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A large diversity of non-rhizobial endophytes found in legume root nodules in Flanders (Belgium)

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ABSTRACT

We analysed the genetic properties of non-rhizobial root nodule endophytes (NRE) isolated from indigenous legumes in Flanders. In total, 654 isolates were obtained from 30 different plant species within the Faboideae legume subfamily. Partial sequencing of the 16S rRNA gene revealed a large diversity of different taxa from the classes Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, Firmibacteria, Flavobacteria and Sphingobacteria. Many of the isolates belonged to the genera *Bacillus* (17.9%) and *Pseudomonas* (15.9%). No symbiosis (*nodC*) or nitrogen fixation related genes (*nifH*) could be detected amongst the isolates, which indicate the endophytic nature of the bacteria. Statistical analysis grouped the investigated plant species into six clusters according to the presence of particular NRE. However, no correlations could be found within these six clusters towards plant tribes or ecoregions the plants had been sampled from. Cluster analysis of the ecoregions according to the presence of NRE, revealed correlations between bacterial genera and those areas. However, groups present in the ecoregions did not correlate with the groups present in the different plant clusters. When combining our previous study on rhizobial diversity recovered from the same sampling campaign (De Meyer et al., 2011) with the current study, 84.1% of the isolates belonged to the traditional rhizobia groups and only 15.9% were NRE. The Loamy ecoregion yielded the lowest number of culturable NRE (8.04%) and the Campine ecoregion the highest number (24.19%). The present study highlights the frequent presence of these NRE in root nodules. The occurrence of certain rhizobia was correlated with the presence of particular NRE, suggesting their presence may not be accidental, however their functions remain unclear at this point.

1. Introduction

Rhizobia are soil bacteria capable of symbiosis with legume plants where they can reside in root or stem nodules and perform nitrogen fixation for the host. While traditionally, rhizobia belonged to the genera, *Azorhizobium*, *Bradyrhizobium*, *Ensifer*, *Mesorhizobium* and *Rhizobium* (Sawada et al., 2003), in recent years nitrogen fixing root nodule bacteria have also been described in other Alphaproteobacterial genera, including *Ochrobactrum* (Trujillo et al., 2005), *Methylobacterium* (Sy et al., 2001), *Microvirga* (Ardley et al., 2012; Radl et al., 2014), *Devosia* (Rivas et al., 2003) and *Phyllobacterium* (Zakhia et al., 2006). Furthermore, so-called Betarhizobia have in the last ten years been described in the Betaproteobacterial genera *Burkholderia* and *Cupriavidus* (Chen et al., 2001; Moulin et al., 2001; De Meyer et al., 2013a; De Meyer et al., 2013b; De Meyer et al., 2014). In addition to strains that can elicit nodules and belong to documented rhizobial species, several other bacterial species have been reported from legume nodules without a clear indication of their role within the host. In the absence of positive nodulation tests, they can be regarded as non-rhizobial endophytes (NRE). These include i.a., Alphaproteobacteria (*Aminobacter* (Estrella et al., 2009), *Ochrobactrum* (Zurdo-Pineiro et al., 2007; Imran et al., 2010), *Methylobacterium* (Palaniappan et al., 2010), *Devosia* (Bautista et al., 2010) and *Phyllobacterium* (Mantelin et al., 2006)), Betaproteobacteria (*Herbaspirillum* (Valverde et al., 2003) and *Shinella* (Lin et al., 2008)), Gammaproteobacteria (*Pantoea*, *Enterobacter* and *Pseudomonas* (Benhizia et al., 2004; Ibáñez et al.,

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Table 1
Genera recovered in this study with their rep-clusters, identification according to partial 16S rRNA gene sequencing and the number of isolates.

