

Characterisation of the spermosphere of the common bean seed (Phaseolus vulgaris)

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Characterisation of the spermosphere of the common bean seed (*Phaseolus vulgaris*)

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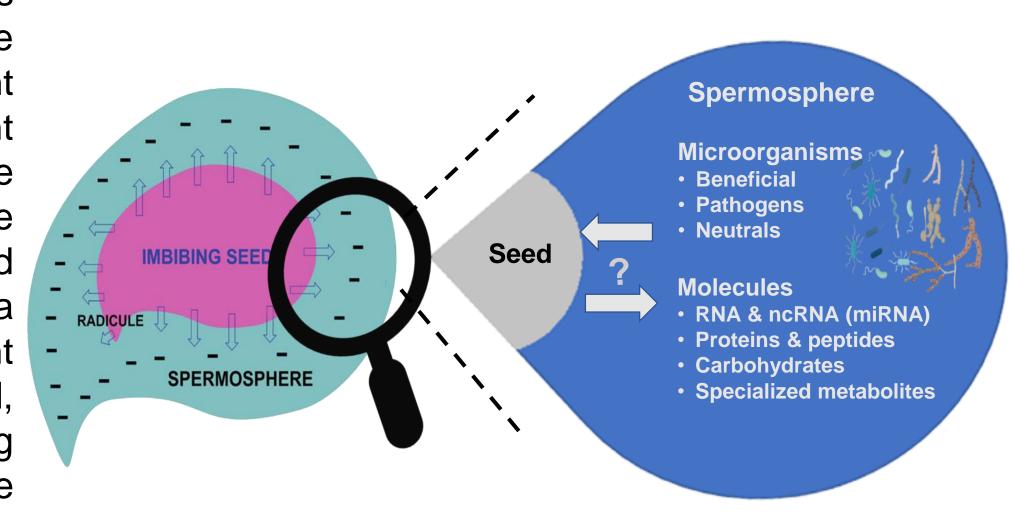
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Sphere-**Phyllosphere** microbes assemblies during plant life cycle Seed production and dispersal Microorganisms **Germination and Plant** seedling development establishment

Introduction

Seed plants have co-evoluted with microorganisms by forming beneficial relationships in order to facilitate their adaptation to terrestrial lifestyle^[1]. Indeed, more and more studies are demonstrating the inter-relationships of the microbiome and their hosts and the resulting impacts of such relationships in terms of the host's fitness, environmental response, adaptation and evolution^[2]. These studies have given rise to a relatively new concept of the holobiont which views the host organism and its associated microbiota as a single functional unit, where evolutionary selection occurs between both the host and its microbiota, as well as among different microbial species within the microbiota^[3].

While plant microbiome in the phyllosphere and rhizosphere has been well studied, seed spermosphere and microbiome have remain relatively unnoticed^[4]. Yet, seeds have an important distinctive function in the inheritance process by connecting plant generations and serving as a critical point in the continuity of the plant microbiome^[4]. However, the fate microorganisms after germination is relatively unexplored. Seed germination, a transient process giving rise to a microenvironment known as the spermosphere, is a critical point for the establishment of the future plant microbiome. Indeed, seed exudation after imbibition is hypothesized to be the driving force for microbial selection, growth and interaction in the spermosphere^[5]. The aim of this study is to characterize the germinating bean spermosphere with multi-omics technologies to understand the complex interactions between seed borne microorganisms and seed exudates.

