Microbiology and bioactivity of the biodynamic preparation 500

Extensive microbiological studies of the characteristic bacterial composition of the horn manure preparation show specific enzyme activities. The latter appears to have an effect comparable to the plant's own hormone auxin. P500 the efficiency of soil bacteria to increase.

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In contrast to organic farming, biodynamic farming uses characteristic preparations such as field spray preparations or compost additives, as also described in EU Regulation 834/2007. The Biodynamic Preparation 500 made from fermented cow dung is used to improve soil fertility and stimulate a strong root system. Over

We reported its chemical composition in a previous publication. Results from long-term field tests, such as: B. von Mäder and Kol-legen showed that biodynamic practices such as the regular use of preparation 500 improve overall soil quality: In particular, parameters such as organic matter, microbial biomass and diversity were significantly higher in biodynamic variants in comparison to organic ones (7, 8).

The basis for these effects can be found in the field spray preparations.

Shorter field trials have shown that the use of both preparations (P500 and P501) on lentils leads to higher yields in relation to plant biomass, but also to lower and higher carbon and crude protein contents in the grain Nitrate retention in spring soft wheat as well as increased ammonia (NH_4) levels in the soil. (3). Their application was also higher

Mineral carbon content - which is considered an indicator of microbially available carbon - as well as differences in soil microbial fatty acid profiles in the first year of the study.

We recently examined the molecular composition of preparation 500 using nuclear magnetic resonance spectroscopy and thermochemolysis and found an enrichment of biolabile components compared to the starting material. Compared to the starting material manure, previous reports showed that preparations had 500 lower values in pH, CO_2 -

respiration and C:N ratio, contained more nitrate and lost less organic matter (1). The present study was intended to provide a microbial characterization of the preparation 500 as well as indications of its biological activity.

material and methods

Purchased items were used for examination Preparation 500 from three known ones

The original article was published in jmb – Journal of Microbiology and Biotechno-logy (http://www.jmb.or.kr) of the Korean Society for Microbiology and Biotechnology (KMB) under the title "Microbiological Features and Bioactivity of a Fermented Manure Product (Prepa-ration 500) used in Biodynamic Agricul-ture" in the issue of May 28, 2013 p. 23(5) 644-651 and translated for LE in an adapted version with the kind permission of the jmb and printed.

Italian manufacturers from three regions. Three preparations from 2010, one from 2011, and its original manure raw material were analyzed.

Carbon, nitrogen and sulfur of the samples and soils were measured, the microbial population was cultured and sorted according to ARDRA, and then DNA from each bacterial isolate was sequenced to determine the species at the molecular level. The diversity of bacteria and fungi from preparation 500 and soils was estimated using the ARISA method. The activity of the preparation 500 and

sample	%N	%C	%S	C/N
Raw material dung (Rome)	2.57	32.02	0.46	12.46
Preparation 500 (Rome)	2.74	24.80	0.58	9.06
Preparation 500 (Bolzano)	2.39	27.06	0.52	11.33
Preparation 500 (Reggio Emilia)	2.21	26.30	0.56	11.89

Table 1: Percentages of carbon, nitrogen and sulfur as well as the C:N ratio P500 origins

The biodynamic one Horn manure preparation (P500) matures in the ground over winter: How does the raw material, cow dung, change?

Enzymes found in other material such as arylsulfatase, ß-gucosidase, acetate esterase, leucine aminopeptidase, alkaline phosphatase and chitinase were measured using the usual methods. Furthermore, the triggering threshold of cell-to-cell communication of gram-negative bacteria was determined based on the presence of N-acyl-homoserine lactone. Components of plant material, including microbially modified ones, which can trigger nodule formation, were measured using the ß-galactosidase test.

