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Lignin Database for Diversity of Lignin Degrading Microbial Enzymes (LD^{2}L)

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Lignin is the second most abundant constituent of the plant cell wall, where it protects cellulose against hydrolytic attack by saprophytic and pathogenic microbes. Lignin degradation plays a major role in carbon recycling in the ecosystem as well as convert plant biomass for second generation biofuel (ethanol) production. This environmentally recalcitrant organic material has been degraded by different microorganisms like, Bacteria, Fungi and Actinomycetes and they are capable of producing various degrading enzymes such as, Aryl oxidase, β-Glucosidse, Cellulase, Endoglucanase, Glycerol oxidase, Hemicellulase, Lignin peroxidase (LiP), Laccase, Manganese peroxidase, Oxylate decarboxylase and Xylanase. But the degradation process is controversial against different lignin due to its complex structure and bonding to carbohydrate complexes. To address this issue, we developed a lignin degrading microbial enzymes database which gives overall information about the diversity of lignin degrading enzymes of reported microbes. This database is made available through official Web server http://lignindegradingenzymesdatabase.zohosites.com/ for common use by the scientific community.

Key words: Lignin degrading microbes, Lignin, Lignin degrading enzymes, Biofuel

Lignin is by far the most abundant aromatic substance present in the biosphere. It is a non-carbohydrate aromatic hetero polymer composed of phenylpropanoid units linked through a variety of *non hydrolyzable* C-C and C-O-C bonds (Martínez *et al*. 2005). Research on lignin is important for conversion of solid organic waste into production of valuable compounds like Biofuel and maintenance of global carbon cycling in the field of Environmental engineering. This recalcitrant organic compound has been effectively degraded by microbes like fungi, actinomycetes and bacteria through oxidation process under aerobic & facultative anaerobic environment (Wong *et al*. 2009). Among the components of wood, cellulose and hemicelluloses are easily subjected to conversion of biofuel & CO_2 under aerobic and facultative anaerobic environment. Lignin, because of its complex structure and interaction with other carbohydrates in plants make the degradation process slow (Timothy *et al.*, 2011).

Fungal (Macro & Micro) Enzymes in Lignin Degradation

Macro & Micro Fungi are the more predominant organisms effectively involved in degradation of lignin in hard and soft wood. The Macro fungi are classified into two types according to their decay pattern: brown rot & white rot. The most effective lignin degraders in nature are white rot fungi and have the ability to degrade complex recalcitrant organic molecules into simple aromatic residues or its constitute compounds under aerobic environment. Micro Fungus degrades the lignin under aerobic & facultative anaerobic environment in soil as a result formation of quality manure and humus. Breakdown of hard and soft wood lignin makes the availability of cellulose and hemicellulose from plant biomass, which can be utilized for the production of bio ethanol. This Fungal lignin degradation process is oxidative and non-specific, which decreases methoxy, phenoxy and aliphatic content of lignin cleaves aromatic rings and forms new carbonyl groups by the action of enzymes namely Laccase, Lignin peroxidase and Manganes peroxidase etc., These changes in the lignin molecule result in depolymerization and carbon dioxide production (Timothy *et al.*, 2011).

Actinomycetes Enzymes in Lignin Degradation

Actinomycetes are one of the contributors involved in lignin degradation. A well-studied lignin degrading actinomycetes are *Streptomycetes* sp, which produces an enzymes namely Laccase, Mangananese peroxidase, Lignin peroxidase etc., But the activity of various enzymes of actinomycetes species on different Lignin linkage types is poorly understood. Hence, an extensive research has to be done for understanding the mechanisms of enzyme action towards enhancing the lignin degradation process. Most of the actinomycetes enzymes degrade Lignin by cleaving the different types of linkages and lead to oxidation of lignin and liberation of available carbohydrates in plant biomass. This oxidation process also helps for the formation of p - Coumaric acid (4-hydroxycinnamic acid), protocatechuic acid (3,4- dihydroxybenzoic acid), gallic acid (3,4,5-trihydroxybenzoic acid), gallic acid methyl ester (methyl-3,4,5-trihydroxy-benzoate) and 4-methoxyphenol in the compost environment, which depends on the type of lignin present in the plant biomass (substrate) (Chen *et al.*,2012).

