/mL) and 20 minutes (40.75±2.47 µg/mL) (Table 2). Meanwhile, contradictory result was obtained in cells c (Table 2) wherein tubes incubated at 10 minutes (1.81±0.44 µg/mL) had the highest tryptophan activity which was followed by 60 minutes incubation (1.00±0.35 µg/mL).

Table 2: One-way ANOVA comparison of indole produced in Cells A (basal medium only), Cells B (basal medium + tryptophan), Cells C (basal medium + glucose) and Cells D (basal medium + tryptophan + glucose) with addition of tryptophan and incubated at different time

Time (min.)	Indole Produced (µg/mL)			
	Cells A	Cells B	Cells C	Cells D
10	13.31±0.62 (tube 2)	33.81±3.27^{b} (tube 4)	1.81±0.44 (tube 16)	0.75±0.18 (tube 8)
20	12.56±2.74 (tube 10)	40.75±2.47^{b} (tube 12)	0.88±0.00 (tube 14)	1.38±0.18 (tube 16)
60	15.38±1.41 (tube 20)	56.88±3.71^{a} (tube 20)	1.00±0.35 (tube 22)	1.63±0.88 (tube 24)

Cells B had the highest tryptophanse activity across time of incubation as compared with Cells A, Cells C and Cells D. During 10 minutes incubation, indole produced in Cells B was three times, 19 times and 45 times significantly higher as compared to indole produced in Cells A, Cells C and Cells D, respectively ($p<0.05$). Lowest tryptophanase activity was recorded in Cells D followed by Cells C. Intermediate tryptophanase activity was observed in Cells A (Table 3). Prolonging the incubation of test tubes (20 minutes) has resulted to the increased gap of tryptophanse activity between Cells B (40.75±2.47 µg/mL) compared to Cells A (12.56±2.74 µg/mL), Cells C (0.88±0.00 µg/mL) and Cells D (1.38±0.18 µg/mL). Tryptophanse activity in Cells B was three times, 46 times and 30 times significantly higher as compared to Cells A, Cells C and Cells D (Table 3). During 60 minutes incubation, indole produced in Cells B was four times, 56 times and 35 times significantly higher as compared to indole produced in Cells A, Cells C and Cells D, respectively. Still, Cells C (1.00±0.35 µg/mL) had the lowest tryptophanase activity followed by Cells D (1.63±0.88 µg/mL). The presence of glucose in Cells C and Cells D tend to repress the activity of tryptophanase. Tryptophan in Cells D was not enough to neutralize the repressor effect of glucose.

Table 3: One-way ANOVA comparison of indole produced of *E. coli* grown in different media (Cells A = basal medium only; Cells B = basal medium + tryptophan; Cells C = basal medium + glucose; Cells D = basal medium + tryptophan + glucose) with addition of tryptophan and incubated at 10, 20 and 60 minutes

Media	Indole Produced (µg/mL)		
	10 mins. incubation	20 mins. incubation	60 mins. incubation
Cells A	13.31±0.62^{b} (tube 2)	12.56±2.74^{b} (tube 10)	15.38±1.41^{b} (tube 18)
Cells B	33.81±3.27^{a} (tube 4)	40.75±2.47^{a} (tube 12)	56.88±3.71^{a} (tube 20)
Cells C	1.81±0.44^{c} (tube 6)	0.88±0.00^{c} (tube 14)	1.00±0.35^{c} (tube 22)
Cells D	0.75±0.18^{c} (tube 8)	1.38±0.18^{c} (tube 16)	1.63±0.88^{c} (tube 24)

Different letter shows significance at $p<0.05$

Monod and Bazire [5] found out that tryptophanase was increased more than 40-fold in the presence of 5 x 10^{-3} M DL-tryptophan. Also, excellent tryptophanase activity was obtained when the bacterial cells were grown in basal media containing indole, L-, DL-, or glycyl-L-tryptophan. Some tryptophanase formation was also evident when 5 hydroxytryptophan, anthranilic acid, shikimic acid, or indole-3-acetic acid were included in the growth medium. The authors also found out that little or no enzyme activity was manifested unless L-tryptophan was present, suggesting that the capacity of these compounds to induce tryptophanase during growth is due to their conversion by the bacterial cells to indole or tryptophan.

Direct measurements of the amino acid pool of *E. coli* showed that the free-tryptophan content of cells grown without glucose was considerably higher than of those grown with the carbohydrate [6]. The enzyme tryptophanase, which degrades tryptophan to indole, pyruvic acid and ammonia, is repressed by glucose [7]. According to Freundlich and Lichstein [8], glucose normally exhibits the most marked inhibition of tryptophanase activity. The inhibition by glucose and other carbohydrates of induced enzyme formation has been attributed to repression by intermediates of carbohydrate metabolism [9]. Levin and Magasanik [10] have mentioned that these compounds are thought to be the "metabolite moieties" of the repressor molecule which prevent enzyme synthesis by competing with the inducer at the enzyme-forming site. Yanofsky[6] has hypothesized two possible reasons for the depletion of tryptophan in the presence of glucose: (i) glucose is metabolized to a compound which blocks the endogenous formation of tryptophan, and (ii) tryptophan is metabolized so rapidly in the presence of glucose that there is little chance for the amino acid to accumulate. There would be an increased requirement for protein building materials in culture containing glucose, thus, it is acceptable that there would be a smaller pool of free tryptophan [11].

