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Research Article

EVALUATION OF TYROSINASE PRODUCING INDIGENOUS BACTERIAL STRAINS WITH REFERENCE TO THEIR EFFICACY IN L-DOPA PRODUCTION

SHARMA ANJANA*[1], VYAS PRASHANSA[1] AND REHMAN MEENAL BUDHOLIA[2]
[1]Bacteriology Laboratory, Department of P. G. Studies and Research in Biological Science, Rani Durgavati University, Jabalpur, 482001, Madhya Pradesh, India
[2]Department of Botany, Mata Gujri Women's College, Jabalpur, Madhya Pradesh, 482001, India
*Corresponding Author: Email - anjoo1999@gmail.com

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Abstract- The present study is focussed on isolation and screening of tyrosinase enzyme produced by bacterial isolates and its application in the production of L-Dopa. L- Dopa is an amino acid which is used as a drug for the treatment of Parkinson's disease. 112 bacterial isolates have been screened which were collected from soil of different gardens of Jabalpur, Madhya Pradesh. Tyrosinase activity of all bacterial isolates was determined qualitatively and quantitatively. The primary screening of all the isolates for Tyrosinase production was carried on Tyrosine agar medium. Among them 21 isolates showed brown pigmented colonies indicating Tyrosinase activity. Secondary screening was done on the basis of extracellular enzyme activity. Based on the result of secondary screening, out of 21 isolates 5 were reported as potential Tyrosinase producers which were further checked for L-Dopa production. On the basis of morphological, cultural and biochemical characteristics the potent L-Dopa producing bacterial isolate was identified as *Pseudomanas sp* which could be a promising source for the production of L-Dopa.

Keywords- *Tyrosinase, L-Dopa, Drug, L- Tyrosine, Soil, identification*

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Introduction

L-Dopa (3,4 dihydroxyphenylalanine) is an amino acid which is used as a drug for the treatment of Parkinson's disease [1]. Parkinson's disease affects approximately 1.5 million people worldwide and the cost of the treatment is more than 14 billion a year according to research published in Movement disorder 2014 [2]. This disease is caused due to insufficiency of the neurotransmitter dopamine [3]. Mosanto first developed the asymmetric hydrogenation method for the synthesis of L-Dopa which showed many limitations such as poor conversion rate, low enantioselectivity and high production cost [4]. In contrast biotechnological approaches have been explored as potential alternative not only for improving the poor conversion rate but also for economizing the process. L- Dopa is biotechnologically synthesized from L- Tyrosine by one step reaction catalyzed by tyrosinase [5]. Tyrosinase (1.14.18.1) is a copper containing enzyme which is widely distributed in plants, animals and microorganisms. This enzyme has both monophenolase and diphenolase activity and responsible for the biosynthesis of melanin. It has been produced from various sources such as *Escherichia intermedia* [6], *Bacillus thuringiensis* [7], *Banana* [8], *Aspergillus oryzae* [9], *Portulaca grandiflora* [10], *Yarrowia lypolytica* [11], *Acremonium rutilum* [12], *Brevundimonas sp* [13] *and Bacillus sp JPJ* [1] *and Hypericum lariciforium juss* [14] Although, it has been observed that very few attempts have been made to produce L-Dopa using bacterial sources. Therefore, the present study is undertaken to isolate and explore L-Dopa producing bacteria present in the soil.

Materials and Methods

Chemicals

L-tyrosine, Tryptone, L-Dopa and all other chemicals were procured from Himedia, India. All chemicals were of the highest purity available and of analytical grade.

Isolation of bacterial isolates

Soil samples were collected from different gardens of Jabalpur (M.P) and all samples were enriched in Tyrosine broth medium containing (Yeast extract 0.3g/l, Peptone 0.5g/l, NaCl 0.5g/l and L-Tyrosine 1g/l) pH 7 at 37˚C and 120 rpm for 24 h. Thereafter enriched samples were serially diluted, plated and incubated in incubator at 37˚C for 24 hours. All tyrosinase producing isolates were maintained on nutrient agar slants and stored at 4˚C for further use.

Primary screening

Primary screening of all the bacterial isolates was carried on tyrosine agar medium containing (NaCl 5g/l, Beef Extract 3g/l, Peptone 5g/l, Agar 20g/l) pH 7.0 supplemented with 1g/l L- Tyrosine. All the isolates were streaked on to tyrosine agar medium and incubated in incubator at 37˚C for 24hours [1].

Secondary screening

Preparation of crude enzyme extract

24hour old culture was inoculated on to sterilized broth containing (Tryptone 4g/l, Beef extract 0.5g/l, and L-Tyrosine 1g/l) pH 7and incubated at 37˚C in orbital shaker incubator at 120 rpm for 24 hours. After the growth of 16-18 hour having absorbance 0.75 were harvested by centrifugation at 8000rpm for 15 minutes at 4˚C and the cell free supernatant was used further for Tyrosinase activity and Protein content.

