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Growth Pattern of Laccase from *Bacillus licheniformis* cloned in *E.Coli* Rosetta 2 Strain

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ABSTRACT:
Laccasegene from *Bacillus licheniformiswas* cloned and expressed in *E. coli* Rosetta2 strain under the control of T7 promoter. The molecular weight of expressed Laccase was observed to be 65KDa. The *E. coli* Rosetta 2 strain expressing Laccase was grown in presence of different types of media and it was observed that expression of Laccase protein can be doubled in presence of TB media resulting in overall enhancement of Laccase expression.

BACKGROUND:
Textile, pulp and paper industries discharge a huge quantity of waste in the environment, and the disposal of this waste is a big problem. To solve this problem, work has done to discover such an enzyme, which can detoxify these wastes and is not harmful to the environment.
Laccase belongs to those enzymes, which have innate properties of reactive radical production, and its utilization in many fields has been ignored because of its unavailability in the commercial field (Imran et al 2012). Laccase are widely distributed among fungi, higher plants, insects, and bacteria (Koschorreck et al 2009 and Vivekananda et al 2014). Laccase have the ability to detoxify a range of environmental pollutants. Laccase reduces dioxide to two molecules of water and simultaneously performs one-electron oxidation of many aromatic substrates (Bourbonnais et al 1995).
Their property to act on a range of substrates and also to detoxify a range of pollutants have made them to be usable for several purposes in many industries including paper, pulp, textile and petrochemical industries (Imran et al 2012).

INTRODUCTION:
Laccase belong to the multi-copper oxidase (MCO) enzyme family. These enzymes are classified as blue copper proteins and contain between one and six copper atoms. They are produced by various fungi, plants, insects and bacteria. Laccase are considered to be ideal "*green catalysts*" since they are capable of oxidizing a wide variety of compounds in a straightforward manner, using O_2 from the air and releasing H_2O as the only by-product (Lahtinen et al 2013 and Bourbonnais et al 1995).Numerous studies have shown that Laccase catalyzed decolonization of textile dyes.
Fungal Laccase have difficulties in the overproduction of their recombinant forms and thus the lack of sufficient enzyme stocks is the most important obstacles to commercial application of Laccase. However, bacterial Laccase can be overproduced more easily in heterologous host like *Escherichia coli*, because of their intracellular localizations and development of host-vector system for prokaryotic expression (Wu Jet al 2010).
In most fungi, Laccase are produced in the native hosts at levels that are too low for commercial purposes. Therefore, improving the productivity and reducing the production cost are the major goals for the current studies on Laccase production. Cloning of the Laccase genes followed by heterologous expression may provide higher enzyme yields. In order to improve Laccase production, fungal Laccase have been expressed heterologously in *Saccharomyces cerevisiae*, *Trichodermareesei*, *Aspergillusoryzae*, *Pichiapastoris*,

Yarrowialipolitica, Aspergillussojae, Aspergillusniger, Aspergillusnidulans, Aspergillusficuum, tobacco and maize. Bacterial Laccase from *B.subtilis, Thermusthermophilusand Streptomycin lavendulaehave* been expressed in *Escherichia coli* but successful expression of fungal Laccase in E. coli has not been reported (Pannuand Kapoor 2014). In spite of the fact that Laccase production levels have often been improved significantly by expression in heterologous hosts, the reported levels are still rather low for industrial applications (Pannuand Kapoor 2014).

Discovery of novel Laccase with different substrate specificities and improved stabilities is very important for industrial applications, besides developing an effective, high yielding and economic production medium to enhance its utility (Desai and Nityanand 2011). Thus the goal of the present study is to determine effect of different media for enhancing the Laccase expression for industrial use.

MATERIALS AND METHODS:

The materials used to perform experiments were obtained from NEB, Fermentas, Qiagen and Himedia

CLONING OF THE *B. licheniformiswas* Laccase Gene:

The organism selected for present research study is ***Bacillus licheniformiswas*** **ATCC 14580**(MTCC No. 429).

The 1542bp Laccase gene of *Bacillus licheniformiswas* cloned into MCS of pET28 vector. The cloned gene was initially transformed in *E. coli* DH5 α cells and confirmed by restriction digestion with appropriate enzymes. Finally the pET28- Laccase gene was transformed in *E. coli* Rosetta2 strain. The clones were confirmed by PCR.