Bacterial Enzymes in Lignin Degradation

There are numerous reports available for lignin degradation by various bacterial enzymes: however, their molecular mechanism is still obscure and need to be explored. Four classes of bacteria are effectively involved in lignin degradation, they are α - proteo bacteria, β proeteo bacteria, γ proteo bacteria and actinobacteria. They can degrade the bi-phenyl structures of various Lignin to low molecular wt components namely 3-methylgallic acid, syringic acid, vanillate and other lignin dimeric model compounds as a sole carbon source. This process is a primary metabolic process, where further molecular & biochemical analysis of degradation pathway helps to overcome the complexities in lignin degradation by bacterial enzymes. In addition, enhancement of lignin degradation process has tremendous industrial applications. Hence, finding novel enzymes for lignin degradation process helps utilization of bulk lignin containing plant biomass as feedstock for sustainable production of energy

Materials and Methods

Data collection & Curation

A literature search was done using PubMed and the journals like Science Direct, Springer link. From that all the available information is retrieved till date. Search terms included lignin degrading microbes, (Bacteria, Fungi and Actinomycetes), Lignin degrading enzymes, Laccase, and Lignin peroxidase etc., Only lignin related data were collected from

Brenda (an enzyme database). All the information has been curated manually.

Database structure

This database is the source that can provide comprehensive information related to the microbes & macro fungal resources of enzymes involved in lignin degradation, which is a unique public domain web-interface that elaborates database exploring diversity of lignin degrading enzymes alone (http://lignindegradingenzymesdatabase.zohosites.com/). This provides access to the classification, Common Name of fungi, Habitat, Environment, Enzyme activity on various kind of lignin bonding, Reported Enzyme, Cofactors for enzyme activity in Macro & Micro fungi, Actinomycetes and Bacteria.