Genera	Representative strains	Rep-clusters	# isolates
Actinobacteria			
Actinoplanes sp.	R-45801	Alone	1
Aeromicrobium sp.	R-45950, R-45951	411	13
Arthrobacter sp.	R-45644, R-45645,R-45677, R-45678, R-45679, R-45733, R-46132, R-46278, R-46319	Alone, 317, 403, 443	24
Brevibacterium sp.	R-45585, R-45586	Alone	2
Corynebacterium sp.	R-45865, R-45902, R-45903, R-45927, R-46008	Alone, 172	5
Curtobacterium sp.	R-46162, R-46314	Alone, 69	10
Kocuria sp.	R-45655, R-45665, R-45689, R-45691, R-45692, R-46308	Alone, 257, 442, 448	20
Leifsonia sp.	R-45745, R-46062, R-46076, R-46167, R-46259	Alone, 427	7
Microbacterium sp.	R-45570, R-45573, R-45658, R-45659, R-45676, R-45694, R-45704, R-45758, R-45762, R-45772, R-45841, R-45861, R-45991, R-46024, R-46029, R-46031, R-46041	Alone, 79, 185, 275, 324, 325, 404, 415, 444	43
Microbispora sp.	R-45698	Alone	1
Micromonospora sp.	R-45554	Alone	1
Moraxella sp.	R-45536, R-45904	Alone, 24	10
Mycobacterium sp.	R-45620, R-46056, R-46330	Alone, 77, 180	5
Oerskovia sp.	R-45820	Alone	1
Plantibacter sp.	R-46164	Alone	1
Promicromonospora sp.	R-45862, R-45892, R-46030, R-46035	Alone, 326, 379	16
Rhodococcus sp.	R-45548, R-45551	Alone	2
Sphaerisporangium sp.	R-46174	Alone	1
Streptomyces sp.	R-45560, R-45795, R-45838, R-45839, R-45840, R-45852, R-45853, R-45856, R-45857, R-45858, R-45880, R-46032, R-46033, R-46034, R-46036, R-46037, R-46057, R-46058, R-46156, R-46264, R-46282, R-46320	Alone, 280, 321, 336	30
Alphaproteobacteria			
Ancylobacter sp.	R-45799, R-45800	Alone	2
Bosea sp.	R-45681, R-46060, R-46070, R-46073	Alone, 6	5
Caulobacter sp.	R-46323	Alone	1
Inquilinus sp.	R-45827, R-46318	Alone, 323	3
Novosphingobium sp.	R-45660	329	2
Paracoccus sp.	R-46302, R-46307	Alone	2
Phyllobacterium sp.	R-45564, R-45798, R-46124, R-46157	Alone, 129, 130, 181	9
Sphingomonas sp.	R-46285, R-45731, R-45732	Alone, 211	3
Sphingomonadaceae sp.	R-46192	Alone	1
Betaproteobacteria			
Herbaspirillum sp.	R-45723	Alone	1
Massilia sp.	R-45804, R-45805, R-45830	99, 100, 335	6
Roseateles sp.	R-45571	Alone	1
Variovorax sp.	R-46208	33	10
Firmibacteria			
Bacillus sp.	R-40421, R-45534, R-45535, R-45537, R-45540, R-45543, R-45549, R-45607, R-45608, R-45628, R-45640, R-45650, R-45656, R-45667, R-45669, R-45671, R-45672, R-45706, R-45708, R-45775, R-45785, R-45787, R-45792, R-45793, R-45794, R-45824, R-45833, R-45837, R-45842, R-45851, R-45859, R-45885, R-45890, R-45942, R-45943, R-45944, R-45945, R-45947, R-45997, R-46011, R-46013, R-46020, R-46025, R-46141, R-46144, R-46146, R-46152, R-46169, R-46176, R-46193, R-46216, R-46226, R-46228, R-46238, R-46245, R-46246, R-46263, R-46279, R-46280	Alone, 23, 30, 161, 170, 188, 303, 362, 375, 376, 377, 378, 380, 420, 421, 422, 423, 424, 425, 432, 434, 435, 439, 440	117
Brevibacillus sp.	R-45680	Alone	1
Cohnella sp.	R-45709	428	1
Exigobacterium sp.	R-45918	Alone	1
Lysinibacillus sp.	R-45541, R-45670, R-45748, R-46326	Alone	4
Paenibacillus sp.	R-45550, R-45610, R-45623, R-45647, R-45649, R-45664, R-45673, R-45674, R-45675, R-45701, R-45776, R-45786, R-45807, R-45812, R-45813, R-45814, R-45815, R-45816, R-45939, R-45993, R-46010, R-46038, R-46080, R-46203, R-46243, R-46244, R-46251, R-46252, R-46257, R-46269, R-46305	Alone, 2, 4, 5, 12, 101, 111, 169, 171, 238, 357, 370, 381, 431	84
Staphylococcus sp.	R-45577, R-45580, R-45641, R-45663, R-45688, R-45690, R-45693, R-45875, R-46012, R-46052, R-46142, R-46143	Alone, 7, 406, 412, 413, 419	20
Flavobacteria			
Chryseobacterium sp.	R-45581, R-46064	Alone, 429	7
Gammaproteobacteria			
Acinetobacter sp.	R-45867	313	4
Buttiauxella sp.	R-45774	110	5
Enhydrobacter sp.	R-45682, R-45683, R-45684, R-45685, R-45686, R-45687	Alone, 426, 449	11
Enterobacter sp.	R-45810	82	7
Erwinia sp.	R-45811	Alone	1
Pantoea sp.	R-45539, R-45717, R-45789, R-45806, R-46081, R-46239, R-46301	Alone, 16, 281, 333, 347	27

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