Optimisation of nutritional requirements for dopamine synthesis by calcium alginate-entrapped mutant strain of \textit{Aspergillus oryzae} EMS-6

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The optimization of nutritional requirements for dopamine (DA) synthesis by calcium alginate entrapped mutant variant of \textit{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 variante 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: \textit{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 (± sd) among parallel replicates. The values given in each set vary significantly at p≤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, peptone 0.1%. Y-bars represent standard deviation (± sd) among parallel replicates. The values in each set differ significantly at p≤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 (± sd) among parallel replicates. The values given in each set vary significantly at p≤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, 120 rpm, 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 (± sd) among parallel replicates. The values given in each set vary significantly at p≤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 (± sd) among parallel replicates. The values given in each set vary significantly at p≤0.05.
| Fungal isolates | Enzyme activity (U/mg) | Protein content (mg/ml) | DA activity (µg/ml) | Yield (%) | Mycelial morphology |
|-----------------|------------------------|-------------------------|---------------------|-----------|---------------------|
| 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 | Enzyme activity (U/mg) | Protein content (mg/ml) | DA activity (µg/ml) | Yield (%) | Mycelial morphology |
|----------------------|------------------------|-------------------------|---------------------|-----------|---------------------|
| 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. ± indicate standard deviation (± sd) among the three parallel replicates.
| Nutritional requirements | Enzyme activity (U/mg) | Protein content (mg/ml) | 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 µM, alginate 3%.*0.1% for all bacterial cultures. ± indicate standard deviation (± sd) among the three parallel replicates.