Tyrosinase activity

Tyrosinase activity of all the selected isolates was done by following the method of [10].

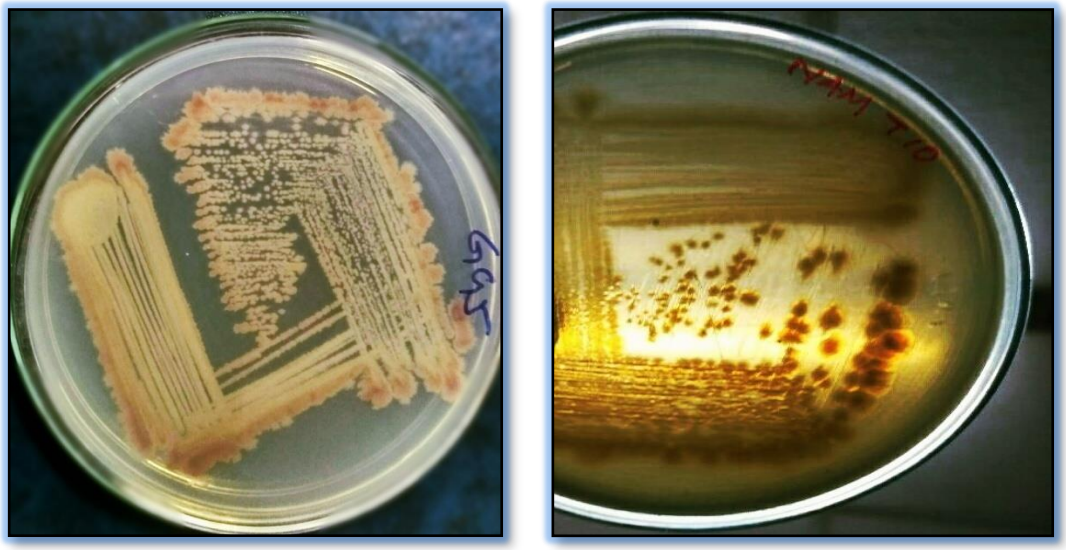

Fig-1 Primary screening of Tyrosinase producing Bacterial isolates

The final assay concentration in 3ml reaction mixture contained 1ml of 50mM Pottasium Phosphate buffer (pH 7.4), 1ml of L-tyrosine, 0 .8 ml of distilled water, 0.1 ml L-ascorbic acid and 0.1ml of culture supernatant of Tyrosinase positive isolates. The reaction mixture without culture supernatant was used as blank. Tyrosinase activity was estimated at A280 nm for 10 minutes using UV-Vis spectrophotometer. One unit of tyrosinase activity was equal to A280 nm of 0.001/min at pH 7.4 at 25°C in 3ml reaction mixture containing l-tyrosine. Protein content was estimated using Lowry's method [15]. The enzyme activity can be calculated by using following formula:

Units of enzyme/ml

$$\frac{\Delta A280\text{nm/min Test} - \Delta A280\text{nm/min Blank (df)}}{(0.001)\ \ (0.1)}$$

L-Dopa assay

The L-dopa assay was determined according to Arnow's method [16]. 1 ml of supernatant was added with 1ml of 0.5NHCL, 1ml of nitrite molybdate reagent and 1ml of 1N NAOH then final volume was adjusted to 5ml by distilled water. The absorbance was measured at 530 nm using UV- Vis spectrophotometer and concentration of L- Dopa was determined from Arnow standard curve of L- dopa. All the experiments were carried out in triplicates.

Y = 1.892x + 0.009/ R2=0.996

Identification of Bacterial Isolate

L-Dopa producing bacterial isolate was characterized on the basis of Morphological, Cultural and Biochemical following Bergeys Manual of Systematic Bacteriology [17] and Probabilistic Identification of Bacteria (PIB) [18] computer kit.

Results and Discussion

Soil is a source of indigenous bacteria therefore, in this study, for the isolation of tyrosinase producing bacterial isolates we have collected soil samples from different gardens of Jabalpur (M.P) and enriched in tyrosine broth for 24 h. Enriched samples were serially diluted and inoculated in a plate containing media, 112 bacterial isolates were isolated. These isolates were further purified in Nutrient agar medium. Thereafter, bacterial isolates were primarily screened for tyrosinase production by using tyrosine agar medium. Out of 112 bacterial isolates 21 isolates showed brown pigmentation indicating tyrosinase activity. Likewise, Raval, *et al*., also isolated 10 isolates of Actinomycetes and used tyrosine agar medium for primary screening of tyrosinase enzyme and also reported that Formation of brown color was mainly due to melanin [19]. After primary screening, these isolates were further checked for extracellular tyrosinase activity. Among 21, 5 isolates *i.e*., TG10, TG7, GG5, GG6, BP8 showed maximum extracellular tyrosinase activity as shown in [Table-2].

