Applied Soil Ecology 94 (2015) 72–79

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Enhancement of soil suppressiveness against *Rhizoctonia solani* in sugar beet by organic amendments

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ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form 17 April 2015
Accepted 4 May 2015
Available online 23 May 2015

Keywords:
Rhizoctonia solani
Soil suppressiveness
Organic amendments
Chitin
Animal by-products
Lysobacter spp

ABSTRACT

The efficacy of different organic soil amendments on disease suppression to *Rhizoctonia*solani AG 2-2IIIB was tested in a bio-assay with sugar beet as a test plant. *Lysobacter* populations in soil were quantified as a possible mechanism for disease suppression. Disease spread through the bio-assay tank was significantly reduced up to 86, 83, 52, and 48% after amending the non-sterilized soil with yeast or chitin at a rate of 0.3% (w/w) in consecutive experiments. Inexpensive protein-rich waste products from food industry (i.e., feather, hoof, meat, blood and fish meal) also effectively increased *Rhizoctonia*-disease suppression. Several plant-derived products (e.g., spent mushroom compost, dried algae, spent brewer's grain, *Brassica* seed meal) were not effective. *Lysobacter* populations naturally present in the soil were increased 3–10 fold (measured by a TaqMan quantitative PCR) in soils amended with organic compounds that stimulated *Rhizoctonia*-disease suppression. The role of *Lysobacter* as a key factor in *Rhizoctonia*-disease suppression, however, could not be confirmed by adding *Lysobacter* isolates to a sterilized soil amended with yeast or chitin. Hence, we hypothesize that unexplored biological factors were involved in disease suppression, since the tested soil became conducive after gamma-sterilization. The consistent enhancement of *Rhizoctonia*-disease suppression in sugar beet with yeast and chitin amendments, and the efficacy of inexpensive protein-rich waste products such as feather meal and hoof meal in our bio-assays, warrants further study in field experiments.

1. Introduction

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is a soil-borne fungal pathogen, which causes economic yield losses in many different agricultural crops worldwide (González García et al., 2006). *R. solani* AG 2-2IIIB severely hampers sugar beet cultivation, with an estimated affected area of 70,000 ha in Europe (i.e., 5% of the cropping area) (http://www.kwsbenelux.com/global/show_document.asp?id=aaaaaaaaaaaeonn). Partial resistant varieties are widely grown, but their yields are in general lower in comparison with susceptible cultivars. For an optimal yield, additional control measures remain necessary. The enhancement of soil suppressiveness would be a profitable strategy for farmers to control *Rhizoctonia* diseases.

The addition of organic compounds to soil has been described as a method to stimulate disease suppression of soil-borne pathogens, including *R. solani* (Bonanomi et al., 2010; Bonilla et al., 2012). However, especially for *R. solani* the effects of organic amendments on suppression rates are rather unpredictable (Bonanomi et al., 2010; Termorshuizen et al., 2006). On the one hand, organic amendments can inhibit pathogen development by enhancing competition with antagonistic populations (Hallmann et al., 1999; Sato et al., 2010; Wongkaew and Homkratoke, 2009) or by production of fungistatic volatiles which inhibit the germination of fungal resting bodies (Garbeva et al., 2011; Papavizas, 1976). On the other hand, *R. solani* is a plant pathogen with saprotrophic capacities. Consequently, it may be able to proliferate on the added organic compounds. Indeed, increased disease incidence has been described for *Rhizoctonia* after soils were amended with organic compounds (Bonanomi et al., 2010; Termorshuizen et al., 2006; Tuitert et al., 1998). Organic amendments also directly affect plant growth by influencing nitrogen availability and soil pH. Thus, the effects of organic amendments on plant health and *Rhizoctonia* development are the result of complex soil factors which can reduce or increase crop yield.

Previous research has shown that three closely related species of *Lysobacter* (*L. antibioticus, L. capsici,* and *L. gummosus*) were present in *Rhizoctonia* suppressive soils (Postma et al., 2010, 2008). Isolates of these species strongly inhibited *R. solani* growth in *in*

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http://dx.doi.org/10.1016/j.apsoil.2015.05.002
0929-1393/

vitro assays. Natural populations of *Lysobacter* spp. are not very abundant in most soils. *Lysobacter* populations in arable fields range from approximately 0.3% of the culturable bacterial population (Postma et al., 2008) to an average of 0.6% of the bacterial cells when detected with molecular methods (TaqMan) (Postma et al., 2011). In other suppressive soils, *Lysobacter* spp. have been detected by the isolation of antagonists or by pyrosequencing, at low population densities (Adesina et al., 2007; Rosenzweig et al., 2012). Stimulation of antagonistic bacteria in the soil environment is a promising method to boost disease suppression. *Lysobacter* spp. are an interesting group of bacteria to stimulate to higher population densities in soil, because of their large potential to inhibit fungal pathogens and their low abundance. No data are available yet about the enhancement of indigenous *Lysobacter* populations in the soil ecosystem. We hypothesized that *Lysobacter* populations might be increased by amending the soil with organic compounds resulting in increased soil suppresiveness.