The biological activity of preparation 500 in a solution of 200 mg in 60 ml of water - comparable to field application - was measured by means of the growth reduction of the roots of watercress (Lepidium sativum L.) to an auxin -effect to be determined. The increase in length of seed sprouts (Lactuva sativa L.) was also measured with regard to possible gibberelin effects. The Adus method was used for this purpose. Watercress and lettuce seeds were superficially sterilized, rinsed intensively and ten seeds each were aseptically placed on filter paper in a Petri dish. The seeds germinated in special solutions at 25 °C in the dark, 48 hours for watercress and 72 hours for lettuce, before roots or seedlings were measured.

Results

Material analysis from preparation and Source material: The measurement of the three macroelements carbon, nitrogen and sulfur and the C/N ratio (Table 1) indicate a fermentation process below - in comparison to Starting material – volatilization of carbon without nitrogen losses. This suggests that the mineralized nitrogen is absorbed by microbial biomass.

Analysis of culturable microbial Population: The aerobic fraction from preparation 500 that could be cultivated on the PCS nutrient medium achieved 2.38 x 108 colony-forming units per g dry weight, the anaerobic incubation resulted in 7.85 x 107 colony-forming units. Fungal colonies amounted to 1.2 x 106 units. Table 2 shows the taxonomic composition of the bacterial population: gram-positive species dominate with lower occurrences of actinobacteria and gammapro-teobacteria. There were no significant differences between the samples and origins. The composition corresponds to the transformation potential of low redox conditions that promote slow proteolysis and fermentation of organic matter, i.e. the ripening conditions during the creation of preparation 500.

The biodiversity fromBacteria and mushrooms, Detected using the ARISA method, which is accurate down to individual nucleotides, resulted in between 58 and 65 indicated bacterial species and between 32 and 60 fungal species, with a bacteria to fungi ratio of 1.6 to 1. This is consistent with the chemical characterization, which showed an increase in lignin components as the preparation matured, the decrease of which is linked to fungal activity.

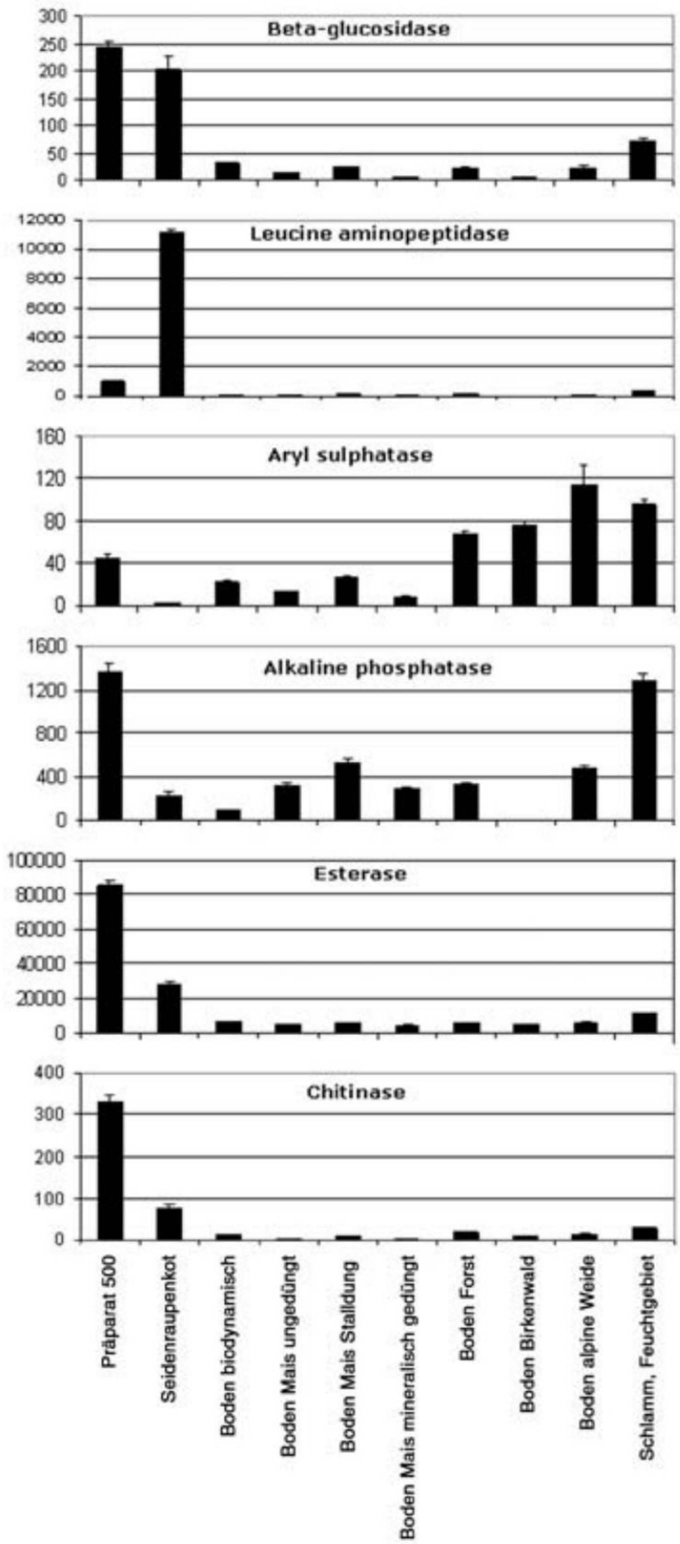
Here, however, there were differences between the years. This was also confirmed by the statistical NMDS analysis.

