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Vanillin: A Wonder Molecule with Diverse Application and Alternative Manufacturing Strategies

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ABSTRACT

Vanillin, an aromatic aldol compound and an extensively used flavouring agent that has been used throughout the world for decades in various sectors, primarily food industries. Lately, its use had also been explored in other sectors. The natural source of Vanillin or flavour Vanilla (whose major constituent is Vanillin) is the cured pod of the Orchid plant of genus *Vanilla*. However, limited yield, environmental impact, and pest infestation necessitate the need to explore other alternative sources. There are two other approaches used to produce Vanillin, the major constituent of flavour Vanilla, viz. chemical or synthetic route, and microbial biotransformation. The synthetic route is used to produce Vanillin in a cost-effective manner but is associated with the health impact on prolong use by humans. Recent advancements are focused on exploring the use of the biotechnological route for the production of Vanillin. One of the well-explored biotechnological routes is via biotransformation using microbes. Researchers have also explored the production of Vanillin from compounds commonly eugenol, isoeugenol, and ferulic acid, via biotransformation, using microbial enzymes. Research is underway to explore novel biotransformation agents and optimization of strategies to develop a sustainable economic Vanillin production strategy. Moreover, the researchers have expended the horizon of the application of Vanillin beyond flavour, by exploring photophysical properties and as protective agents against radiation-induced injury. The article highlights the recent advancement in Vanillin production strategies and its application in different sectors.

INTRODUCTION

The global impetus toward acceptance of natural products had shown a rapid surge in the demand of natural flavours and fragrances in the last two decades, with Vanilla being the second most demanded flavour, after saffron (Banerjee and Chattopadhyay, 2019). The major constituent of the flavor Vanilla is Vanillin is an aldol compound chemically characterized as '4-hydroxy-3methoxybenzaldehyde', a white crystalline powder used for its flavour and aroma. It is due to these properties that Vanillin is often interchangeably used with Vanilla, being the major constituent of it and responsible for flavour and aroma (Hoffman and Zapf, 2019). The application of Vanillin is not only limited to aroma and flavour but has been reported as a bio-preservative, antimutagenic agent, raw material for pharmaceutical drugs including dopamine and L-Dopa that are relevant in regulating neurological disorder (Kaur and Chakraborty, 2013). Recent studies have also reported the application of vanillin derivatives in different sectors. Wu et al. (2020) reported enhanced antibacterial properties of Vanillin derivatives containing a 1,3,4-thiadiazole, against some plant pathogens (Wu et al, 2020). Tumer et al. (2020) explored the redox and photophysical properties of vanillin derivative. On the contrary, Li et al. (2020) reported the role of Vanillin derivative VND3207, in protecting against radiation-induced intestinal epithelial cells injury.

The natural source of Vanillin is the plant of genus *Vanilla* sp. Studies have shown the existence of around 110 species of genus *Vanilla* (Family: *Orchidaceae*). Out of these, three species of Vanilla, most commonly used for extraction of Vanillin, are *Vanilla planifolia*, *Vanilla tahitensis*, and *Vanilla pompona*, and the aldol compound is extracted from the cured pods from these species (Khoyratty et al, 2018; Soto-Arenas and Cribb, 2010). One of the major concerns with the production and extraction of Vanillin from plant sources is low yield and slow plant growth. Other factors that had affected the yield of Vanillin, in producer countries like Mexico, are drought, high temperatures during fruiting seasons, fungal infestation and high production cost than market value (Hernandez-Hernandez, 2019).

Researchers have explored tissue culture mediated Vanillin production as an alternative to conventional field cultivation but the strategy had the limitation of comparatively slow plant growth. Moreover, Vanillin biosynthetic pathway is not very actively expressed under tissue culture conditions (Rao and Ravishankar, 2000).

Such issues are trivial and necessitate the need to explore alternative sources for the production of Vanillin. Other sources for the production include the use of synthetic route and biotechnological means. Artificial Vanilla, produced via synthetic route dominated the market due to its low cost but may cause adverse effects after prolonged consumption due to other by-products. The process is also not environmentally friendly. Microorganisms that produce Vanilla through biotransformation from natural raw materials or substrates can be employed (Gallage and Møller, 2015). The studies are still underway to explore a suitable microbial system, native or recombinant, for higher yield and process optimization.

