1. INTRODUCTION

It has been customary for many years to dye human hair to conceal the grey. In Conventional hair dyeing process, there are three components

- Ammonia; for maintaining alkaline pH (França et al., 2015). It is known that pH plays significant role in the process of hair dyeing. Under acidic and neutral pH conditions, hair apparently gets colored but easily decolorizes on washing. Whereas at alkaline pH hair swells which enhances the penetration of dyes hence sufficient color fastness is obtained (Cheng et al., 2013).
- Oxidizing agent; in the developer for the oxidation of dye precursors, H_2O_2 is the most commonly used oxidizing agent (Takada et al., 2003).
- Dye precursors; like p-diamines and p-aminophenols, which on mixing with oxidizing agent generate color (Nohynek et al., 2004). The most widely used dye precursor is p-phenylenediamine (PPD) (Wall, 1972).

The harmful effects of chemical based hair dyeing process are associated with all of these components:

- Ammonia is harmful for hair and it can cause some allergic reactions to skin (Fujita et al., 2010)
- Chemical based dyes such as PPD apart from affecting skin and hair, they are also known to be carcinogenic (Prabhakaran, 2012)
- Health concerns associated with the use of H_2O_2 have also been widely recognized. As oxidation power of H_2O_2 is very strong it causes severe damage to hair and skin (Takada et al., 2003)

Limitation of ammonia in hair dyeing has been widely discussed and some alternative have been explored. A number of ammonia free formulations are now available in the market (França et al., 2015). Although, equally important, but the concerns associated with chemical based dye and developer have been ignored. Therefore, alternative processes need to be developed in this regard enzymes are employed in many industries to replace chemical based processes (Choi et al., 2015). In many industrial processes oxidation reactions are essential. Laccase/s are one of the oldest enzymes which can be applied for wide range of processes requiring oxido-reductase reactions (Brijwani et al., 2010; Monssef et al., 2016). The origin of present study was to develop a chemical free, user friendly hair dyeing process using laccase.

Laccases (benzendiol oxygen oxidoreductases, EC 1.10.3.2) are polyphenol oxidases which are capable of oxidizing wide range of phenolic and non phenolic substrates (Buddolla et al., 2013; Sondhi et al., 2015). Laccase/s are ubiquitous in nature, they are found in plants, insects, archea, bacteria, and fungi (Couto and Herrera, 2006a; Chauhan et al., 2017). First report of laccase obtained was from the sap of Japanese lacquer tree, *Rhus vernicifera,* (Yoshida, 1883). Laccases are widely distributed in various fungal species (Yang et al., 2017; Agrawal et al., 2018). Mostly white rot fungal species have been reported to produce laccase/s (Hatakka, 1994); basidiomycetes, is the most widely known laccase producing fungi. A number of bacterial laccases have also been reported since last decade (Chauhan et al., 2017), but most of the bacterial laccases have been reported to be intracellular (Galai et al., 2009; Zheng et al., 2013). There are very few reports which have reported extracellular laccases (Chauhan et al., 2017).

Although laccase/s have been employed in number of industrial processes such as pulp and paper industry for biobleaching of pulp and bioremediation of effluent; textile industry for denim bleaching and decolorization of effluent, food industry for bread making etc. (Murugesan and Kalaichelvan, 2003; Mate and Alcalde, 2017; Chauhan et al., 2017). Despite such wide range of application, laccases have not been explored much for their application in hair dyeing. Use of fungal laccases for the oxidation of hair dyes precursors has been suggested in some reports (Takada et al., 2003; Saito et al., 2012; Chen et al., 2013). But fungal laccases are mostly active at acidic/neutral pH (Buddolla et al., 2014); whereas, hair coloring reaction has to be carried out at alkaline pH for effective penetration and retaining of dye (Gavazzoni Dias, 2015). As bacterial enzymes are known to be active and stable under extreme temperature and pH conditions (Kumar et al., 2011); therefore major objective of this study was to isolate an extracellular alkali stable bacterial laccase and to standardize a process for its use as oxidizing agent to replace H_2O_2 in the hair dyeing.

Other limitation of hair dyeing process is the use of chemical based hair dye precursors (Jeon et al., 2010). Because of ability of laccase/s to act on wide range of substrates (Morozova et al., 2007a) they can also been used for synthesis of dyes from natural precursors. Although there are number of reports on the use of laccase/s for textile dye/s synthesis (Polak and Jarosz-Wilkolazka, 2012; Wang et al., 2018a) but there use for synthesizing hair coloring dye/s has not been explored. Only report where polymerization of natural precursors and synthesis of hair coloring dyes by laccase has been shown is by Jeon et al., (2010). Laccase used was from *Trametes versicolor* and reaction was carried out for 22h at pH-5. As hair coloring process is generally done for only 15 to 30 min and at alkaline pH, therefore application of this enzyme is not feasible at commercial scale. Therefore, preliminary studies were also done to use laccase/s for synthesis of chemical free hair dye/s from natural precursors.

Thus, major objectives of the present study in brief were:

1. Isolation and screening of extracellular laccase producing bacteria from environmental samples capable of oxidizing a wide range of substrates
2. Selection of the isolates capable of oxidizing dyes like PPD (p-phenylenediamine) in alkaline range.
3. Identification of isolate by microscopic, biochemical tests and 16S rDNA sequence analysis.
4. Process optimization for increasing the yield of laccase by one variable at a time and statistical methods.
5. Purification and characterization of the laccase with respect to temperature optima/stability, pH optima/stability, Kinetics etc.
6. Standardizing the conditions for the optimal oxidation of dye precursors such as PPD with laccase.

7. Dyeing of hair with enzymatically oxidized dye.

8. Oxidization of commercial hair dyes with enzyme instead of H_2O_2

9. Formulation of laccase based developer for oxidizing hair dyes.

10. Comparison of dyes oxidized with laccase based developer verse H_2O_2 based developer

a) Coloring ability of enzymatically versus H_2O_2 oxidized dye.

b) Retaining ability of the enzymatically versus H_2O_2 oxidized dye on hair with respect to repeated washing.

c) Evaluation of enzymatically versus H_2O_2 oxidized dye with respect to effect on hair.

Apart from the above objective in the original plan, use of laccase for the synthesis of hair coloring dye/s from natural precursors was also explored.