Improving Ability of *Pseudomonas putida* Strain to Biosynthesis of L-Tryptophan

Nourah H.M Al-Zahrani

Department of Biology, Faculty of Science for Girls, King Abdulaziz University, Jeddah, Saudi Arabia
nhalzhrani@kau.edu.sa

Abstract: This study conducted to test and improve the ability of biosynthesis of L-Tryptophan by *Pseudomonas putida*. First It was tested for producing amino acid L-Tryptophan, then exposed to UV radiation *get mutants produce L* – Tryptophan. Tow strains were obtained after exposure 20 min to U V *P. putida* AI and *P. putida* BI produce 2, 0%mg and 1.4%mg% L-Tryptophan respectively biosynthesis of Tryptophan ability of *P. putida* AI was improved by adding cacium carbonate 0.3g % anthranillic acid. Anew mutant strain resistant to anthranillic acid P. putida AII was obtained from P. *putida* AI, daily after 18hrs addition of anthranillic acid and Ammonium sulphate to the fermentation medium increasing the biosynthesis of L- Tryptophan to 117.39 mg% by this isolate *P.putida*.
[Nourah H.M Al-Zahrani**. Improving Ability of *Pseudomonas putida* Strain to Biosynthesis of L-Tryptophan.** *Life Sci. J* 2014; 11(4):476-481] (ISSN: 1097-8135). http://www.lifesciencesite.com. 70

Key words: L-Tryptophan, *Pseudomonas putida,* U.V. Radiation, Anthranillic acid.

1. Introduction

Among all standard amino acids, L-Tryptophan (L-Trp) is one of the least abundant and most expensive to produce (Anyanful *et al.,* 2005). It is an aromatic amino acid with the unique in dole side-chain that makes it a fundamental precursor in brain **(**Bauer *et al.,* 2000). It is a safe and reasonably effective sleep aid (Riemann, 2002) probably due to its ability to increase brain levels of serotonin (Le Floc'h Nathalie & Seve Bemard,2007 and Laddha *et al*.2012). It is the third limiting essential amino acid required in the diet of protein deficient population and livestock after methionine (Bidisha *et al.,* 2013). It can be used as food additive, infusion liquids, pellagra treatment, sleep induction and nutritional therapy(Bongaerts *et al.,* 2001 and Gosset, 2009).It is also the precursor of a number of plant metabolites like IAA (Sahasrabudhe, 2011). Mostly L-Tryptophan is produced industrially for feed and pharmaceutical purposes. Since the chemical synthesis of L-Tryptophan has many disadvantages such as nonrenewable toxic raw materials and racemic mixtures of products, microbial fermentation of L-Tryptophan has become attractive alternative. Attempts have been made to overproduce this important amino acid in a wide number of bacteria including *Bacillus subtilis* (Gollnick *et al.,* 2005) and *Pseudomonas aeruginosa* (Pasupuleti *et al.,* 2009)

Industrial production of L- Tryptophan is mostly for feed and pharmaceutical purposes. A shift towards microbial processes for L-Tryptophan production by microbial fermentation began in the early 1960s.Now a days L-Tryptophan production is carried out through enzymatic (Mateos *et al.,* 2009) and fermentative processes using metabolically engineered *Pseudomonas* sp. 023K (Bidisha, *et al.*(2013). Enzymatic methods use enzyme or whole cells of microorganisms with TPase (Chant and Summers, 2007) or Tease (Chaudhry *et al.,* 1998) activity. Since the chemical synthesis of L-Tryptophan has many disadvantages such as nonrenewable toxic raw materials and racemic mixtures of products, microbial fermentation of L-tryptophan has become attractive alternative (Berry, 1996).