DEMOGRAPHIC DISTRIBUTION OF NATURAL VANILLIN

Natural Vanillin is majorly extracted from pods of *Vanilla planifolia* and *Vanilla tahitensis* using different extraction and refining processes which make it a costly process (Hernandez-Hernandez, 2019). As per the FAOSTAT (2018), the major global share of natural Vanillin is produced in Madagascar (3102 tons) followed by Indonesia (2259 tons), Mexico (495 tons), Papua New Guinea (493 tons) and China (459 tons) (Figure No. 1 and 2). The total amount of natural Vanillin is 7.94 kilotons globally with the cost of $951.45 Million (Food and Agriculture Organization, 2020). Due to less production and high cost, Vanillin from the plant is often not the preferred choice among manufacturers.

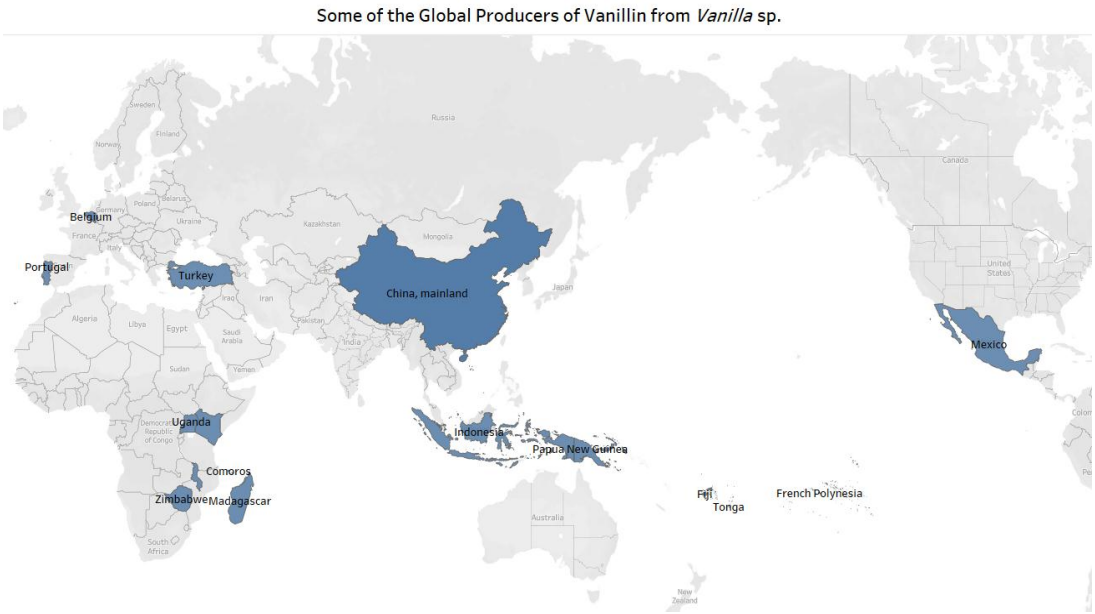

Figure No. 1: Major global producers of Vanillin (Data retrieved from Food and Agriculture Organization of United Nation (Food and Agriculture Organization, 2020,) represented using software tool Tableau)

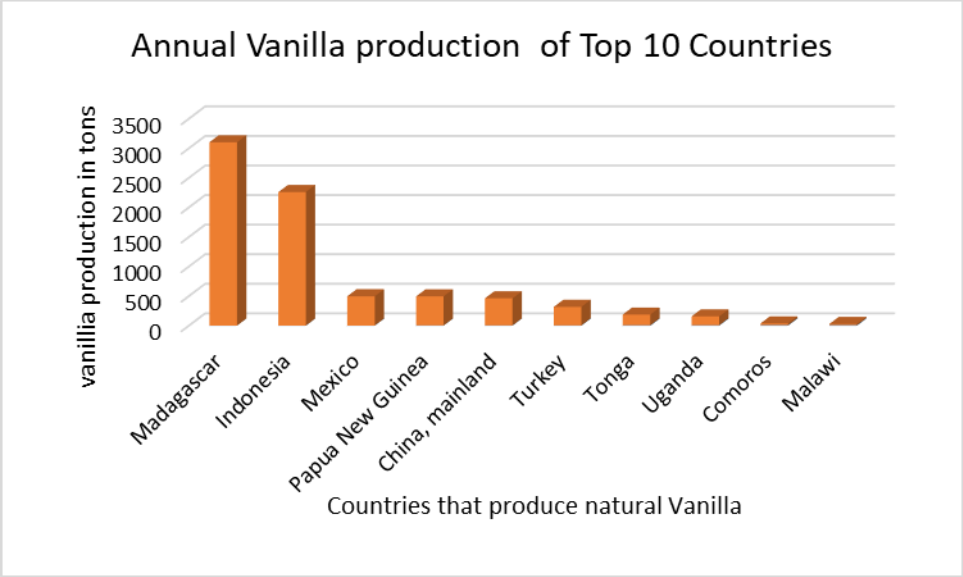

Figure No. 2: Top 10 Countries that produce natural Vanilla (graph plotted based on data for the year 2018, Food and Agriculture Organization, 2020)