EXPRESSION OF LACCASE:

E. coli Rosetta 2 cells carrying pET28-laccase plasmid was grown in 5ml LB medium and TB medium respectively supplemented with kanamycin (30 g/ml) at 37°C and 200rpm for overnight. The conditions for expression in *E. coli Rosetta* 2 strain were designed on the basis of protocol followed by Reiss et al 2011. The overnight culture was inoculated in 30ml LB &TB medium respectively with cells equivalent to 0.2 OD. When OD_{600} of culture was reached 0.3, cells were induced by IPTG. At the time of induction 0.25mM $CuCl_2$ was added and temperature was decreased to 30°C. The cells after induction were further incubated for 4 hr. at 120rpm Cells were harvested by centrifugation at 4°C for 10 min at 10000rpm. Pellet was washed and re-suspended in 1X PBS. Pellet volume corresponding to 0.8nm OD was run on SDS-PAGE.

Expression Study: LB medium

Strain	OD_{600nm} before induction (Dil 1:10)	OD_{600nm}4 hrs after induction (Dil 1:10)
Rosetta2 (DE3)	0.343	0.508

Expression study: TB medium

Strain	OD_{600nm} before induction (Dil 1:10)	OD_{600nm}4hrs after induction (OD_{600nm} (Dil 1:100)
Rosetta2 (DE3)	0.411	0.128

RESULTS:

GROWTH PATTERN OF LACCASE EXPRESSION:

To compare growth pattern of *E. coli Rosetta* 2 cells expressing Laccase gene, cells were grown in LB and TB medium (Koschorrecket al 2008 and Sherifet *al* 2013). More growth was observed with more enriched

TB medium i.e., two fold increase in OD values in TB medium as compared to LB medium were observed (Fig.1). The increased OD resulted in increased expression of Laccase enzyme (Fig.2).

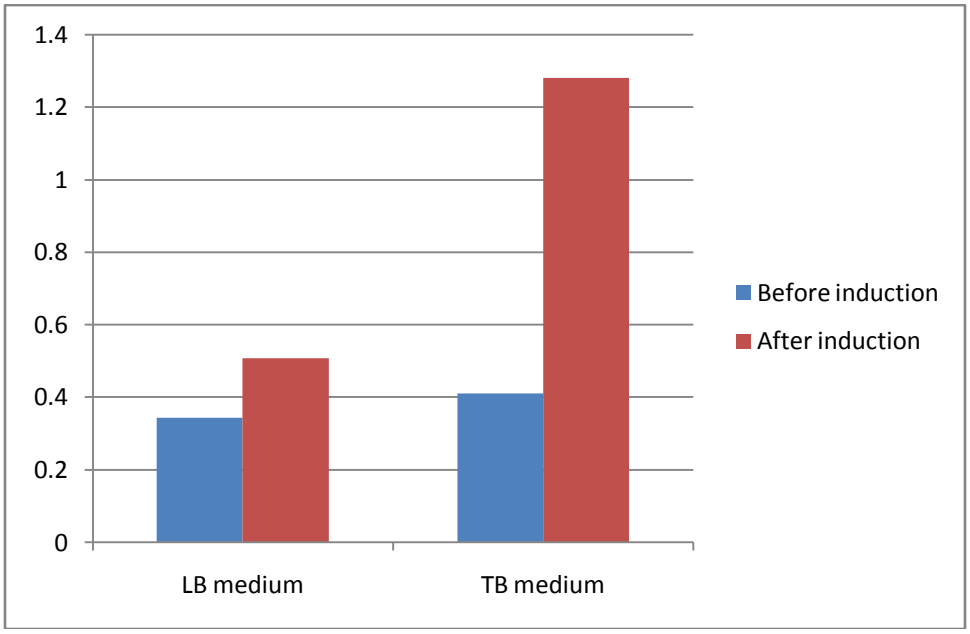

Fig1: Comparison of growth pattern of E.coli Rosetta 2 cells expressing laccase enzyme in LB & TB medium respectively.