Rao *et al.* (1975) found after exposing *Aspergillus fumigates* to ultraviolet radiation which produced mutants with the change of its biosynthesis of tryptophan activity. Lawrance (1971) reported after exposing cells to ionizing radiation produced a series of reactions that raise the proportion of chemical substances and lead to physiological alterations.Habbs and McClellan (1975) observed that radiation affects the proteins and enzymes, nucleic acids, lipids, carbohydrates, and thus shows the effect on the cells. A number of researchers they reported that Suitable (pH) for the production of L-Tryptophan occasion ranging from 5–7. (Ajinomoto, 1984; Ishida *et al.,* 1986; Takamatsu *et al.,* 1986), and provide fermentation medium with materials that operate on the equivalent medium considered an important process to increase production of L –Tryptophan, when using glucose as the carbon source is causing the low pH to less than five and adding calcium carbonate works to maintain the pH at the medium (Ajinomoto, 1980; Shiio *et al.,* 1983 and Nyester, 1983).

Bacillus subtitles strain resistant to acid anthranillic acid Was obtained maximum amount after addition of 0.8 g% anthranillic acid to fermentation medium ranged to 10.4mg/ml.(Arima *et al.,* 1972). The 0.5 gm % of the anthranillic acid was the inhibitory concentration of L-Tryptophan production by *P. putida* and the addition of anthranillic acid in installments over of fermentation period and the strain did not suffer from the inhibitor effect of the acid (Yamada *et al.,* 1972), also it's possible that the acid

works as an induction in the way of constructed of L-Tryptophan when anthranillic acid provide limited quantities prohibitive for growth (Ishida *et al.,* 1986 and Takamatsu *et al.,* 1986).

The aim of the study was to improve ability biosynthesis of the amino acid L-Tryptophan by *P. putida*, using different chemical and irradiation treatments.

2- Material and Methods:-

2.1Material:-

2.1.1. Microorganisms:-

Pseudomonas putidda was obtained from Mircen, Fac. of Agric., Ain Shams Univ., Egypt.

2.1.2. Media:-

2.1.2.1. Complete Medium: (Nieder and Shapiro, 1975):-

(composed of 10g Ttrypton; 5g yeast extract; 5g sodium chloride; 5g Glucose; pH7-.2)

2.1.2.2. Minimal Medium:(Ornston *et al.,* 1969):-

10mM potassium dehydrogenate phosphate; 50mM Sodium monohydrate phosphate; 2g Ammonium sulfate; 100uM Calcium chloride;1mM Magnesium sulfate; 10uM Iron sulfate; Glucose; pH6.2).

2.12.3.Seed Medium: (Nieder and Shapiro,1975):

Treptone10g; Yeast extract5g; Sodium chloride5g; Anthranillic acid 0.8g; pH7-7.2.

2.1.2.4.. Fermentation Medium:-

Minimal medium modified by addition of 50g/l glucose, 3g/l $CaCO_3$ and 0.8g/l anthranillic acid.

2.2.Methods:-

2.2.1.**Studying the Ability Biosynthesis of The Amino acid L-Tryptophan by The Wild Organism *P. putida*.**

The wild type of bacteria *P. putida* inoculated on seed medium, after 18 hrs of incubation at 35°C this culture was used as an inoculums for fermentation medium and incubated for 96 hrs, free cell filtrate was used to determine glucose, total amino acids and L-Tryptophan residual in the medium.

2.2.2. Selection Mutants of *P. putida* by Penicillin G:-

Bacteria *P. putidda* inoculated in liquid Minimal Medium with addition of 410 units / ml penicillin to select of mutant anti penicillin bacteria and then incubated at 35°C for 16hours in shaking incubator (100 rev/min), cells separated and washed with a saline solution (0.8%), and then re-incubated in an a complete medium with addition of 410 units / ml penicillin for 24 hours to isolate mutant cells using the method of gradual dish to work the following experiments (Ornston *et al.,* 1969 and Fusho *et al.,* 1986).

