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# Outstanding impact of soil tillage on the abundance of soil hydrolases revealed by a metagenomic approach

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### ABSTRACT

The soil represents the main source of novel biocatalysts and biomolecules of industrial relevance. We searched for hydrolases *in silico* in four shotgun metagenomes (4,079,223 sequences) obtained in a 13-year field trial carried out in southern Brazil, under the no-tillage (NT), or conventional tillage (CT) managements, with crop succession (CS, soybean/wheat), or crop rotation (CR, soybean/maize/wheat/lupine/oat). We identified 42,631 hydrolases belonging to five classes by comparing with the KEGG database, and 44,928 sequences by comparing with the NCBI-NR database. The abundance followed the order: lipases > laccases > cellulases > proteases > amylases > pectinases. Statistically significant differences were attributed to the tillage system, with the NT showing about five times more hydrolases than the CT system. The outstanding differences can be attributed to the management of crop residues, left on the soil surface in the NT, and mechanically broken and incorporated into the soil in the CT. Differences between the CS and the CR were slighter, 10% higher for the CS, but not statistically different. Most of the sequences belonged to fungi (*Verticillium*, and *Colletotrichum* for lipases and laccases, and *Aspergillus* for proteases), and to the archaea *Sulfolobus acidocaldarius* for amylases. Our results indicate that agricultural soils under conservative managements may represent a hotspot for bioprospection of hydrolases.

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## Introduction

The soil is the richest habitat in microbial diversity, with estimates of containing about 10 billion micro-organisms per gram, encompassing thousands of species of bacteria, fungi and archaea,<sup>1</sup> corresponding to approximately 1000 Gbp of microbial genomes per gram of soil.<sup>2</sup> With this microbial arsenal, the soil represents the main source for isolation of novel biocatalysts and other biomolecules of industrial relevance, such as antibiotics and enzymes. Soil micro-organisms and microbiomes have been studied in a variety of conditions, including undisturbed,<sup>3,4</sup> agricultural,<sup>4-6</sup> polluted or contaminated.<sup>7</sup> Studies in several agroecosystems have allowed not only to get a better understanding of the microbial composition and functioning, but have also provided important tools for searching new molecules with potential biotechnological use.<sup>5,8,9</sup>

The intensification of land use for food production often results in negative impacts on soil quality, mostly caused by incorrect managements, including heavy use of fertilizers and pesticides, and practices that result in erosion and losses of soil organic matter. Therefore, soil and crop managements greatly influence the soil quality and may impact microbial community, affecting long-term sustainability.<sup>4,5,10-12</sup> Soil management known as no-tillage (NT), in which sowing is performed directly into the crops' residues, results in higher contents of soil organic matter, water content, and lower temperature oscillations, improving the conditions for micro-organisms acting on the biogeochemical processes, and allowing higher yields. On the contrary, the conventional tillage (CT) imposes constant soil stirring by plowing and disking, resulting in fast oxidation of soil organic matter, erosion, loss of water and biodiversity.<sup>4,5,10-12</sup> Besides soil management, crop rotations including legumes and green manures are key for improving soil quality and reducing pests and diseases, increasing the sustainability of the cropping systems.<sup>4,5,10-12</sup>

The probability of finding micro-organisms with biotechnological potential for degradation of agrochemicals in agricultural soils is high.<sup>13</sup> However, due to limitations of the classical methods for microbial isolation and growth, only about 1% of the soil microbial diversity is known.<sup>14,15</sup> Nevertheless, the metagenomics approach independent of cultivation has revealed the hidden microbial potential, such as new microbial species, genes, and biomolecules.<sup>16</sup>

Hydrolases are among the most searched microbial molecules in soils; they compose a class of enzymes that catalyze the hydrolysis of covalent bonds, and have large industrial uses.<sup>17</sup> Micro-organisms use hydrolases for degradation of natural organic polymers as source of energy; in addition, they are also involved in the metabolism of xenobiotics such as pesticides. New hydrolases with biotechnological potential, for example, lipases, amylases, proteases and cellulases, have been isolated by using different metagenomic strategies<sup>18,19</sup>; screening of enzymes in metagenomes is usually performed *in silico*, based on comparisons of gene sequences in databases.

The aim of this study was to identify hydrolases with potential industrial applications in an agricultural soil under

different soil and crop managements in southern Brazil, through an *in silico* screening of shotgun DNA sequences obtained in four metagenomes.

## Materials and methods

### Description of the field trial and soil sampling

Soil metagenomes were obtained from soil samples of a 13-year-old experiment at the experimental station of Embrapa Soja, in Londrina, north of Paraná State, southern Brazil (23°11'S, 51°11'W, elevation of 620 m). The soil is classified as Latossolo Vermelho Eutroférrico (Brazilian system), corresponding to Rhodic Eutrudox (US taxonomy). Soil chemical and physical properties and climatic conditions were given elsewhere.<sup>5</sup> The treatments consisted of conventional tillage (CT) and no-tillage (NT), each under crop succession (CS) [soybean (*Glycine max* L. Merr.) in the summer and wheat (*Triticum aestivum* L.) in the winter], or crop rotation (CR) [soybean or maize (*Zea mays* L.) in the summer and wheat, lupine (*Lupinus angustifolius* L.) or oat (*Avena strigosa* Schreb.) in the winter]. The four treatments are designated as NTS, NTR, CTS and CTR. Other information such as cropping history, plot size, experimental design and replicates were given elsewhere.<sup>5</sup> Soil samples were collected from the 0 to 10 cm layer, in the rainy season, before sowing soybean (summer crop), three weeks after harvesting the winter crop, wheat. The great benefit of long-term experiments is that the effects of soil and crop managements reflect a large period, and the effects observed are cumulative, for example, as shown with samplings in different times of the year and different times of implementation performed in previous studies performed by our group.<sup>10,12</sup>

DNA extraction, shotgun sequencing and data processing have been described before.<sup>5,8</sup> Shotgun sequencing resulted in about 1 million sequences for each treatment,<sup>5</sup> and the datasets are deposited in the NCBI-SRA (National Center for Biotechnology Information- Sequence Read Archive) with the submission Accession Number SRA050780.

### *In silico* screening based on nucleotide sequences

The four soil metagenomes, totaling 4,079,223 sequences, were compared against sequences of hydrolases deposited at the NCBI-NR and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases, and assigned according to the highest similarities. First, sets of data were created with the DNA sequences for each hydrolase (amylase, cellulase, lactase, lipase, pectinase and protease) from microorganisms (bacteria, archaea, fungi and virus) extracted from the NCBI database. The DNA sequences of each hydrolase were then compared with the sequences of the four soil metagenomes, by using the BlastX tool against the NCBI-NR and the KEGG databases.

### Statistical analysis

The datasets were normalized by using the MG-RAST tools, as described before<sup>5</sup>; and analyzed with STAMP<sup>20</sup> to identify differences in frequencies of DNA sequences coding for

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