

PREDICTION OF HORIZONTAL GENE TRANSFER OF LACCASE GENE; A COMPARATIVE STUDY OF 30 PROKARYOTIC GENOMES

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ABSTRACT

Horizontal Gene Transfer (HGT) plays an important role in microbial evolution. Present study provides an in silico evidence for horizontal gene transfer of laccase gene. Laccases are multi-copper oxidases widely distributed among plants, fungi and bacteria. These enzymes are responsible for oxidation of a wide variety of organic and inorganic substrates and can be used for degradation of micropollutants in wastewater. In the present paper computational methods were applied across 30 completely sequenced Laccase producing bacterial genomes of different subtypes of E coli, Salmonella, Serratia, Pseudomonas, Bacillus and Vibrio for studying the prospects of horizontal gene transfer of Laccase Gene. Genome sequences, Laccase gene sequences and 16S rRNA sequences were retrieved from Kyoto Encyclopedia of Genes and Genomes. Genome and Laccase gene sequences were further analyzed and compared for their % GC content. Multiple sequence alignment was done and phylogenetic trees were constructed from 16S rRNA and Laccase gene sequences using Mega-6. Differing patterns of 16S rRNA gene tree and the Laccase gene tree supported by high bootstrap values and discrepancies in %GC content of complete genome and of Laccase gene led to the conclusion that Horizontal Gene Transfer events might have taken place during the evolution of this gene.

INTRODUCTION

Laccases are benzene diol: oxygen oxidoreductase, (EC 1.10.3.2) found in higher plants and microorganisms. They are copper containing enzymes that carry out one electron oxidation of phenolic compounds and reduce oxygen to water [Couto *et al.*, 2006; Gianfreda *et al.*, 1999], this reaction generates free radicals which may further cause hydration, disproportionation, and polymerization of substrate [Faccello *et al.*, 2008]. Laccases are commonly used in food, paper, pulp, textile and cosmetics industries. It also plays an important role in soil bioremediation and biodegradation of environmental phenolic pollutants thus allowing microorganisms to expand their habitat and proliferate in the presence of hazardous substances. [Gianfreda *et al.*, 1999] Extra chromosomal genetic elements, such as plasmids and transposons which are transferred from one bacterium to another through transformation, conjugation, and transduction play an important role in HGT. Acquiring new genes, in the course of evolution develops the adaptability of bacteria to survive in new environment. An evolutionary force has been suggested to enhance bacterial adaptation to environment contaminated with heavy metals and toxic compounds [Top, 2003; Walsh, 2006]. Bacteria can also acquire resistance to antibiotics by HGT [Walsh, 2006] It has been shown by genomic studies that a considerable proportion of genes in prokaryotic genomes have been subjected to horizontal gene transfer. [Koonin, 2001] A detailed study is required to study HGT which plays a crucial role in microbial evolution. Over 3000 (bacterial and archaeal)

genomes have been made publically available today, providing an unprecedented opportunity to examine evolutionary genomic trends and offering valuable reference data for a variety of other studies such as metagenomics. The utility of these genome sequences is greatly enhanced when we have an understanding of how they are phylogenetically related to each other. [Lang *et al.*, 2013] In the present study genomes of 30 prokaryotic organisms from different taxonomic groups are chosen to study HGT of Laccase gene and an in depth bioinformatics analysis is performed by using various parametric and phylogenetic methods to assist evolutionary biology. Available DNA data and computational methods like building trees are used to evaluate the evolutionary changes and for predicting gene transfer events of Laccase gene amongst different prokaryotic organisms.

MATERIALS AND METHODS

Sequences of Laccase gene as well as 16S rRNA from 30 prokaryotes from different taxonomic groups were retrieved from KEGG (Kyoto Encyclopedia of Genes and Genomes) (<http://www.genome.ad.jp>) Retrieved sequences were aligned using the CLUSTAL W (<http://www.genome.jp/tools/clustalw/>) and phylogenetic trees for Laccase Gene and 16S rRNA gene sequences were constructed using Mega6 version (www.megasoftware.net/) Evolutionary history was inferred using the Neighbor-Joining method [Saitou *et al.*, 1987]. The optimal tree with the sum of branch length = 0.76812088 is shown in species tree and 4.55681384 in gene tree. The percentage of replicate trees in which the associated taxa