2.2.3. Selection of *P. putida* Mutants producing L-Tryptophan Resistant to Ultraviolet Radiation (UV):-

P. putida strain resistance to penicillin treated with UV. In liquid Minimal Medium with addition of 410 units / ml penicillin, 30 cm distance, samples were obtained at intervals (5-10-15-20 minutes), and then inoculated at 35°C and incubated for 24 hours in a shaking incubator, samples were used to inoculate complete medium that containing penicillin in petri dishes and incubated at 35°C for 48 hours. This mutant was used in the following studies.

2.2.4. Effect of Incubation Period on L-Tryptophan Biosynthesis by *P. putida* AI in Presence of $CaCO_3$:-

Fermentation medium was inoculated by strain ***P. putida* AI** mutant resistant to UV incubated in shaking incubator at35°C, cells collected by centrifuge for several intervals after 18 hrs of incubation, then estimated the amount of glucose, tryptophan and total amino acid residual in the medium.

2.2.5. Effect of The Presence of Anthranilic acid on L- Tryptophan Biosynthesis by ***P. putida* AI** (Ruban and Lobireva,1965**)**

Fermentation medium containing 0.8g/l acid anthranillic acid inoculated by *P. putida* AI strain resistance to penicillin and UV gave higher production L-Tryptophan was incubated in shaking incubator at 35°C,

2.2.6. Effect of Daily Addition of Antheranillic acid + Ammonium sulphate on L-Tryptophan Biosynthesis by *P. putida* AII.

strain resistant to anthranillic acid was obtained of *P. putida* AI by sing gradient plate method,, this strain named *P. putida* AII, it was tolerated to 0.5 g% anthranillic acid, this strain resistant to penicillin and UV and anthranillic acid.

P. putida AII inoculated in seed medium and incubated for 24hours at 35°C. and used to inoculate the fermentation medium that containing of 0.8 g/ l anthranillic acid and incubated 24 hours at 35°C, daily addition every 18hrs of 50mg% anthranillic acid dissolved in ethanol and 10 mg% ammonium sulfate dissolved in distilled water and sterilized as a source of nitrogen were added to the fermentation medium to increase the biosynthesis of tryptophan by this isolate.

2.2.7.Chemical Determination:-

2.2.7.1.Colorimetric Determination of Glucose:

To estimate glucose with colorimetric methods Cooper and Daniel, (1970) method was used. The control sample was prepared by taken 0.1ml of the samples with a reagent solution 3 ml (1.5 thiourea in 940ml of glacial acetic acid, 60ml o -toluidine) requested the samples well and incubated in a water bath for 8 minutes at the boiling point, then refrigerate for 10 minutes and determined by using

spectrophotometer at at 630nm. Calculated the amount of glucose in the sample compared to the standard curve for glucose (1 g/ glucose in benzoic acid solution (1 g/l), and used the following dilutions: 20, 40, 60, 80, 100 g/l).

2.2.7.2. Colorimetric Determination of Total Amino acid:-

Muting and Kaiser, (1963) method was Followed, Mixing 0.1 ml of the sample + 1.5 ml mixture of ethanol and acetone (1:1) + 0.1 ml phosphate buffer (0.1m,pH6.5)+ 2 ml ninhydrin in test tubes 15 ml x 200 ml, and placed in a water bath at boiling degree for ten minutes, then transported directly to the ice bath, and then added to a 10 ml ethanol and determined the total amino acids by using spectrophotometer at at 580nm. Using known amount of glycine as a control sample was determined the total concentration of nitrogen or total quantity of amino acids.

2.2.7.3.Colorimetric Determination of L-Tryptophan:

The acid ninhydrin method were used (Molnar and Pinter, 1989),when 1 ml of the sample added to 2 ml ninhydrin reagent and incubated in a water bath at a temperature of 35 ° c for 90 minutes with the use of a control sample of water, then distilled water added to samples, bringing the volume to 10 ml, and measured by spectrophotometer at 390 nanometers.

Statistical Analysis:-

Anova Analysis was used.

Conversion (%) = tryptophan quantity/ Consumed sugar ×100.

Yield (%)=L-Tryptophan quantity/The amount of sugar added to the medium ×100.