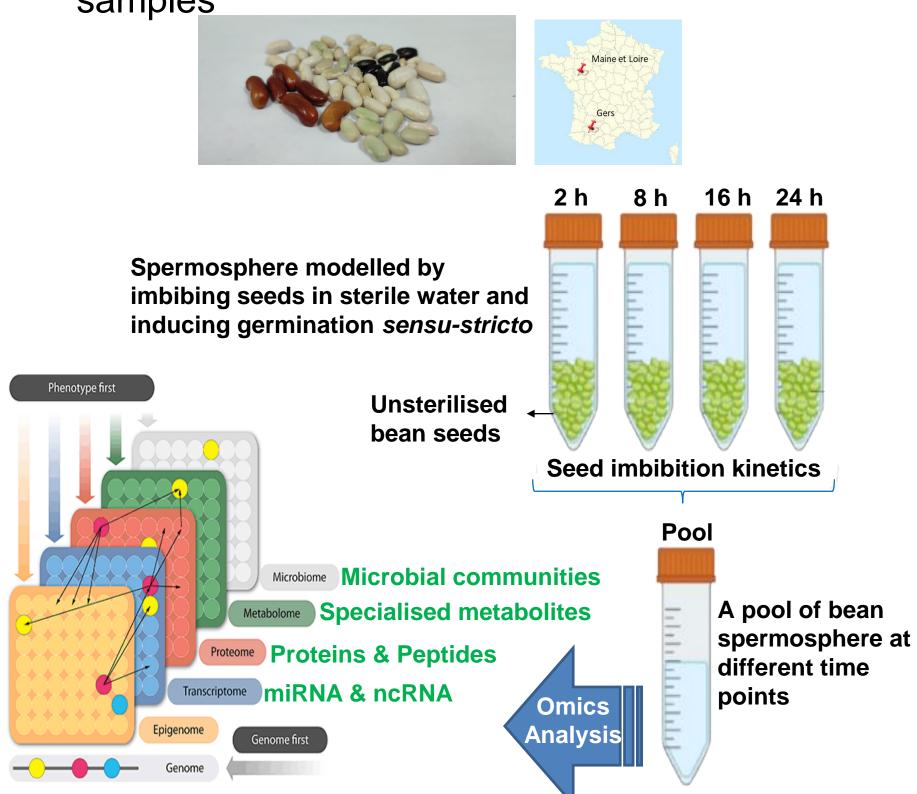


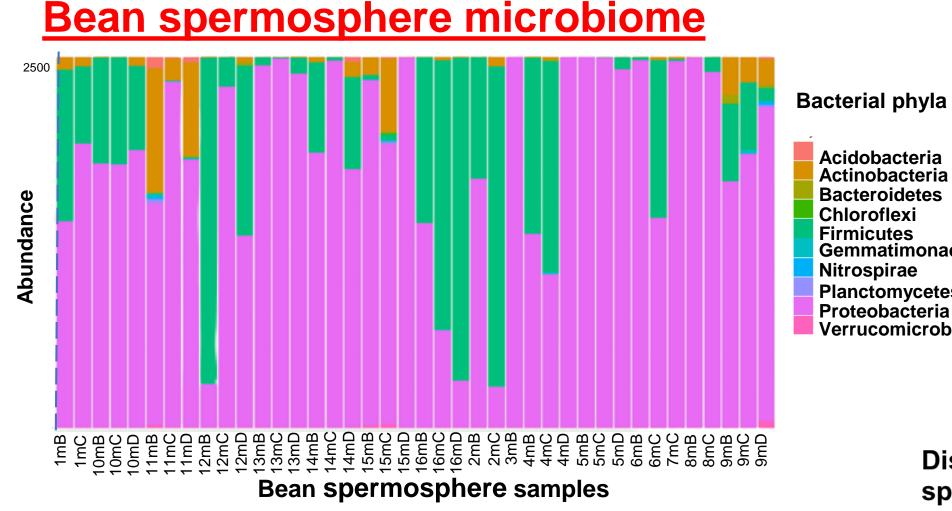
Experimental design

Microorganisms

Spermosphere

- 8 different common bean (*Phaseolus vulgaris*) genotypes, cultivated in France, produced in 2 different locations were used in the experiment
- Bean genotypes were selected based on their contrasting phenotypes (germination vigor, size, colour, weight)
- Metabarcoding, untargeted metabolomics (LCpeptidomics MS/MS), and transcriptomics (smallRNAs) were carried out on spermosphere samples





- 2467 metabolite features
- Metabolite annotation using biometabolomic libraries and informatic tool MetGEM based on clustering methods using spectral fragmentation
- Genotype is the major effect driving metabolite accumulation (results not shown)

Results

Acidobacteria Actinobacteria

Bacteroidetes

Gemmatimonadetes

Chloroflexi

Firmicutes

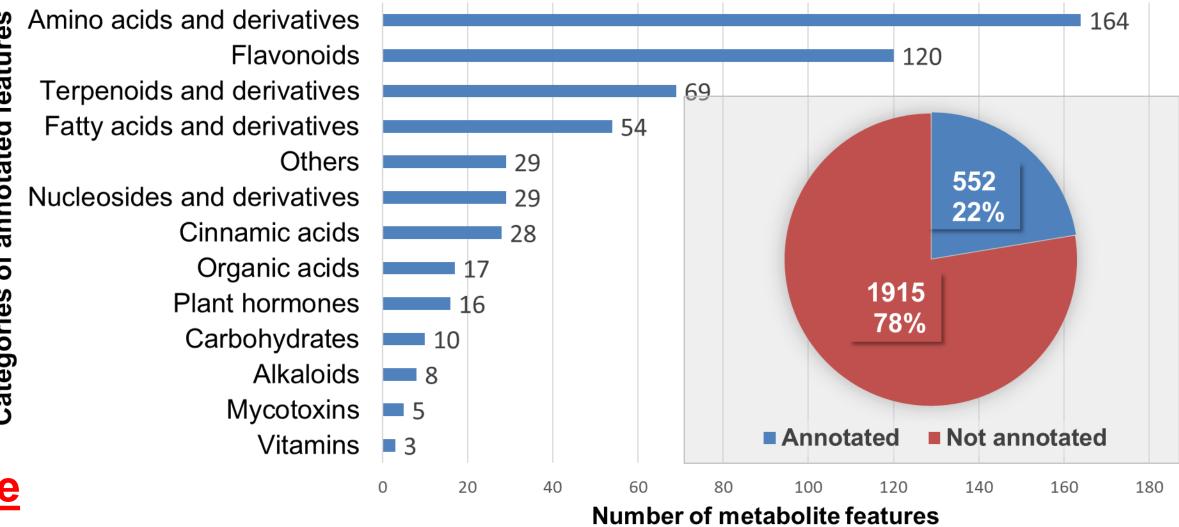
Nitrospirae

Planctomycetes Proteobacteria

- 10 identified bacterial phyla
- Ascomycota & Basidiomycota (not shown) only identified fungal phyla
- Genotype is the major effect driving bacterial communities
- Environment of seed production is the major effect driving fungal communities (results not shown)