Enzymatic Test: The six enzyme activities examined were selected as representative indicators for the four main nutrient cycles of carbon, nitrogen, phosphorus and sulfur and as indicators of the degradative potential by fungi and arthropods (chitinase). the general enzyme activity in the form of esterase. Preparation 500 had high specific activities, especially for degrading substances

Art	Taxonomic Group	Percent CFU*
Bacillus megaterium	Firmicutes	46.83
Bacillus safensis	Firmicutes	44.99
Rhodococcus coprophilus	Actinobacteria	3.65
Pseudoxanthomonas dajeonensis	Gammaproteobacteria	1.42
Microbacterium sp.	Actinobacteria	1.06
Aeromonas rivuli	Gammaproteobacteria	0.74
Bacillus pumilus	Firmicutes	0.46
Nocardia globerula	Actinobacteria	0.39
Agromyces fucosus	Actinobacteria	0.32
Sphingopyxis macrogoltabida	Alphaproteobacteria	0.11
Pseudomonas fulva	Gammaproteobacteria	0.04

Table 2: Type and percentage of culturable bacterial species in P500

*(CFU = Colony Forming Unit)



Enzyme activity of preparation 500 compared to soils

Tendencies in the form of ß-glucosidase or chitinase (degradation of complex polymers). The potential for hydrolysis of organic phosphorus esters is also high, but the activity of leukine aminopeptidase, an enzyme in the nitrogen cycle, is low. In comparison to the increased values of uncultivated soils, which are higher than those of cultivated soils, regardless of whether dynamic or conventional, the activity of the preparation 500 was significantly higher (Fig. 4). This suggests that organic farming practices stimulated with Preparation 500 balance activity levels towards a natural context, towards a more intense carbon cycle and more efficient biological turnover or sustainable fertility in relation to the nitrogen cycle -ensure running. Conventionally managed systems, on the other hand, respond to the addition of organic material with very high levels of activity.
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Gene-activating ingredients, e.g. B. the genes for nodule formation in legumes, there was no enzymatic evidence: triggering the flavonoid -similar activities or flavones could not be determined.

