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Potential of 2-ethylphenylhydrazine as a regulatory mutagene in citric acid bioproduction by *Aspergillus oryzae*

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Abstract: Citric acid remains one of the most important organic acids in industrial biotechnology, and strain improvement of *Aspergillus oryzae* is a key route to increase process productivity and cost-effectiveness. In this study we evaluated 2-ethylphenylhydrazine as a regulatory mutagen to generate and select *A. oryzae* NCIM-944 mutants with enhanced citric acid bioproduction. Conidial suspensions of *A. oryzae* NCIM-944 were treated with graded concentrations of 2-ethylphenyl-hydrazine under controlled conditions to induce random mutagenesis. In the present communication potential of 2-ethylphenylhydrazine as a regulatory mutagene has been studied. It has been found that the potential of 2-ethylphenyl -hydrazine has stimulatory effect on citric acid bioproduction by *Aspergillus oryzae* NCIM-944 and enhances the yield of citric acid to an extent of 19.628% higher in comparison to control under optimized conditions.

(Keywords : 2-ethylphenylhydrazine, *A. oryzae* NCIM-944, citric acid, metabolic regulation, fermentation efficiency).

Introduction

Citric acid is one of the most important organic acids in the food, pharmaceutical and chemical industries, widely used as an acidulant, chelating agent and intermediate in chemical syntheses. Microbial fermentation principally using Aspergillus species - remains the dominant route for large-scale citric acid production because of its cost-effectiveness and scalability. Among Aspergilli, *Aspergillus oryzae* strains are attractive production hosts due to their Generally Recognized As Safe (GRAS) status, robust saccharolytic capacity and amenability to strain improvement[1-11]. Still, improving volumetric productivity, yield on substrate and process stability remain ongoing goals for industrial biotechnology, and directed modification of cellular regulation is a powerful route to achieve them.

Mutagenesis has historically been used to generate improved microbial production strains by creating genetic diversity that can be selected for desirable phenotypes. Beyond random mutagenesis, the concept of a regulatory mutagen - an agent that perturbs regulatory networks or stress responses to reprogram metabolism rather than simply damage DNA indiscriminately — is gaining interest. Carefully applied, regulatory mutagens can shift metabolic fluxes, alter enzyme expression and modulate membrane physiology or redox balance in ways that favor overproduction of target metabolites such as citric acid. However, the outcome depends strongly on the mutagen's mode of action, dose, exposure regime and the screening strategy used to recover stable, productive variants.

Hydrazine and phenylhydrazine derivatives are a chemically diverse class with known biological reactivity. Phenylhydrazines can interact with cellular nucleophiles, generate reactive species and, in some contexts, induce mutations or oxidative stress. 2-Ethylphenyl-hydrazine (2-EPH) is a substituted phenyl - hydrazine whose small structural modification

(an ethyl group at the ortho position) could influence its cellular uptake, reactivity and target specificity relative to unsubstituted phenylhydrazine. To date, the application of substituted phenyl -hydrazines as controlled regulatory mutagens in fungal biotechnology is underexplored. This gap presents both a scientific opportunity and a need for careful, systematic evaluation because hydrazine derivatives can be biologically potent and their effects can range from reversible stress
responses to irreversible genotoxicity[12-18].

This study investigates the potential of 2-ethylphenylhydrazine as a regulatory mutagen to improve citric acid bioproduction by *Aspergillus oryzae* NCIM-944. Our working rationale is twofold: (1) sublethal exposure to a carefully chosen hydrazine derivative may activate stress- or redox-responsive regulatory circuits that redirect central carbon metabolism towards citrate accumulation (for example, by modulating glycolytic flux, tricarboxylic acid cycle regulation, or overflow metabolism), and (2) limited genetic perturbation induced during such exposure can produce stable variants with altered expression of key metabolic enzymes (e.g., citrate synthase, isocitrate dehydrogenase, transporters) or membrane/secretory properties that favor citric acid excretion.