Bean spermosphere metabolome

Distribution of different categories of annotated (552) features in bean spermosphere



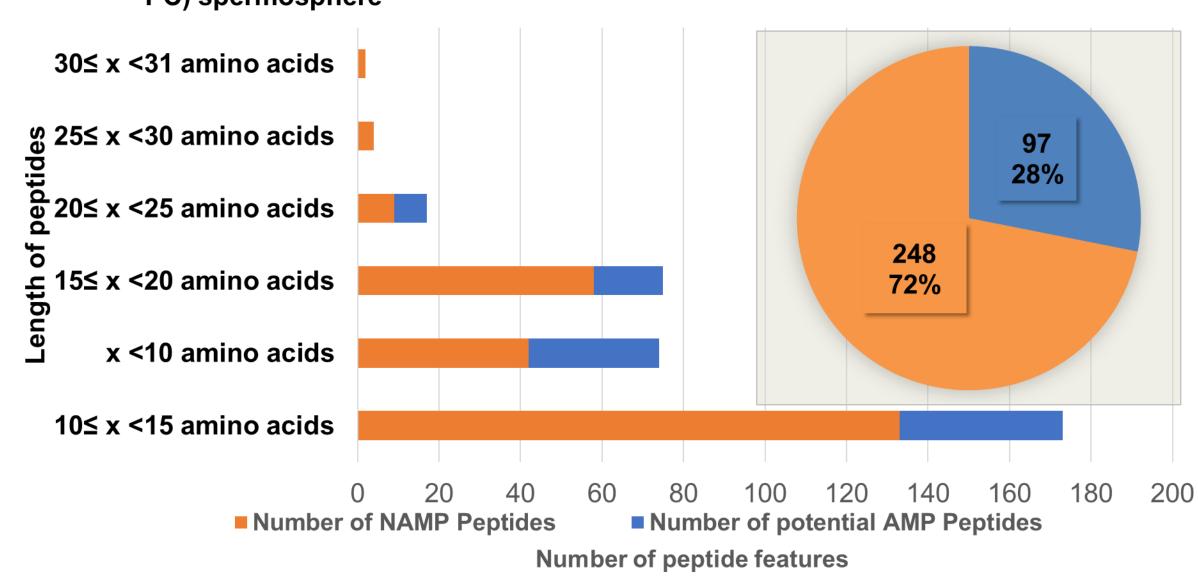
Bean spermosphere transcriptome

- 20 0 m B 3 m B 3 m B 3 m B 3 m B 4 m C 5 m B 6 m B 6 m B 6 m B 6 m B 6 m C 5 m C 7 m B 7 m C 6 m B 6 m C 6 m B 6 m C 6 m B 6 m C 6 m B 6 m C 6 m C 6 m B 6 m C
 - ☐ Insects ☐ Dicots Bean spermosphere samples
 - 345 peptides from 1 bean genotype (H-CON-PC)
 - Peptide lengths: 7 to 32 amino acids
- Most abundant: categories of 10 to 20 amino acids
- Anti-microbial (AMPs) peptides predicted using 4 different prediction models.
 - ➤ 3 AMPs were identified by all 4 models

- Small Non Coding RNAs: Ribosomal RNAs (rRNA), Transfer RNAs (tRNAs) and Micro RNAs (miRNAs)
- 15 to 25 nucleotides long (results not shown)
- rRNAs most abundant (>80%) while miRNAs least abundant (<5%)
 - > 14 miRNA families from common bean
 - > 8 miRNA families from insects

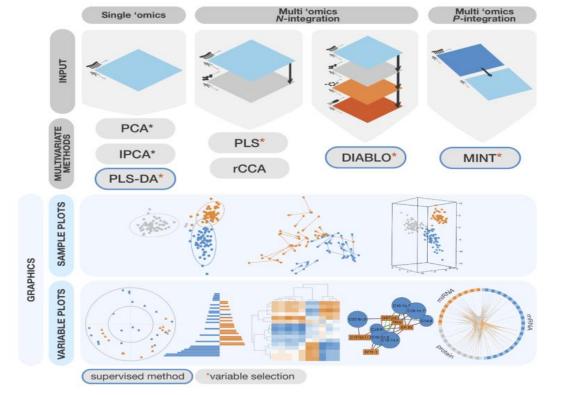
Bean spermosphere peptidome

Distribution of 345 identified peptides in a single bean genotype (H-CON-PC) spermosphere



Future perspectives

of multi-omics^[7] data identify Integration to correlations between spermosphere molecules and macromolecules and microbiome



Development of biobased alternatives to synthetic pesticides to protect and stimulate seeds in a context of agro-ecological transition

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