3. Results and Discussion:-

P. putida resistance to penicillin exposure to ultraviolet radiation resulted in obtaining 2 resistant *P. putida* A1 and *P. putida* B1 after 20min, where they tested their ability of biosynthesis L-Tryptophan compared to the original strain and found the following. It is possible to get tow isolates of *P. putida* as a result of exposing the microbe (for 20 minutes) to UV radiation produced a small amounts of L-Tryptophan and this agreed with results that Rao *et al.* (1975) found. Estimated amount of glucose consumed by *P. putida* wild strain of 3.28 mg % of the total amount, while the percentage in *P. putida* A1 3.86 % and 3.62 mg 5 mg in *P. putida* B1, of 4.83 mg % race total amount in the medium Table (1). Total amount of amino acids produced by *P. putida* A1 was 36 mg %, and 37.5 mg % by *P. putida* B1, and (25 mg %) by wild type (Table1). Amino acids were estimated by wild strain *P. putida* -matched controls and proven lack of ability of biosynthesis L-Tryptophan. While *P. putida* A1 produced 2.0 mg % and 1.4 % by *P. putida* B1 (Table1) (Fig 1), this results agree with that found by Lawrance(1971) and Habbs and McClellan (1975).

Table(1):Effect of ultraviolet radiation on L-Tryptophan biosynthesis by *P. putida*.

Organism	Biomass (mg%)	Consumed sugar(g%)	Sugar consump./ unit weight	L-Tryptophan (mg%)	T.A.A. (mg%)	Conversion (%)	Yield (%)
Control	740	3.28	4.43	-	25	-	-
Colony AI	320	3.86	12.06	2.0*	36	0.05	0.04
Colony BI	210	3.62	17.24	1.4	37.5	0.04	0.03

Initial amount of glucose=4.83g%

*Rate of increase in tryptophan biosynthesis 42.86% over colony.

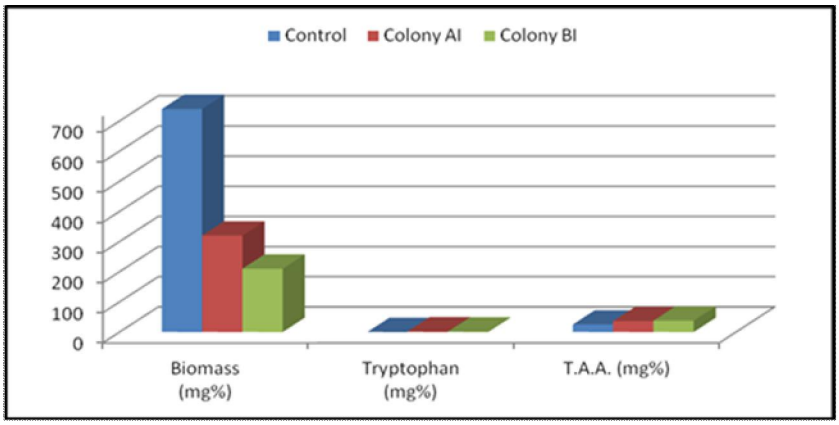

Fig.(1): Effect of ultraviolet radiation on L- Tryptophan biosynthesis by *P. Putida*

Studying the effect of inoculation period in presence of $CaCO_3$ fermentation medium gave a higher amount of extracellular L-Tryptophan after 108hrs days of fermentation (26%) using *P. putida* AI either percentage increase of 160% over the control (Table2) (Fig2). A number of researchers they reported that best degree pH of the production of L-Tryptophan occasion ranging from 5-7 (Ajinomoto, 1984; Ishida *et al.,* 1986 and Takamatsu *et al.,* 1986) and provide fermentation medium with materials that operate on the equivalent medium considered an important process to increase production of L-Tryptophan, when using glucose as the carbon source is causing the low pH to less than five, but adding calcium carbonate works to maintain the pH in the medium (Ajinomoto, 1980; Shiio *et al.,* 1983 and Nyester, 1983), or because that the biosynthesis of L-tryptophan from glucose involves a long metabolic pathway, there are several regulatory circuits which affected the accumulation of L-tryptophan such as transcriptional repression, attenuation, feedback inhibition and so on (Bongaerts, *et al.,* 2001 and Bidisha *et al.*, 2013).