BIO-VANILLIN PRODUCTION FROM MICROBES

Since natural Vanillin is not able to fulfil the market demand and artificial Vanillin has its own drawbacks, researchers in the quest to explore alternative Vanillin producers identified and explored different microbial systems as suitable alternatives to plant systems. Bacteria like *Bacillus* sp. and *Pseudomonas* sp. and fungi like *Saccharomyces* have been reported for their ability to convert substrate like eugenol, isoeugenol, ferulic acid and glucose into vanillin by biotransformation (Gallage and Moller, 2015). Vanillin produced using microbes is very much similar to natural Vanillin and thus it is also a safe alternative to plant derived counterpart (Kaur and Chakraborty, 2013). Several endogenous enzymes are employed in this biotransformation. Studies have highlighted the role of *β-glucosidase* in *E.coli* as the enzyme involved in the biotransformation of Glucovanillin into Vanillin (Snell et al, 1996). Microbes can be easily grown and can produce a higher yield of Vanillin in comparatively less time due to a higher growth rate (Table No. 1). Various molecular tools are recently used to enhance the yield and further optimization and yield improvement are in progress.

Table No. 1: **Potential microbial strains used for the production of Vanillin by biotransformation**

Substrate	Microorganism	Yield	Reference
Ferulic acid	*Pseudomonas putida*	>10 g/L	Plaggenborg et al., 2003
Eugenol	*Pseudomonas* sp. HR199	2.6 g/L	Overhage et al., 1999
Isoeugenol	*Bacillus subtilis*	1.36 g/L	Zhang et al., 2006
Glucose	Recombinant *S. cerevisiae*	>0.5 g/L	Hansen et al., 2009
Ferulic Acid	*Bacillus subtilis*	0.64 g/L	Yan et al., 2016
Ferulic Acid	Recombinant *E.coli* FR13	28.1 mM	Luziatelli et al., 2019

The existing studies have indicated the advancement in the research toward exploration of alternative strategies and host for the production of natural or bio-vanillin from alternative sources for developing a sustainable strategy for the production of the wonder molecule Vanillin in a cost-effective way. The approach is not only limited to exploring new organisms but also optimizing bioprocess conditions for improving microbial growth and Vanillin yield (Yan et al., 2016). Recent studies have also suggested alternative cost-effective methods of Vanillin production using recombinant, plasmid-free system (Luziatelli et al., 2019). It will be important to observe the scalable transitions of these technological advancements to meet the growing need.

DISCUSSION AND CONCLUSION

Vanillin, the major constituent of Vanilla flavour is a white crystalline aldol organic compound, well known for its use in flavour and aroma. Apart from this, Vanillin and its derivatives have also reported for their use as a precursor for therapeutic agents, antimicrobial agents, bio-preservative, antimutagenic agents and as a protective agent against radiation. With the plethora of applications, the conventional way of harvesting Vanillin is from the pod of the orchid plant of genus *Vanilla* sp. However, low yield, poor or slow plant growth, damage due to environmental changes, and infestation by pathogen necessitate the need to explore alternatives. Microbes obtrude as a suitable alternative that biotransforms various precursors such as eugenol, isoeugenol, and ferulic acid into *V*anillin. Alternatively, synthetic Vanillin production is also practiced commercially. However, artificial Vanilla causes several health issues. But due to the low cost, the consumption of artificial Vanilla is at its peak. And that is the only reasonable option for fulfilling the market demand. Current research in

progress is focused on exploring various microbes as alternative bio-vanillin producers. However, the exploration of novel strain or their recombinant derivatives also demands the development of robust optimized strategies for vanillin production. Studies underway are exploring cheaper raw material for bio-vanillin production using microbial systems. There is a need to explore a potential strain and optimize cultivation strategy for improving yield and minimizing production cost concomitant with reduced time and higher productivity.

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