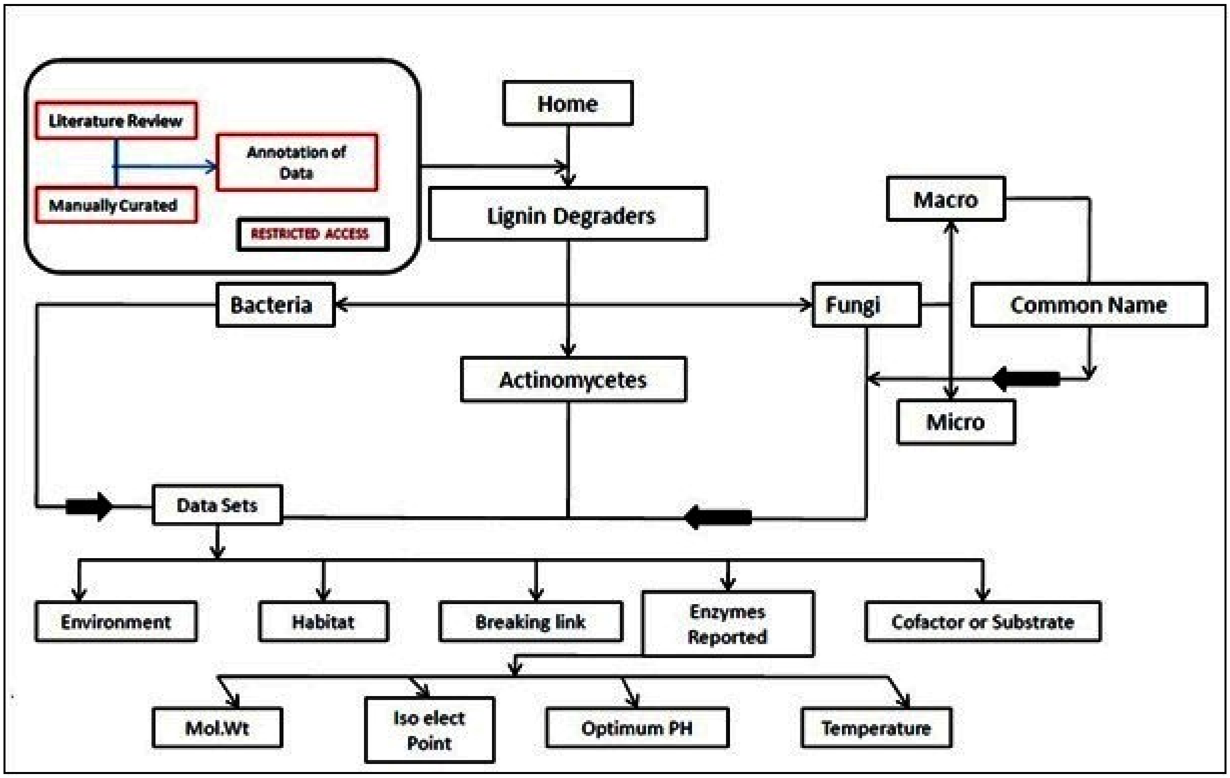


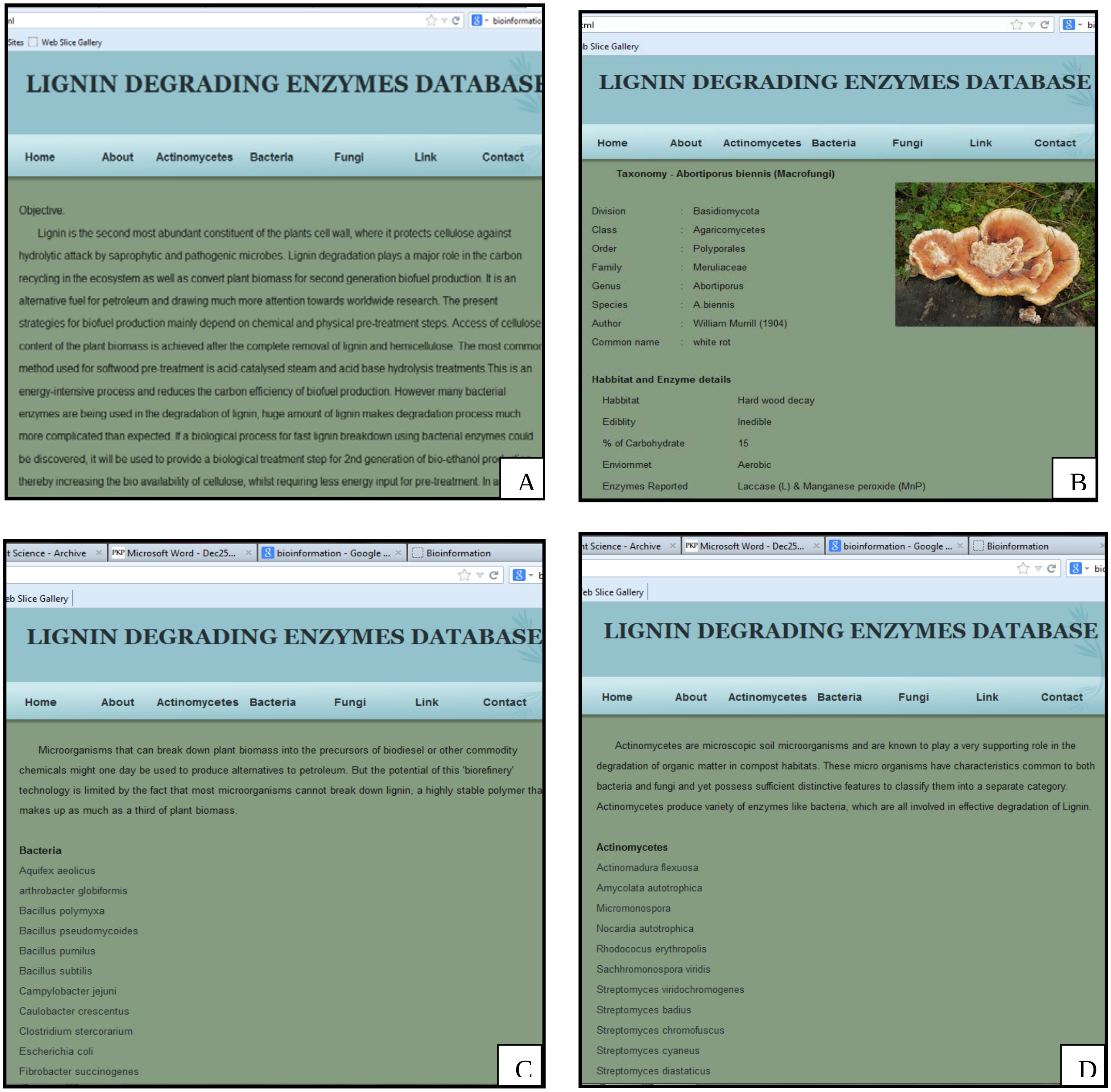
Fig :1 Data flow in LD^{2}L data Base while search in progess