Table(2): Effect of incubation period on L-Tryptophan biosynthesis by *P. putida* AI in presence of $CaCO_3$.

Incubation period (hrs)	Biomass(mg%)	Consumed sugar(g%)	Sugar cunsump/unit weight	Tryptophan (mg%)	T.A.A. .(mg%)	Convesion (%)	Yield (%)
Control	430	4.53	10.54	10	19	0.22	0.20
18	250	3.53	14.12	11	80	0.31	0.23
36	290	3.99	13.76	19	95	0.48	0.39
54	340	4.35	12.79	20	80	0.46	0.41
72	390	4.49	11.51	21	70	0.47	0.44
90	410	4.63	11.29	24	56	0.52	0.50
108	405	4.78	11.80	26*	45	0.54	0.54
116	400	4.81	12.03	19	44	0.40	0.39

P. putida AI.=Resistant to UV radiation; Initial amount of glucose=4.83g%.
The results were significant at 5 %; *Rate of increase in L-Tryptophan biosynthesis 160%.

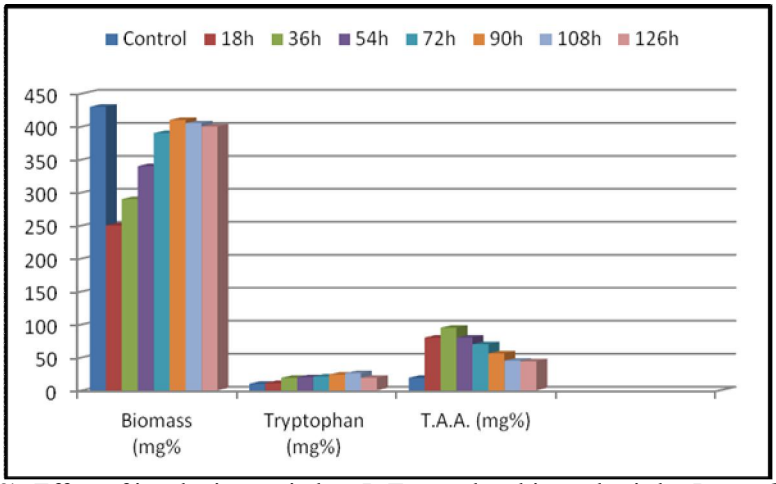

Fig.(2): Effect of incubation period on L-Tryptophan biosynthesis by *P. putida* AI.

The addition of 0.8mg% anthanillic acid in culture medium increased the ability of *P. putida* AI biosynthesis L-Tryptophan 8.9mg% compared with control, this result agreed with that found by arima, et al., (1972) (Table3) (Fig3).

Table(3):Effect of Presence of Anthranilic acid in inoculation medium on Tryptophan biosynthesis by *P. putida* AI.

Inoculum medium	Biomass (mg%)	Consumed sugar(g%)	Sugar cunsump/unit weight	L-Tryptophan (mg%)	T.A.A. (mg%)	Conversion (%)	Yield (%)
Control (No anthranillc acid)	190	4.01	21.11	2.2	30	0.06	0.05
0.8mg% anthranillc Acid	450	3.65	8.11	8.9*	98	0.24	0.18

P. putida AI.=Resistant to UV radiation; Initial amount of glucose=4.85g%
*Rate of increase in tryptophan biosynthesis 3.4.55%.