Since glucose is a principal product of the β-galactosidase reaction, a hexose proximally derived from glucose would be most likely to be responsible for effecting catabolite repression. Similarly, since pyruvate is the product of catabolic significance in the tryptophanase reaction, pyruvate or a closely related compound would play a primary role in the catabolite repression of tryptophanase [12]. In addition, data of Freundlich and Lichstein [12] has suggested that glycerol, pyruvate and glucose can provide a compound which can cause catabolite repression either directly or indirectly. Alternatively, the mechanism for catabolite repression of tryptophanase may be multivalent with a whole variety of compounds capable of causing repression. The addition of glucose to cultures of *E. coli* causes the excretion of cAMP[13]. It has been suggested that the loss of cAMP is responsible for catabolite repression [14, 15] and that various physiological conditions affect the internal levels of cAMP. Pastan and Perlman [16] found out that cyclic AMP enhanced tryptophanase production and relieved transient repression when it was present before RNA synthesis was inhibited, but not when it was added in the presence of the inhibitor. Those results suggested that cyclic AMP stimulated the transcription of the tryptophanase gene; however, since translation was also operating during induction the stimulation of transcription by cyclic AMP might have been secondary to the effect on translation.

3.2. Induction/repression of synthetase

Since the initial concentrations of indole (Cells A = 33.89 µg/mL; Cells B = 35.61 µg/mL; Cells C = 27.61 µg/mL and Cells D = 15.28 µg/mL) were most of the time lower as compared to the final concentrations, negative values of tryptophan synthetase activities were obtained in most of the time. It was hypothesized that something was done wrong in the conduct of this part of exercise. In order to have a better discussion, the data were expressed as unutilized indole rather than utilized indole wherein the final indole was not deducted to the initial indole.

In Table 4a, it appeared that tubes without DL-serine had higher tryptophan synthetase activity (lower unutilized indole) as compared with tubes with DL-serine. There was no significant difference when the two groups were compared ($p>0.05$). For Table 4b, tubes with DL-serine in general had higher synthetase activity as compared with tubes without DL-serine. Significant difference was observed in tubes incubated at 20 and 60 minutes but results were contradicting. Synthetase activity was generally higher in tubes with DL-serine in Table 4c but opposing result was obtained in tubes with DL-serine in Table 4d. It was expected that higher synthetase activity should be present in tubes with DL-serine because together with indole, they served as reactants in the activity of the enzyme synthetase. Indole and serine are being catalyzed by tryptophan synthetase to produce tryptophan and water.

Table 4a: Pairwise comparison of unutilized indole in Cells A (basal medium only) with (tubes 2, 10, 18) or without (tubes 1, 9, 17) addition of DL-serine

Without Ser	Unutilized Indole (µg/mL)	With Ser	Unutilized Indole (µg/mL)
1	33.72±7.46	2	28.17±0.24
9	28.67±0.409	10	47.39±0.55
17	23.28±3.38	18	27.56±0.00

Table 4b: Pairwise comparison of unutilized indole in Cells B (basal medium + tryptophan) with (tubes 4, 12, 20) or without (tubes 3, 11, 19) addition of DL-serine

Without Ser	Unutilized Indole (µg/mL)	With Ser	Unutilized Indole (µg/mL)
3	40.94±7.94	4	35.11±1.73
11	39.78±0.16	12	43.33±0.00*****
19	41.67±0.00*****	20	20.00±0.94

Asterisk shows significance at $p<0.05$

Table 4c: Pairwise comparison of unutilized indole in Cells C (basal medium + glucose) with (tubes 6, 14, 22) or without (tubes 5, 13, 21) addition of DL-serine

Without Ser	Unutilized Indole (µg/mL)	With Ser	Unutilized Indole (µg/mL)
5	33.78±0.00	6	27.33±4.71
13	27.78±8.64	14	30.44±3.77
21	23.78±0.63	22	23.56±0.00

Table 4d: Pairwise comparison of unutilized indole in Cells D (basal medium + tryptophan + glucose) with (tubes 8, 16, 24) or without (tubes 7, 15, 23) addition of DL-serine

Without Ser	Unutilized Indole (µg/mL)	With Ser	Unutilized Indole (µg/mL)
7	33.00±5.19	8	39.89±9.74
15	32.00±15.24	16	40.06±2.44
23	30.33±8.64	24	25.00±2.67

In Cells A, Cells B, Cells C and Cells D (Table 5), it was observed that tryptophan synthetase activity has increased as the time of incubation increases. This will give more time for the enzyme synthetase to catalyze serine and indole to produce tryptophan. The synthetase activity during 60 minutes incubation was significantly higher as compared to 20 minutes incubation but not with 10 minutes incubation. Meanwhile, synthetase activity in Cells B during 60 minutes incubation was significantly higher as compared to 10 and 20 minutes incubation ($p<0.05$). Highest synthetase activities in Cells C and Cells D were also recorded during 60 minutes incubation but not significantly different when compared to 10 and 20 minutes incubations ($p<0.05$) (Table 5).