Development and Website Structure

The 'LD^{2}L' database is developed using MySQL (http://www.mysql.com/) a relational database management system that serves as the backend for storing data. APACHE 2.2 (Apache HTTP Server) is used as the web server and PHP5 (Hypertext preprocessor) (http://php.net/) a widely used scripting language driven by Zend engine is used as the web interface. For the process of database creation in the PHP admin, the MySQL client version: 5.0.24a is used. The localhost of Server version: 5.0.24 a, Protocol version: 10, Server: localhost via TCP/IP is used for the database creation. The local host for the process is php Myadmin of 2.9.0 as shown in the (Figure 1).

Utility of Database

LD^{2}L database contains the detailed information about all the microorganisms such as bacteria, fungi and actinomycetes that contain the lignin degrading enzymes. The properties of enzymes like Lacasses, MnP, Lip, Cellulase, Xylanase, Glyoxyl oxidase, Aryl oxidase, Hemicullase, Oxylate decarboxylase, Beta- Glucosidase and Endogulcanse in those organisms like specificity of the enzymes, molecular weight, iso electric point, optimum temperature and pH required for the enzyme to degrade the recalcitrant lignin.



Objective:

Lignin is the second most abundant constituent of the plants cell wall, where it protects cellulose against hydrolytic attack by saprophytic and pathogenic microbes. Lignin degradation plays a major role in the carbon recycling in the ecosystem as well as convert plant biomass for second generation biofuel production. It is an alternative fuel for petroleum and drawing much more attention towards worldwide research. The present strategies for biofuel production mainly depend on chemical and physical pre-treatment steps. Access of cellulose content of the plant biomass is achieved after the complete removal of lignin and hemicellulose. The most commo method used for softwood pre-treatment is acid-catalysed steam and acid base hydrolysis treatments This is an energy-intensive process and reduces the carbon efficiency of biofuel production. However many bacterial enzymes are being used in the degradation of lignin, huge amount of lignin makes degradation process much more complicated than expected. If a biological process for fast lignin breakdown using bacterial enzymes could be discovered, it will be used to provide a biological treatment step for 2nd generation of bio-ethanol pro thereby increasing the bio availability of cellulose, whilst requiring less energy input for pre-treatment. In a

Taxonomy - Abortiporus biennis (Macrofungi)

Division : Basidiomycota
Class : Agaricomycetes
Order : Polyporales
Family : Meruliaceae
Genus : Abortiporus
Species : A.biennis
Author : William Murrill (1904)
Common name : white rot

Habbitat and Enzyme details

Habbitat	Hard wood decay
Ediblity	Inedible
% of Carbohydrate	15
Enviornmet	Aerobic
Enzymes Reported	Laccase (L) & Manganese peroxide (MnP)

Microorganisms that can break down plant biomass into the precursors of biodiesel or other commodity chemicals might one day be used to produce alternatives to petroleum. But the potential of this 'biorefinery' technology is limited by the fact that most microorganisms cannot break down lignin, a highly stable polymer tha makes up as much as a third of plant biomass.