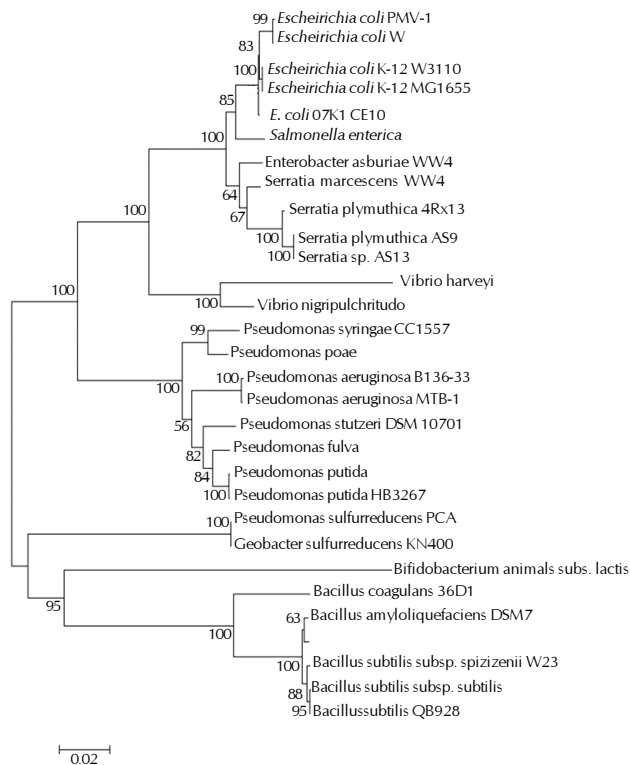


Figure 1: Species Tree

clustered together in the bootstrap test (500 replicates) is shown next to the branches [Felsenstein, 1985]. Evolutionary distances were computed using the p-distance method [Nei *et al.*, 2000] and are in the units of the number of base differences per site. The analysis involved 30 nucleotide sequences. Codon positions included were 1st+ 2nd+ 3rd+ Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1476 positions in the final dataset for species tree and 623 in the final dataset of gene tree. Evolutionary analyses were conducted by MEGA6 [Tamura *et al.*, 2011] (www.megasoftware.net/). The GC Content of Laccase gene for each organism was calculated using the online program Oligo Calc Software (<http://www.basic.northwestern.edu/biotools/oligocalc.html>) [Kibbe, 2007]. The GC Content for the whole genomes was obtained from NCBI (<http://www.ncbi.nlm.nih.gov/genomes/>).

RESULTS

Different branching patterns of species tree and gene tree shown in Fig. 1 and 2 indicate the possibility of horizontal gene transfer. Some organisms were found closer to evolutionary distant organisms. For example, Vibrionales is clustered with Enterobacteriales. Similarly Desulfuromonadales was observed with Bacillales. Such observations also indicated the possibility of HGT between these organisms. Their unusual placement was further confirmed when same topology is seen with more than one tree building algorithm (DNA Parsimony and Maximum Likelihood Method of Mega6 package, version. www.megasoftware.net/) To further study this discrepancy

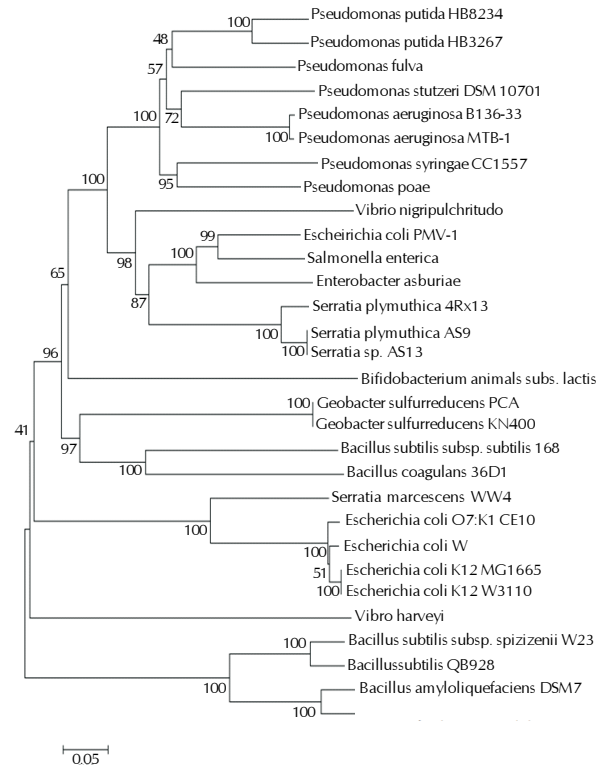


Figure 2: Laccase Gene Tree

between the species tree and gene trees and to estimate HGT, T-REX software is used. A bootstrap validation procedure is also employed by this software to check the reliability of a specific gene transfer. (<http://www.fas.umontreal.ca/biol/casgrain/en/labo/t-rex>) To further support these phylogenetic discrepancies and HGT of Laccase gene, parametric method comprising of detailed comparative analysis of %GC of laccase gene and the respective host genome was conducted. GC content of newly acquired gene often differs from the GC content of whole genome as shown in Table 1 this difference is also indicative of HGT.

