SUPPLEMENTARY MATERIAL

Optimisation of nutritional requirements for dopamine synthesis by calcium alginate-entrapped mutant strain of *Aspergillus oryzae* EMS-6

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The optimization of nutritional requirements for dopamine (DA) synthesis by calcium alginate entrapped mutant variant of *Aspergillus oryzae* EMS-6 using submerged fermentation technique was investigated. A total of 13 strains were isolated from soil. Isolate I-2 was selected as a better producer of DA and improved by exposing with ethyl methylsulphonate (EMS). EMS-6 was selected as it exhibited 43 µg/ml DA activity. The mutant variable was further treated with low-levels of L-cysteine HCl to make it resistant against diversion and environmental stress. The conidiospores of mutant variant were entrapped in calcium alginate beads for stable product formation. EMS-6 gave maximum DA activity (124 µg/ml) when supplemented with 0.1% peptone and 0.2% sucrose, under optimized parameters viz. pH 3, temperature of 55°C, and incubation time of 70 min. The study involves the high profile of DA activity and is needed, as DA is capable to control numerous neurogenic disorders.

Keywords: *Aspergillus oryzae*; dopamine; submerge fermentation; calcium alginate

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Figure S2: Effect of concentration of peptone on DA activity by calcium alginate entrapped spores of EMS-6. L-tyrosine 2.5 mg/ml, L-ascorbic acid 5 mg/ml, acetate buffer (50 mM) pH 3.5, 50°C, 60 min, 120rpm, EMS conc. 2.5 mM, exposure time 5 min, L-cysteine HCl 0.2 µM, alginate 3%. Y-bars represent standard deviation ($\pm$ sd) among parallel replicates. The values given in each set vary significantly at $p \leq 0.05$.

Figure S3: Effect of concentration of sucrose on DA activity by calcium alginate entrapped spores of EMS-6. L-tyrosine 2.5 mg/ml, L-ascorbic acid 5 mg/ml, acetate buffer (50 mM) pH 3.5, 50°C, 60 min, 120rpm, EMS conc. 2.5 mM, exposure time 5 min, L-cysteine HCl 0.2 µM, alginate 3%, peptone 0.1%. Y-bars represent standard deviation ($\pm$ sd) among parallel replicates. The values in each set differ significantly at $p \leq 0.05$.

Figure S4: Effect of pH of buffer on DA activity by calcium alginate entrapped spores of EMS-6. L-tyrosine 2.5 mg/ml, L-ascorbic acid 5 mg/ml, 50°C, 60 min, 120rpm, EMS conc. 2.5 mM, exposure time 5 min, L-cysteine HCl 0.2 µM, alginate 3%, peptone 0.1%, sucrose 0.2%. Y-bars represent standard deviation ($\pm$ sd) among parallel replicates. The values given in each set vary significantly at $p \leq 0.05$.

Figure S5: Effect of temperature of the reaction procedure on DA activity by calcium alginate entrapped spores of EMS-6. L-tyrosine 2.5 mg/ml, L-ascorbic acid 5 mg/ml, acetate buffer (50 mM) pH 3, 60 min, 120rpm, EMS conc. 2.5 mM, exposure time 5 min, L-cysteine HCl 0.2 µM, alginate 3 %, peptone 0.1%, sucrose 0.2%. Y-bars represent standard deviation ($\pm$ sd) among parallel replicates. The values given in each set vary significantly at $p \leq 0.05$.

Figure S6: Effect of incubation time of the reaction procedure on DA activity by calcium alginate entrapped spores of EMS-6. L-tyrosine 2.5 mg/ml, L-ascorbic acid 5 mg/ml, acetate buffer (50 mM) pH 3, 55°C, 120rpm, EMS conc. 2.5 mM, exposure time 5 min, L-cysteine HCl 0.2 µM, alginate 3 %, peptone 0.1%, sucrose 0.2%. Y-bars represent standard deviation ($\pm$ sd) among parallel replicates. The values given in each set vary significantly at $p \leq 0.05$.

<i>A. oryzae</i>	Enzyme activity (U/mg)	Protein content (mg/ml)	DA		Mycelial morphology
			DA activity (µg/ml)	Yield (%)	
Fungal isolates					
I-1	13±0.65	60±3	11±0.55	0.44	Viscous
I-2	28±1.40	112±5.6	21±1.05	0.84	Small round pellets
I-3	21±1.05	91±4.55	10±0.5	0.4	Large pellets
I-4	8±0.4	35±1.75	6±0.3	0.24	Dumpy mass
I-5	12±0.6	58±2.90	15±0.75	0.6	Intermediate pellets
I-6	16±0.8	87±4.35	2±0.1	0.08	Dumpy mass
I-7	5±0.25	22±1.10	13±0.65	0.52	Large pellets
I-8	11±0.55	46±2.30	8±0.4	0.32	Broken mycelia
EMS treated variants					
EMS-1	24±1.20	109±5.45	22±1.1	0.88	Gelatinous
EMS-2	17±0.85	78±3.70	20±1	0.8	Fine pellets
EMS-3	21±1.05	95±4.75	30±1.5	1.2	Fine pellets
EMS-4	14±0.7	82±4.10	29±1.45	1.16	Intermediate pellets
EMS-5	29±1.45	102±5.10	38±1.9	1.52	Elongated mycelia
EMS-6	41±2.05	154±7.70	43±2.15	1.72	Small round pellets
EMS-7	12±0.6	61±3.05	36±1.8	1.44	Dumpy mass

Table S1: Biotransformation of L-tyrosine to DA using dry mycelia of isolates of *A. oryzae* and EMS treated variants. L-tyrosine 2.5 mg/ml, L-ascorbic acid 5 mg/ml, 50 mM acetate buffer (pH 3.5), dry mycelia 7.5 mg/ml, 50°C, 60 min, 120 rpm, EMS conc. 2.5 mM, exposure time 5 min. $\pm$ indicate standard deviation ($\pm$ sd) among the three parallel replicates.

Nutritional requirements	Enzyme activity (U/mg)	Protein content (mg/ml)	DA	
			DA activity(μg/ml)	Yield (%)
Nitrogen sources*				
Peptone	79±3.95	177±8.85	76±3.80	3.04
Urea	29±1.45	125±6.25	34±1.70	1.36
Ammonium nitrate	59±2.95	160±8	69±3.45	2.76
Ammoniumchloride	38±1.90	129±6.50	45±2.2.5	1.8
Sodium nitrite	45±2.25	136±6.8	48±2.40	1.92
Sodium nitrate	50±2.50	151±7.55	58±2.90	2.32
Carbon sources*				
Glucose	45±2.25	140±7	51±2.55	2.04
Fructose	62±3.1	155±7.75	66±3.3	2.6
Sucrose	79±3.95	195±9.75	84±4.2	3.3
Maltose	56±2.80	148±7.4	60±3	2.4
Starch	24±1.20	104±5.2	25±1.25	1
Cellulose	70±3.5	162±8.1	68±3.4	2.72

Table S2: Effect of additional nitrogen and carbon sources on DA activity by calcium alginate entrapped spores EMS-6. L-tyrosine 2.5 mg/ml, L-ascorbic acid 5 mg/ml, 50 mM acetate buffer (pH 3.5), 50°C, 60 min, 120 rpm, EMS conc. 2.5 mM, exposure time 5 min, L-cysteine HCl 0.2 μ M, alginate 3 %.*0.1 % for all bacterial cultures. $\pm$ indicate standard deviation ($\pm$ sd) among the three parallel replicates.