Table 5: One-way ANOVA comparison of indole produced in Cells A (basal medium only), Cells B (basal medium + tryptophan), Cells C (basal medium + glucose) and Cells D (basal medium + tryptophan + glucose) with addition of DL-serine and incubated at different time

Time (min.)	Unutilized Indole (µg/mL)			
	Cells A	Cells B	Cells C	Cells C
10	28.17±0.24^{b} (tube 2)	35.11±1.73^{b} (tube 10)	27.33±4.71^{a} (tube 16)	39.89±9.74^{a} (tube 8)
20	47.39±0.55^{a} (tube 10)	43.33±0.00^{a} (tube 12)	30.44±3.71^{a} (tube 14)	40.06±2.44^{a} (tube 16)
60	27.56±0.00^{b} (tube 18)	20.00±0.94^{c} (tube 20)	23.56±0.00^{a} (tube 22)	25.00±2.67^{a} (tube 24)

Different letter shows significance at $p<0.05$

During 10 minutes incubation, Table 6 showed that Cells C had the highest tryptophan synthetase activity as compared to Cells A, Cells B and Cells D. No significant difference was observed when the amount of unutilized indole was compared ($p>0.05$). Meanwhile, tryptophan synthetase activity in Cells C during 20 minutes incubation was significantly higher as compared to Cells A, Cells B and Cells D (Table 6; $p<0.05$). During the 60 minutes incubation, it appeared that cells b had the highest tryptophan synthetase activity and it was significantly higher as compared to Cells A only (Table 6). Result of Table 6 was not ideal since tryptophan is known repressor of synthetase activity.

Table 6: One-way ANOVA comparison of unutilized indole of *E. coli* grown in different media (Cells A = basal medium only; Cells B = basal medium + tryptophan; Cells C = basal medium + glucose; Cells D = basal medium + tryptophan + glucose) with addition of DL-serine and incubated at 10, 20 and 60 minutes

Media	Unutilized Indole (µg/mL)		
	10 mins. incubation	20 mins. incubation	60 mins. incubation
Cells A	28.17±0.24 (tube 2)	47.39±0.55^{a} (tube 10)	27.56±0.00^{a} (tube 18)
Cells B	35.11±1.73 (tube 4)	43.33±0.00^{a} (tube 12)	20.00±0.94^{b} (tube 20)
Cells C	27.33±4.71 (tube 6)	30.44±3.77^{b} (tube 14)	23.56±0.00^{ab} (tube 22)
Cells D	39.89±9.74 (tube 8)	40.06±2.44^{a} (tube 16)	25.00±2.67^{ab} (tube 24)

Different letter shows significance at $p<0.05$

According to Pardee [1], the physiological significance of the glucose inhibition of tryptophanase becomes clearer when this is considered in conjunction with the stimulatory effect of glucose on tryptophan synthetase. Thus, the function of repression in tryptophan biosynthesis appears not to decrease the amount of tryptophan produced in the presence of excess exogenous tryptophan, but, rather, to provide for economy [1] and flexibility [17] in the formation of the synthetase enzyme. It is likely that cells growing in the presence of glucose would need more of an enzyme synthesizing an amino acid essential for increased protein formation. This argument is supported further by the fact that other environmental factors that increased growth also increased the level of tryptophan synthetase.

In the case of the degradative enzyme, it is speculated that repressors of tryptophanase formation are produced from carbohydrate dissimilation. On the other hand, the increased metabolic rate caused by glucose and other carbon sources reduces the intracellular level of tryptophan, the repressor of tryptophan synthetase [18]. In study conducted by Freundlich and Lichstein [12], it was revealed that compounds which increased the level of tryptophanase decreased the formation of synthetase; L-, DL-, and glyCyl-L-tryptophan were equally effective in repressing the enzyme.

4. Conclusion

The tryptophanase activity in general was higher in tubes with added L-tryptophan during the assay. It was also observed that tryptophanase activity has increased as the time of incubation increases. It appeared that tubes without DL-serine had higher tryptophan synthetase activity as compared with tubes with DL-serine. There was also observed increase in tryptophan synthethase activity as the time of incubation was prolonged.

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