Lysobacter spp. are known to degrade various biomacromolecules; they proliferate on chitin and decompose nematodes, bacteria, yeasts, and fungi (Reichenbach, 1992). Organic amendments from microbial origin to plant and animal derived materials were selected to test their capacity to stimulate *Lysobacter* populations in soil. Purified products such as chitin, and heterogeneous inexpensive waste products were also evaluated. For a practical application in agriculture, an inexpensive, safe, and effective waste product will be needed.

This study addresses the following questions: (i) which types of organic compounds increase soil suppressiveness to *Rhizoctonia,* (ii) can *Lysobacter* populations be stimulated in their natural soil environment, and (iii) is there a correlation between soil suppressiveness and the abundance of *Lysobacter* populations? Knowing the soil factors (biological or chemical) that contribute to soil suppressiveness to *R. solani* would be a significant step forward in developing soil management strategies for a sustainable agriculture.

In the present paper we describe three experiments where different organic amendments were tested for their efficacy to increase disease suppression to *R. solani* AG 2-2IIIB in sugar beet. Experiments were performed in a climate room with a non-sterilized marine clay agricultural soil and with a known indigenous *Lysobacter* population. Two additional experiments were carried out with sterilized soil with or without added *Lysobacter* populations and with or without selected organic compounds. Finally, several chemical and biological soil characteristics, including disease suppression, were analyzed in control and disease-suppressive soil samples, comparing sterilized with non-sterilized treatments. The different experiments and their main research questions are summarized in Table 1. To determine the effect of the organic amendments on *Lysobacter* populations in soil, and the correlation between *Lysobacter* populations and *Rhizoctonia* suppressiveness, *Lysobacter* populations were quantified in soil samples with a TaqMan assay detecting mixed populations of *L. antibioticus, L. capsici* and *L. gummosus*.

2. Materials and methods

2.1. Origin of soil

A marine clay soil with sandy loam texture (Fluvisol) was collected at Zwaagdijk, in the province of North Holland, the Netherlands (Tóth et al., 2008). Approximately 1000 L was collected from a field where cauliflower had continuously been grown for more than 40 years. The soil was stored outdoors under fluctuating weather conditions. Soil from this batch was used for all experiments described in this study. This soil was previously found to be suppressive against *R. solani* AG 2-1 and it contains antagonistic *Lysobacter* species (Postma et al., 2010).

Part of the soil was sterilized by gamma radiation (60 kGy) and used as a positive control for the development of the pathogen (i.e., conducive control soil). The sterilized soil was also used in experiments IV and V to test the effect of *Lysobacter* presence or absence in combination with organic amendments. After gamma radiation, the soil was always stored for at least 4 days to avoid potential negative effects by the sterilization treatment (Mahmood et al., 2014). In experiment VI a shorter time of exposure to gamma radiation was applied (40 kGy) in order to have a similar incubation period with the organic amendments as in the other experiments.

2.2. Organic amendments

Standard organic amendments used in all experiments were yeast (active dried baker's yeast, Dr. Oetker, Ede, NL) and chitin (practical grade, C71700; Sigma–Aldrich, USA). These amendments comprised 8 and 7 % nitrogen (N), respectively. In order to find products that are relevant for practical application in agriculture, a variety of amendments with different origins was selected and tested (Table 2).

The test soils were amended with the organic products, mixed well and incubated in plastic bags for 1 week at room temperature, until testing for disease suppressive properties and sampling for *Lysobacter* detection was performed. The dosage was 0.3% (w/w), except for spent mushroom compost which was applied as 3% moist product. This higher dosage of spent mushroom compost was applied, since compost is normally used at higher dosages to be effective.

2.3. Soil suppressiveness assays

Soil suppressiveness of differently treated soil was assessed in three independent experiments in September 2009 (Experiment I), April 2010 (Experiment II) and March 2011 (Experiment III) (see Table 1). All experiments included a sterilized control, non-sterilized non-amended soil, non-sterilized soil with yeast, and non-sterilized soil with chitin. Several other amendments added to non-sterilized soil were included in these experiments (see Table 2). The experiments were carried out in four replicates in a randomized block design, with each replicate soil sample in a different block.

Table 1
Overview of experiments with their main objectives.

Experiments	Treatments	Objectives
I, II, III	Non-sterilized soil unamended or amended with yeast, chitin and other products	Evaluate the possibility to enhance disease suppression with organic amendments
I, II, III	Non-sterilized soil unamended or amended with yeast, chitin and other products	Test which products stimulate *Lysobacter* populations in soil
IV, V	Sterilized soil +/− inoculated *Lysobacter* populations and +/− organic products	Evaluate causative relationship between *Rhizoctonia* control and *Lysobacter*
VI	Control and suppressive soil samples, which were sterilized or left non-sterilized	Assess the role of different chemical and biological soil characteristics in soil suppressiveness to *Rhizoctonia* disease