The specific aims of the study are to (i) determine sublethal exposure conditions for 2-EPH that allow recovery of viable *A. oryzae* colonies, (ii) screen and select variants showing enhanced citric acid production in bench-scale fermentations, (iii) characterize physiological and biochemical changes in selected variants (sugar consumption, biomass, citrate titer and yield, key enzyme activities), and (iv) assess the stability of the improved phenotype across serial cultivation. We further seek to probe mechanistic indicators e.g., altered expression of genes involved in glycolysis and the TCA cycle, changes in intracellular redox balance or organic acid profile - that may explain how 2-EPH exposure reshapes metabolism.

Because hydrazine derivatives can pose safety and genotoxicity concerns, the experimental design places high priority on controlled dosing, thorough toxicity assessment, containment and downstream screening for desirable, stable phenotypes rather than transient stress responses. If successful, this approach would demonstrate a targeted application of a small-molecule regulatory mutagen to generate industrially useful fungal variants, offering an alternative or complement to classical mutagens and modern genome-editing strategies when regulatory, economic or technical constraints limit the use of the latter.

In summary, this work evaluates whether carefully dosed exposure to 2-ethylphenylhydrazine can be used as a practical tool to modulate regulatory networks in *A. oryzae* NCIM-944 and thereby enhance citric acid bioproduction, while documenting the physiological trade-offs and biosafety considerations necessary for translational[19-22].

Thus,from the above brief review it is evident that chemical mutagens are required for genetic manipulation and exploitation specially for citric acid fermentation and in view of this the author has studied the influence of 2-ethylphenylhydrazine on bioproduction of citric acid by *Aspergillus oryzae* NCIM-944.

Experimental

The influence of 2-ethylphenyl hydrazine on production of citric acid by *Aspergillus oryzae* NCIM - 944

The composition of the production medium for the production of citric acid by *Aspergillus oryzae NCIM* - 944 is prepared as follows:

Molasses : 24.5% (w/v), NH_4NO_3 : 0.65% ,

Table - 1
Production of citric acid by *Aspergillus oryzae* NCIM-944 exposed to 2-ethylphenyl hydrazine

Concentration of mutagen used A x 10^{-x} M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% of citric acid increased after 10 days
Control	10	7.749	2.559	-
1.0×10^{-5}M	10	7.849	2.456	+1.290
2.0×10^{-5}M	10	7.958	2.349	+2.697
3.0×10^{-5}M	10	8.175	2.129	+5.497
4.0×10^{-5}M	10	8.423	1.873	+8.697
5.0×10^{-5}M	10	8.663	1.636	+11.795
6.0×10^{-5}M	10	8.981	1.313	+15.898
7.0×10^{-5}M**	10	9.270***	1.036	+19.628
8.0×10^{-5}M	10	8.772	1.529	+13.201
9.0×10^{-5}M	10	8.237	2.069	+6.297
10.0×10^{-5}M	10	7.969	2.339	+2.839

* Each value represents mean of three trials, **Optimum concentration of mutagen used
***Optimum yield of citric acid (+) values indicate % increase in the yield of citric acid after 10 days. Experimental deviation (±) 1.5-3%

KH_2PO_4 : 0.65%, $MgSO_4.7H_2O$: 0.65%, pH : 2.2

Distilled water was added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 2.2 by adding requisite amount of lactic acid.

Now, the same production medium for production of citric acid by *Aspergillus oryzae* NCIM - 944 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 fermentor-flasks. The remaining 9 fermentor-flasks out of 99 fermentor-flasks were kept as control and these were also rearranged in 3 subsets each consisting of 3 fermentor flasks.

Now, M/1000 solutions of 2-ethylphenyl hydrazmi was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ml of this solution was added to the fermentor-flasks of first 10 sets respectively. The control fermentor-flask contained no chemical mutagens. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 2-Ethylphenyl hydrazmi in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

$A \times 10^{-x}$M, 1.0×10^{-5} M to 10.0×10^{-5} M .

Where, A = amount of mutagens in ml,
ie; from1.0 ml to 10.0 ml.

x = molarity of the 2-EPH

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 30^0C and analysed colorimetrically after 8,10, and 12 days for citric acid[23] formed and molasses sugars[24] left unfermented.