L-Dopa assay

Tyrosinase (1.14.18.1) is a copper containing enzyme and has two catalytic activities. It catalyzes orthohydroxylation of monophenols to diphenols by cresolase activity. It also successively oxidizes diphenols to quinone by catecholase activity [20]. Accordingly, based on tyrosinase activity L-Dopa can be produced by o- hydroxylation of L- Tyrosine by cresolase activity [21] therefore in the present investigation bacterial isolates which exhibited maximum tyrosinase activity were further assayed for L-Dopa production. Nitrite molybdate reagent when added to cell free supernatant gives the formation of yellow colour. Further addition of 1N NaOH resulted in the formation of red colour similar to the one formed at standard L-Dopa. Isolate TG10 produces 0.0385mg/ml, TG7 produces 0.0367 mg/ ml and GG5 produces 0.0372 mg/ml L-Dopa after 18 h of incubation as shown in [Table-3]. Previously, Surwase, *et al*., For the first time reported that the bacterial strain has the ability to utilize L-Tyrosine for producing L-Dopa. They isolated *Bacillus* sp. JPJ & *Brevundimonas* sp. SGJ having the ability to convert L-Tyrosine to L-Dopa from soil samples which can be more favorable for industrial fermentation than plant, fungi and yeast. From the above results, bacterial isolate which have the potential to produce l-Dopa was identified as *Pseudomonas* sp. on the basis of morphological and biochemical characteristics [Table-4].

Table-1 Qualitative Screening of Tyrosinase Producing Bacterial Isolates.

S.No	Code	Brown Pigmented Colonies
1	TG2	+
2	TG7	+
3	TG8	+
4	TG10	+
5	YM6	-
6	YM5	-
7	YM1	+
8	GG7	+
9	GG5	+
10	BP8	+
11	BP11	-
12	BP9	-
13	BP12	+
14	BP13	+
15	GG1	+
16	BP10	+
17	BP1	+
18	BP2	+
19	GG3	+
20	GG6	+
21	GG4	+

Table-2 Quantitative Screening of Tyrosinase Producing Bacterial Isolates

Code	Enzyme Activity (IU/ml)	Protein Content (mg/ml)	Specific Activity (IU/mg)
TG2	2.88	1.95	1.47
TG7	4.53	1.77	2.55
TG8	5.3	2.41	2.19
TG10	11.89	1.96	6.06
YM6	1.47	2.3	0.639
YM5	1.5	2.28	0.657
YM1	2.54	2.3	1.1
GG7	6.95	2.87	2.42
GG5	8.01	2.8	2.86
BP8	7.89	2.77	2.84
BP11	2.34	2.84	0.823
BP9	2.78	2.8	0.992
BP12	2.98	2.87	1.03
BP13	2.99	2.87	1.04
GG1	3.08	2.95	1.04
BP10	2.59	2.99	0.866
BP1	3.51	3.04	1.15
BP2	4.32	2.9	1.48
GG3	3.1	2.75	1.12
GG6	4.1	2.89	1.41
GG4	3.08	3.03	1.01

Table-3 Production of L-Dopa by maximum Tyrosinase producing Bacterial Isolates

S.No	Isolate Code	L-Dopa (mg/ml)
1	GG5	0.0372
2	GG6	0.0343
3	TG10	0.0385
4	TG7	0.0375
5	BP8	0.0252

Conclusion

The indigenous *Psuedomanas* sp. Isolated from soil was found to be a potent L-Dopa producer. For better production of L-Dopa, further work on the optimization, purification and characterization of the tyrosinase enzyme is in progress prior to scale up studies.

Application of research: L-Dopa is a compound of clinical importance, which is produced by the enzyme Tyrosinase. Further, study of an enzyme may enable in developing a simpler and feasible bacterial system for achieving higher yield of L-Dopa.

Research Category: Isolation and screening of tyrosinase enzyme

Abbreviations:

L-Dopa- 3,4 dihydroxyphenylalanine
NaOH- Sodium Hydroxide
NaCl- Sodium Chloride
HCL- Hydrochloric acid
df- dilution factor

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Table-4 Morphological and Biochemical features of potent L-DOPA producing bacteria isolated from garden soil.

Isolate	Morphology	Motility	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	Organism
TG10	*Gram negative bacilli*	+	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	*Psuedomonas* sp.

A-Indole, B-Methyl Red, C-Voges Proskauer, D-Citrate Utilization, E-Sucrose Fermentation, F-Lactose, G-Sucrose, H- Mannitol, I- Gas, J-Hydrogen Sulphide Production, K-Oxidase, L-Catalase, M-Starch Hydrolysis, N-Urease, O-Nitrate Reduction, P- Non Lactose Fermenters, Q-Lactose Fermenters, R-Ornithine Decarboxylse, S-Arginine Decarboxylase