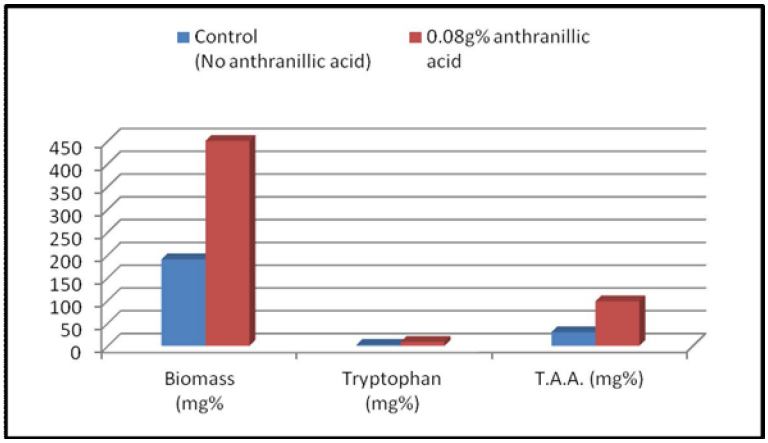

Fig.(3) Effect of presence of anthranillic acid in incoulum medium on Tryptophan biosynthesis by *P. putida* AI.

Daily after 18hr addition of anthranillic acid (50mg% dissolved in 96% ethanol) and 10mg% ammonium sulphate dissolved in distilled water resulted in significant increased amount of tryptophan biosynthesis when isolate *P. putida* AII was used. Further addition of anthranillic acid, tryptophan contents reached117.39mg% after 108hrs incubation when anthranillic acid content was 580mg% with 47.74%increase over the control(Table4), (Fig.4)**,** that can be explained by results obtained confirm that the 0.5 gm % of anthranillic acid was the inhibitory concentration of strain *P. putida* AII, this agreed with (Yamada *et al.,* 1972), also it's possible that the anthranillic acid works as an induction in the way of biosynthesis of tryptophan when anthranillic acid provide limited quantities prohibitive for growth (Ishida *et al.,* 1986 and Takamatsu *et al.,* 1986).

(Table 4.) Effect of daily addition of antheranillic acid + ammonium sulphate on the biosynthesis of Tryptophan by *P. putida* AII.

Fermentation period (hrs)	Amount of anthranillic acid (mg%)	Amount of sulphate (g%)	Biosmass (mg %)	Consumd sugar(g%)	Sugar cunsump/ unit weight	L-Tryptophan (mg %)	T.A.A. (mg %)	Conversion (%)	Yield (%
Control	80	1.20	485	4.55	9.38	80.00	430	1.76	1.70
18	330	1.25	340	4.58	13.47	82.61	470	1.80	1.76
36	380	1.26	315	4.58	14.54	86.95	395	1.90	1.85
54	430	1.27	305	4.59	15.05	95.65	435	2.08	2.04
72	480	1.28	285	4.60	16.14	100.00	475	2.17	2.13
90	530	1.29	265	4.60	17.36	104.34	485	2.27	2.22
108	580	1.30	245	4.61	18.82	117.39*	510	2.55	2.50
126	630	1.30	210	4.62	22.00	113.04	495	2.45	2.41

P. putida AII**.**=Resistant to UV radiation and 30mg% 5MT and 0.5g% anthranillic acid; Initial amount of glucose=4.70g%.
Initial amount of anthranillic=80mg%. Initial amount of ammonium sulphate=1.2 g%.

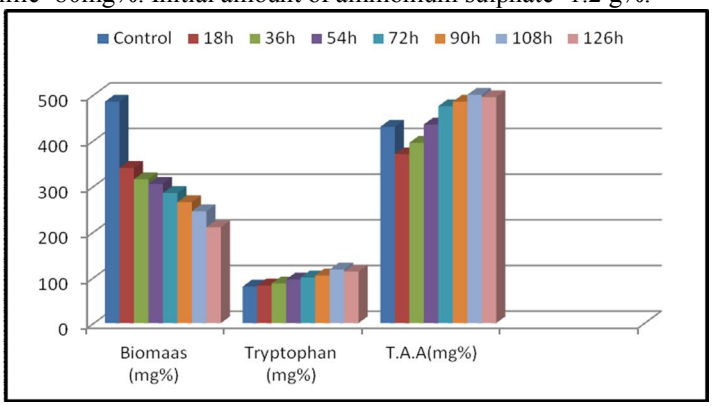

Fig.(4) Effect of daily addition of anthranillic acid + ammonium sulphate on L-Tryptophan biosynthesis by *P. putida* AII