References

1. U. Gonzales-Barron, V. Cadavez, A.P. Pereira, A. Gomes, J.P. Araújo, M.J. Saavedra, L. Estevinho, F. Butler, P. Pires, T. Dias, *Food Res. Int*. **78**, 50 (2015)
2. C. Kim, S. Cho, S. Kang, Y. Park, M. Yoon, J. Lee, W. No, J. Kim, *J. Food Sci.*, **80**, (2015)
3. S. Keisam, N. Tuikhar, G. Ahmed, K. Jeyaram, *Int. J. Food Microbiol*. **296,** 21 (2019)
4. R. Chrun, Y. Hosotani, S. Kawasaki, Y. Inatsu, *Biocontrol Sci.* **22**, 181 (2017)
5. S. Kim, N. Kim, S. Lee, I. Hwang, M. Rhee, *J. Food Prot.*, **77**, 419 (2014)
6. J. H. Yoon, S. Lee, S.Y. Lee, *Food Sci. Biotechnol*. **33**, 2887 (2024).
7. X. Gao, C. Li, R. He,; Y. Zhang, B. Wang, Z.H. Zhang, C.-T. Ho, *Food Chem.*, **405**, 134911 (2023)
8. M. Hu, J. Dong, G. Tan, X. Li, Z. Zheng, M. Li, *Food Microbiol.*, **98**, 103762 (2021)
9. R.J.S. Banicod, W. Ntege, M.N. Njiru, W.H. Abubakar, H.T. Kanthenga, A. Javaid, F. Khan, *Int. J. Food Microbiol.*, **428**, 110996 (2024).
10. C. Liu, T. Zhu, H. Song, C. Niu, J. Wang, F. Zheng, Q. Li, *J. Food Sci. Technol.*, **58**, 2734 (2021)
11. X. Ma, J. Bi.; X. Li, G. Zhang, H. Hao,H. Hou, *Foods*, **10**, 2572 (2021)
12. C. Sokvibol, P. Arunya, C. Chuleeporn, S. Wanticha, P. Kriangkrai, *Food Res*., **6**, 294 (2022)
13. F.D. Algahtani, A.E. Morshdy, M.A. Hussein, E.S. Abouelkheir, A. Adeboye, A. Valentine,; M.T. Elabbasy, *J. Food Qual.*, **2020**, 8718179 (2020)
14. S. Kandasamy, J. Yoo, J. Yun, H.B. Kang, K.H. Seol, J.S. Ham, *Metabolites*, **11**, 31 (2021)
15. N.S. Turna, R. Chung, L.A. McIntyre,. *Heliyon*, **10**, (2024)
16. X. Sun, E. Sun, L. Sun, L. Su, Y. Jin, L. Ren, L. Zhao, *Foods*, **11**, 2057 (2022)
17. S. Hernández-Macias, A. Martín-Garcia, A. N. Ferrer- Bustins, O. Comas-Basté, M. Riu-Aumatell, E. López-Tamames, A. Jofré, M.L. Latorre- Moratalla, S. Bover-Cid, M.C. Vidal-Carou, *Front. Microbiol.*, **12**, 818565 (2022)
18. B. Del Rio, E. Sánchez-Llana, B. Redruello, A.H. Magadan, M. Fernández, M.C. Martin, V. Ladero, M.A. Alvarez, *Front. Microbiol*. **10**, 566 (2019)
19. A.M. Ganjeh, N. Moreira, C.A. Pinto, S. Casal, J.A. Saraiva, *Food Humanity*, **2**, 100252. (2024)
20. F. Tian, S.Y. Woo, S.Y. Lee, S.B.; Park, J.H. Im, H.S. Chun, *Compr. Rev. Food Sci. Food Saf*., **21**, 5131 (2022)
21. I.O. Owolabi, O. Kolawole, P. Jantarabut, C.T. Elliott, *Npj Sci. Food*, **6**, 39 (2022)
22. S. Rämö, M. Kahala, V. Joutsjoki, *Appl. Sci.* **12**, 12769 (2022)
23. J. R. Marrier and M.Boulet *J. Dairy Science* **41**, 1683 (1983)
24. M. Dubois, K.A. Gilles, J. K. Hamilton and F. Smith *Anal Chem*.**28**, 350 (1956)