Bacteria
- Aquifex aeolicus
- arthrobacter globiformis
- Bacillus polymyxa
- Bacillus pseudomycoides
- Bacillus pumilus
- Bacillus subtilis
- Campylobacter jejuni
- Caulobacter crescentus
- Clostridium stercorarium
- Escherichia coli
- Fibrobacter succinogenes

Actinomycetes are microscopic soil microorganisms and are known to play a very supporting role in the degradation of organic matter in compost habitats. These micro organisms have characteristics common to both bacteria and fungi and yet possess sufficient distinctive features to classify them into a separate category. Actinomycetes produce variety of enzymes like bacteria, which are all involved in effective degradation of Lignin.

Actinomycetes
- Actinomadura flexuosa
- Amycolata autotrophica
- Micromonospora
- Nocardia autotrophica
- Rhodococus erythropolis
- Sachhromonospora viridis
- Streptomyces viridochromogenes
- Streptomyces badius
- Streptomyces chromofuscus
- Streptomyces cyaneus
- Streptomyces diastaticus

Figure: 2 Screen shots of web interface of the LD²L. A) Home page describes the database objective B, C, D Alphabetically Pertaining the Detailed description page for every individual selected organisms (Fungi, Bacteria and Acinomycetes) involved in the Lignin degradation and other information.

The present database includes 11 entries of enzymes, 102 entries of fungi, 23 entries of Actinomycetes and 31 entries of Bacterial enzymes involved in lignin degradation from various literatures and journals from 1990 to 2012. The microbial breakdown of lignin is a topic of considerable relevance to the commercial utilization of plant lignin and lignocellulose for production of second generation biofuels and renewable chemicals. Despite extensive work on the fungal degradation of lignin, the catabolic pathways for degradation of lignin are still incomplete in Actinomycetes and Bacteria. It seems likely that there are additional enzymes and pathways yet to be identified, and that the entire lignin catabolism system might have some complexity, but an understanding of this system will be important in the development of lignocellulosic bio refineries for the 21st century. LD^{2}L will simplify the process of utilization of Lignocellulosic/ Ligno biomass waste to produce valuable product synthesis.

Database Access

The lists of Actinomycetes, Bacteria, Fungi (Macro & Micro) involved in the degradation lignin have been alphabetically indexed name in the database. The user can click on the name of the organism, detailed view link and a new page will appear that display the details of the organism. The corresponding page (Fig. 2) for each organism is comprised of (i) general information including scientific name of the organism, taxonomic classification, image of the respective organism, ii) detailed description regarding the Habitat, Edibility along with available carbohydrate (only for fungi), Reported Enzymes, Isoelectric point, Molecular wt, Enzyme breaking point in the lignin, sequence availability, crystallographic solved structure of reported enzymes. Other uses of each organism were included along with further studies on the reported enzymes in the datasets. iii) PDB- ID and crystal structure of solved enzymes can viewed by clicking on hyperlink. LD^{2}L also having cross references to databases like PubMed and NCBI

Further development of Database in future

The current database contains the basic data about nearly 13 enzymes in various microorganisms. In future we plan to add data about crystal solved & Homology Modeled (Theoretically solved) structures of lignin degrading enzymes available from PDB & PMDB. In literature survey we found some of the insects also have the ability to digest the Lignin into simple carbohydrates. We will include such enzymes in future to update the information pertaining various biochemical path way of Lignin degradation and Constituent byproducts of Fungi (Macro & Micro fungi), Bacteria and Actinomycetes. We are working to elaborate the database by incorporating the data on gene which is responsible for enzymes in all selected organisms and it constitutes end products of Lignin degradation. Additional search and retrieval features will also be added to enhance the usability of the web interface.

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