Corresponding author
Nourah H.M Al-Zahrani
Department of Biology, Faculty of Science for Girls, King Abdulaziz University, Jeddah, Saudi Arabia
nhalzhrani@kau.edu.sa

References:-

Ajinomoto Co., Inc. (1980): L-Tryptophan by cultivation of *Brevibacterium flavum*. Jpn. Kokai Tokkyo Koho JP 80 162, 771, cited from Chem. Abst.; (1981)96,33388

Ajinomoto Co., Inc. (1984): L-Tryptophan by fermentation. Jpn.Kokai Tokkyo Koho JP 59, 120, 092 [84, 120, 092] (C1, C12, P13/22), cited from Chem. Abst.;(1984) 101, 149790

Anyanful A, Dolan-Livengood JM, Lewis T, Sheth S, Dezalia M N, Sherman M A, Kalman L V, Benian G M, and Kalman D. (2005): Paralysis and killing of *Caenorhabditis elegans* by enteropathogenic *Escherichia coli* requires the bacterial tryptophanase gene. Mol. Microbiol; 57:988-1007.

Arima, K; Nojami, I, and Yoneda, M.(1972): Fermentation production of L-Trepyophan from anthranillic acid.Japan. Kokai 72,29,584 (C1.36(2)D511.3), cited from Chem. Abst.;(1973) 79,70202b.

Bauer C, Schoonbroodt D, Wagner C and Horsmans Y. Liver abscesses due to *Fusobacterium* species. Liver 2000; 20:267-268

Berry A.(1996): Improving production of aromatic compounds in *Escherichia coli* by metabolic engineering. Trends Biotechnol; 14:250-256.

Bidisha S, Ipsit H., Trinetra M, Anindita R andSubhra K (2013): L-Tryptophan
production by a psychrophilic *Pseudomonas* sp. 023K:US Scholars Research Library, Der Pharmacia Lettre, 2013, 5 (5):35-42.

Bongaerts J, Kramer M, Muller U, Raeven L and Wubbolts M. (2001): Metabolic engineering for microbial production of aromatic amino acids and derived compounds. Metab Eng; 3:289-300.

Chant EL and Summers DK. (2007): Indole signaling contributes to the stable maintenance of *Escherichia coli* multicopy plasmids. Mol. Microbiol., 63:35-43.

Chaudhry R, Dhawan B, Laxmi BV and Mehta VS. (1998): The microbial spectrum of brain abscess with special reference to anaerobic bacteria. Br. J. Neurosurg, 12:127-130.

Cooper, G R and Mc Daniell, V (1970):Standard Methods of Clinical Chemistry, edited by R.P. Mac Donald, 6, 159, Academic Press, New York and London.

Fusho, Y; A Yamashita, A, and Murata, Y. (1986): Selection of microbial mutants producing Amino acids and other compounds. Jpn Kokai Tokkyo Koho JP 61,152, 277, 186,152, 2771.

Gollnick P, Babitzke P, Antson A and Yanofsky C.(1995): Complexity in Regulation of Tryptophan Biosynthesis in *Bacillus subtilis*. Annu Rev. Genet 2005; 39:47-68. Biochemistry, 59:1938-1943.

Gosset G. (2009): Production of aromatic compounds in bacteria. Curr Opin Biotechnol; 20:651-658.

Habbs, C H and McClellan, R O. (1975): In "Toxicology, the basic science of persons" 1st, edition, L.J. Cararett and J. Daull (editors), Macmillan Publishing Co., New York, P. 390.