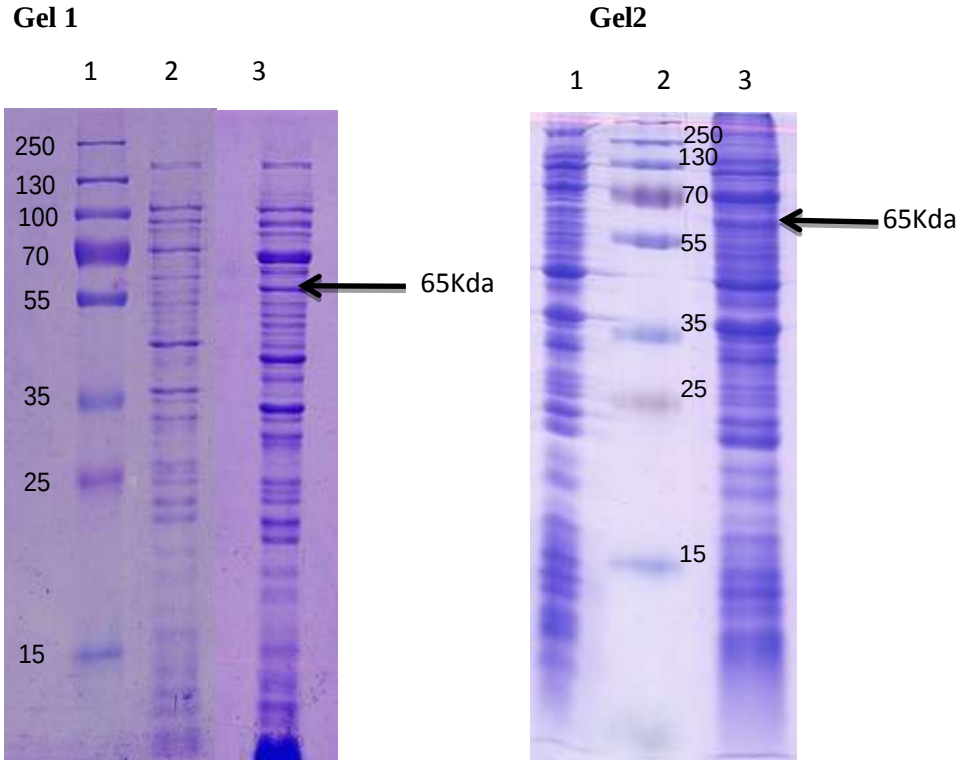

Fig2: Effect of medium on Laccase expression in *E. coli* Rosetta2 strain

Gel 1: *E. coli Rosetta2* strain grown in LB medium; Lane1: Protein Marker, Lane 2: Unindicted sample, Lane 3: Induced sample;
Gel 2: *E. coli Rosetta2* strain grown in TB medium, Lane 1: Unindicted sample, Lane 2: Protein Marker, Lane 3: Induced sample

DISCUSSION:

With the advent of industrialization and urbanization, exploitation and utilization of resources are also reaching to the maximum level. Current population explosion globally urges enlargement of industrial sectors resulting in pollution of water, air and soil. As prevention of pollution is not possible, control of pollution can be practiced effectively. The discharges of pollutants into the environment from various industries possess a threat to living organisms resulting in a great environmental stress. We are facing the problem of pollution and scarcity of water. The biggest problem is of water pollution and 80% of water pollution is due to distillery industries along with textile and fermentation industry.

Laccase are regarded as industrially relevant enzymes due to their broad substrate spectrum and wide range of reactions that they catalyze, Laccase can be used for several industrial applications, such as biological bleaching in pulp and paper industries, dye decolonization in textile industries, detoxification of recalcitrant environmental pollutants, organic synthesis as biocatalyst and bioremediation (Bhuvaneshwari 2015 and, Wu J *et al* 2010).

However, one of the limitations for the large-scale application of Laccase is the lack of capacity to produce large volumes of highly active enzyme at an affordable cost. The use of inexpensive sources for Laccase production is being explored in recent times. In this regard, an emerging field in management of industrial wastewater is exploiting its nutritive potential for production of Laccase.

Present study was designed to enhance the expression of Laccase gene of *Bacillus licheniformiswas*. The industrial potential of Bacillus licheniformiswas first identified by Viet et al 2004 Later Laccase gene from Bacillus licheniformiswas was cloned and expressed in *E. coli* BL21 (DE3) by Koschorrecket al 2008 but expression was very low for commercial application. Further to improve the functional expression of Laccase of *Bacillus licheniformis* was; Koschorrecket al 2009; suggested site directed mutagenesis in the Laccasegene resulting in increased expression.

To enhance expression effect of growth medium on Laccase expression was observed in presence of different media viz., LB and TB medium which showed high cell density in presence of TB media resulting in 2 fold increase in cell density simultaneously increase in Laccase production as compared to LB medium. The recombinant Laccase protein was obtained at ~65kDa size as observed by Koschorreck et al 2008.

CONCLUSION:

In summary, Laccase from *B licheniformiswas* was cloned and expressed in two *E. coli* host strain namely *E.coli* Rosetta2. On comparison of Laccase expression in two different media it was found that *E. coli* Rosetta 2 host strain showed increased cell density in TB medium resulting in enhancement of Laccase enzyme expression.

It was concluded that *E. coli* Rosetta 2 strain is the preferred host for expression of Laccase enzyme from *Bacillus licheniformis*.

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