DISCUSSION

Laccase gene is the suitable model for studying bacterial evolution by horizontal gene transfer mechanism because it allows microorganisms to grow and proliferate in the presence of nasty compounds. Gene transfer experiments are difficult to conduct in natural environment because of the physicochemical variables like pH, temperature, carbon, and nitrogen sources which are quite different in laboratory conditions and in their natural environment. In laboratory genetic transfer is usually done using plasmids and transposons. However, there are chances that the transformed cells may lose the newly incorporated gene by random mutation because they are not providing any adaptive advantage to the host cells or sometimes foreign gene may be toxic to the host cells [Kurland *et al.*, 2003] we can overcome such limitations by using computational methods for predictions of HGT events. At the same time availability of large number of genome and gene sequences has made this

Table 1: % GC Content of Gene and Genome

S. No	Organisms	% GC/Genome	%GC/Gene	Gene length bp
01	E coli 07K12 MG1655	50%	55%	1551
02	Escherichia coli O7:K1 CE10	50%	54%	1551
03	Escherichia coli W	50%	55%	1551
04	Escherichia coli PMV-1	50%	54%	732
05	Escherichia coli K-12 W3110	50%	55%	1551
06	Salmonella enterica	52%	54%	732
07	Enterobacter asburiae	53%	56%	732
08	Serratia plymuthica AS9	56%	60%	732
09	Serratia plymuthica 4Rx13	56%	60%	732
11	Serratia sp. AS13	56%	60%	732
12	Serratia marcescens WW4	59%	63%	1668
13	Vibrio harveyi	45%	45%	1383
14	Vibrio nigripulchritudo	46%	48%	726
15	Pseudomonas putida H8234	61%	68%	741
16	Pseudomonas aeruginosa B136-33	66%	72%	729
17	Pseudomonas aeruginosa MTB-1	66%	72%	729
18	Pseudomonas fulva	62%	70%	741
19	Pseudomonas syringae CC1557	58%	62%	729
20	Pseudomonas poae	61%	63%	726
21	Pseudomonas stutzeri DSM 10701	63%	67%	810
22	Pseudomonas putida HB3267	62%	68%	741
23	Geobacter sulfurreducens PCA	61%	65%	801
24	Geobacter sulfurreducens KN400	61%	65%	801
25	Bacillus subtilis subsp. subtilis 168	43%	46%	837
26	Bacillus subtilis subsp. spizizenii W23	43%	49%	1542
27	Bacillus subtilis QB928	43%	48%	1548
28	Bacillus amyloliquefaciens DSM 7	46%	51%	1539
29	Bacillus amyloliquefaciens subsp. plantarum UCMB5033	46%	50%	1539
30	Bacillus coagulans 36D1	47%	51%	825

possible to study different methods of HGT detection which include parametric and phylogenetic/homology methods [Azad et al., 2011]. In homology

Homology methods, conclusions are drawn from the species distributions of the genes' closest neighbors, also scale to whole genomes (Lander et al., 2001; Podell et al., 2007). The extent and sources of horizontal gene transfer (HGT) in an organism may even affect our ability to reconstruct the entire organism's evolutionary history (Doolittle, 1999). In many cases, the best-performing algorithms use phylogenetic approaches to re-construct the evolutionary histories of genomes and individual genes (Eisen, 2000). Parametric methods compare nucleotide composition of whole genome with the genes in question. As G+C content varies in from one genome to another, the foreign gene with characteristic G+C content can be detected as deviants. Additionally, phylogenetic methods are used to identify similarities/conflicts between the phylogenetic tree for Laccase gene and the corresponding 16S rRNA tree. HGT events in a particular set of organisms can be revealed by comparing gene tree and species tree (Makarenko et al., 2003). Here in the present study Phylogenetic incongruencies are observed between the 'gene tree' and the corresponding 'species tree' predicting horizontal gene transfer events of Laccase Gene in prokaryotic taxa.

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