Ishida, M; Miwa, K; Nakamori, S and Sano, K (1986): L-Tryptophan. Pat. APPL.EP 170,257 (C1, C12N.15/00), GR 84/161, 217. citedfrom Chem. Abst.; (1986) 104, 147154c.

Laddha G P, Vidyasagar G, Bavaskar SR, Baile SB, Suralkar SS, (2012) Serotonin: A Dive of Pleasure and Misery. Der Pharmacia Lettre 4 (2), 443-455.

Lawrance, C W. (1971):Cellular radiobiology, Williams and Sons, Ltd London, P122.

Le Floc'h, N and Bernard Seve, H(2007): Biological roles of tryptophan and its metabolism: Potential implications for pig feeding Ann Biol Res 2(4):460–468.

Mateos SS, Sánchez CL, Paredes SD, Barriga C and Rodríguez AB (2009):Circadian Levels Serotonin in Plasma and Brain after Oral Administration of Tryptophan in Rats. Basic Clin Pharmacol Toxico. 104 (1): 9-52.

Molnar, P I and Pinter, D. (1989): Spectrophotometric determination of tryptophan in intact proteins by the acid ninhydrin method. Anal. BioChem. 177. 16.

Mutting, D and Kaiser, E (1963): Determination of amino nitrogen. Hoppe. Seyler's Zeitschrift fur Physiologische Chemie 332,276.

Nester, E W; Schofer, M. and Lederberg, J.(1983): Gene linkage in DNA transfer: a cluster of genes concerned with aromatic biosynthesis in *Bacillus subtilis*. Genetics,48,529.

Nieder, M and Shapiro, J.(1975):Physiological function of the *Pseudomonas putida* PpG6 (*Pseudomonas olevorans*) alkane hydroxylase. Mono terminal oxidation of alkanes and fatty acids. J. Bacteriol. 122,93.

Ornston, L N; Ornston:, M K and George, C. (1969): Isolation of spontanous mutant strains of *Pseudomonas putida*. BioChem. Biophys. Res. Commun. 36(1),179.

Pasupuleti M, Chalupka A, Mörgelin M, Schmidtchen A and Malmsten M.(2009) Tryptophan End-Tagging of Antimicrobial Peptides for Increased Potency Against *Pseudomonas aeruginosa*. Biochim Biophys. Acta, 1790 (8): 800-8.

Rao, K K; Gupta, A. R. and Singh V. K. (1975): Mutations affecting tryptophan synthetase activity and ergot alkaloid synthesis in *Aspergillus fumigatus*. Proc. Symp. Mutagenicity, Carciong Teraogenicity Chem. 186-200. Bhabha At. Res. Cent.: BombayIndia.

Riemann D, Feige B, Hornyak M, Koch S, Hohagen F and Voderholzer U. The Tryptophan Depletion Test: Impact on Sleep in Primary Insomnia - A PilotStudy. Psychiatry Res 2002; 109 (2): 129-35.

Ruban, E L and Lobireva, L B. (1965):Tryptophan biosynthesis by microorganisms Izv. Akad. Nauk SSSR, Ser. Biol. 30(2),243.

Sahasrabudhe MM (2011) Screening of rhizobia for indole acetic acid production. Ann Biol Res 2(4):460–468

Shiio, I; Sugigmoto, S and Kawamura, K. (1983): L-Tryptophan produced by fermentation. Eur. Pat. Appl. EP.128,637.

Takamatsu, H.; Sakimoto, K.; Takimoto, E; Nakayama, A and Yajima,y. (1986):L-Tryptophan manufacture with transformed Bacillus sp. JPn. Kokai Tokkyo JP 61 85, [86,85,184].

Yamada,K; Kinoshita,S and Aida, K(1972): "The Microbial productio of amino acid "chapter20. Fermintative production of tryptophan from anthranillic acid.P518. Kodansho LTD, Tokyo, A halsted press book, John Willy and Sons.

4/18/2014