Plant hormone-like activation act: When watercress was treated with solution of preparation 500, an auxin-like activity of the order of 0.03 ppm indoleacetic acid was measured. However, no effect was found regarding a gibberelin-like influence on the growth of lettuce. The concentration of auxin-like activity when used as a field spray preparation (200 g dissolved in 60 liters per hectare) is of the same order of magnitude as when using in-dol-3 acetic acid (IAA), which are found in biostimulants such as alfalfa hyd-rolysate or lignosulfin humate occurs. Our measurements support the assumption that spraying preparation 500 causes an active hormone-like effect on the plants.

discussion

Preparation 500 is endowed with significant enzymatic-specific activity, is also rich in microorganisms, especially bacteria, and has an auxin-like effect. The presence of a rich microorganism population is consistent with the parallel chemical characterization as well as with the lower presence of fungi. The high proportion of non-degraded lignin residues is likely to be the reason for the biostimulation of microorganisms and plants, similar to green or vermicompost. (9) In fact, various studies show the auxin-like physiological effect of humus extracts from composts on plants. (2, 5). It is also likely that the high content of carbohydrates and peptides in preparation 500 promotes microbial proliferation and thus the activity of rhizospheres.

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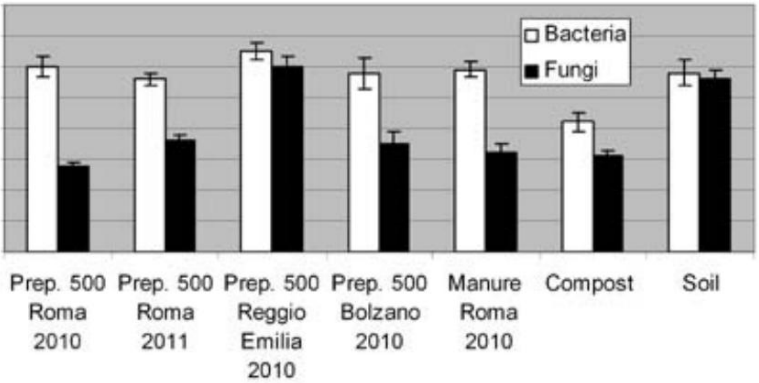
Preparation 500 is reported to promote soil biological activity and root development (6, 13), although it is applied in very small quantities.

Based on the amount of water in a hectare of soil up to a depth of 20 cm - 500,000 liters - the usual amounts of 200 g of preparation per hectare lead to a concentration of 0.004 grams per liter, or to a molecule-related dilution of 2.5 micromoles . This is a very high concentration in the micromolar range in terms of biological activity, as many indications suggest.



The colonization of preparation 500 with fungi and bacterial species in comparison to different origins or to soil and compost.

gen: This is how it starts, for example. B. the nodule formation of legumes stimulated by chito-lipo-oligosaccharides from a concentration of 0.1 nanomole, i.e. a concentration that is 1000 times smaller. Even if the active parts of the preparation 500 are assumed to be significantly lower, for example B. a ten-thousandth of its weight, this is still equivalent to this concentration.

It is therefore unlikely that, at the dosage used, Preparation 500 has an effect as an organic structural fertilizer or microbial inoculation, but it cannot be excluded that it acts by regulating soil bacteria. bacteria recognize and react to extremely low levels of signaling molecules in their environment. Many higher plants have been shown to produce signal-mimicking substances with which they influence the ratio of bacterial density. Preparation 500 is expected to contain abundant potentially bioactive substances, consistent with its microbially mediated slow ripening under low oxygen conditions and resulting proteolytic activity. Similar experiences with other digested substances support this (11).

A complementary, further possible mode of action of Preparation 500 can be hormonal effects on plant growth and development. winding exist. Deffune and Scolfield (4) found that humic acids from this and other biodynamic preparations (505 and 507) elicited a positive growth response in wheat seedlings. A high cytokinin activity was also demonstrated in preparation 500 (10), plus an effective auxin effect, as in the present study. It can therefore be assumed that the biodynamic preparation 500 works by stimulating root development and consequently stimulates the plants to access the soil reserves of nutrients that might otherwise remain untapped. This is consistent with the recommended application, which requires a supply of organic matter in the soil to be effective. It should also be taken into account that auxin stimulates bacterial growth (12), and so acts more through the micro-organisms than directly through the plants.

Studies on further aspects of preparation 500, such as: B. the sequence of the bacterial population during maturity in the soil using metagenomic methods are in progress. I

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