Microbial strains from compost originated from circulated side streams

– Impact on root growth and root hairs

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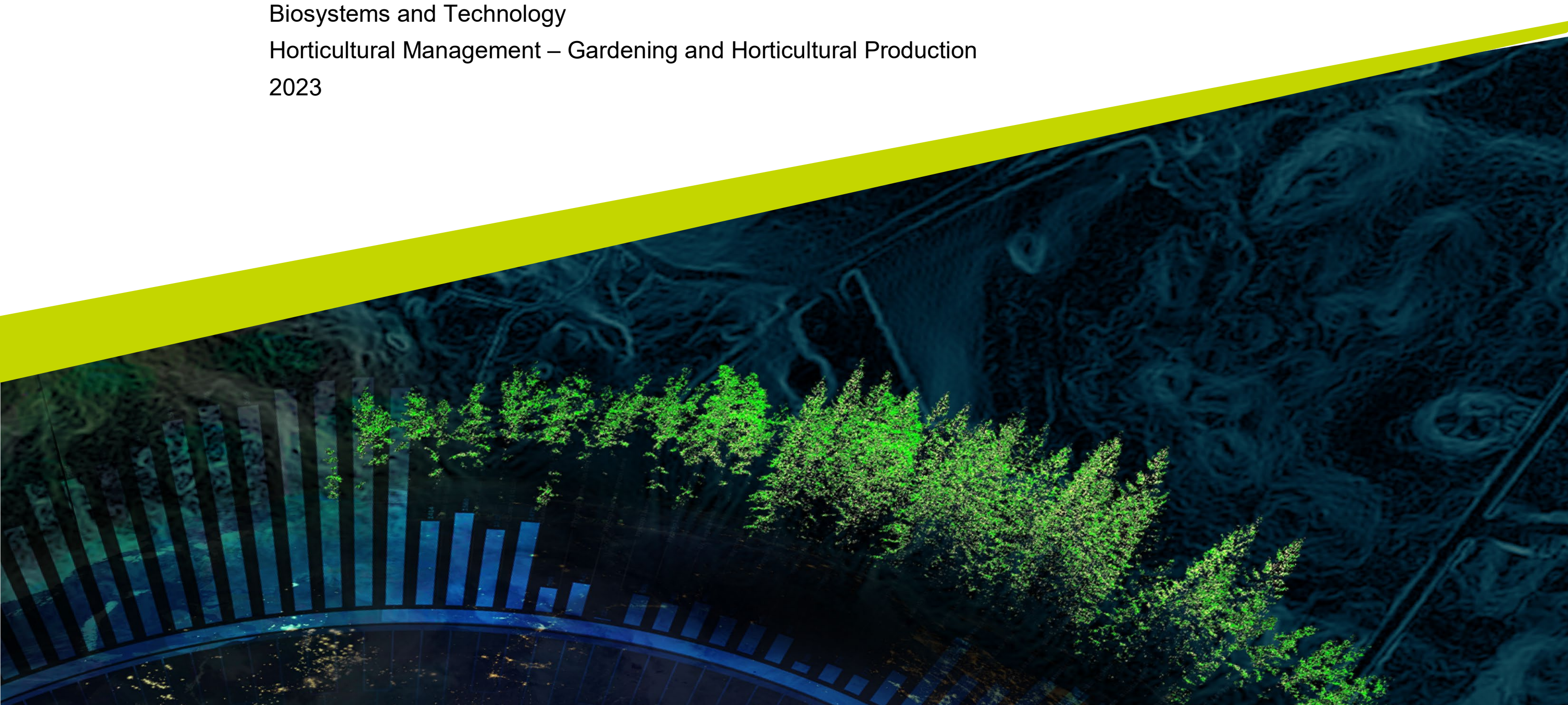

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Abstract

Spent mushroom compost, apple waste and other organic waste materials are of great potential to improve soil health, water holding capacity and to supress plant pathogens. Today compost is used in apple orchards as an additional fertilizer or soil amendment. The aim of the work is to examine the effect of bacterial strains isolated from compost blends containing different fractions of SMC, apple material and woodchips on root growth and root hair formation. Microorganisms play a vital role for growth stimulating effects, formation of root hairs and overall soil health with water uptake and nutrient availability in the soil. Three treatments with different fractions were included in the experiment with microbial enumeration on selective media for the targeted microorganisms. Isolated bacterial strains originated from compost were used in germination, evaluation of root growth and for the formation of root hairs. The results give a preliminary understanding of the microbial content of the compost in the different fractions and their potential to promote root growth and root hair formation.

Keywords: Spent Mushroom Compost, Circulated side streams, Root length, root hairs, Circular Bioeconomy

Svampkompost, restprodukter från äpple branschen och andra organiska material är av intresse för att öka jordhälsan, vattenhållande förmågan och att minska förekomsten av sjukdomar och virus. Idag så används kompost i äppelodlingar som ett extra gödningsmedel eller för jordförbättring. Målet med studien är att undersöka effekten av olika sorters bakterier isolerade från kompost som består av olika fraktioner av SMC, äpple material och träflis på rotlängd och rothårs utveckling. Mikroorganismer har en viktig roll för tillväxt stimulerande effekter, formationen av rothår och för generell jordhälsa med vattenupptag och tillgången på näringsämnen. Tre olika fraktioner undersöktes i studien med mikrobiell räkning på selektiv media för de olika mikroorganismer. Isolerade bakterier som härstammar från kompost användes för grodd, utvärdering av rotlängd och förekomsten av rothår. Resultaten i denna studie ger en inblick på den mikrobiella halten i komposten i de olika fraktioner och deras potential att öka rottillväxten och förekomsten av rothår.

Nyckelord: Svampkompost, cirkulerade sidoströmmar, Rotlängd, rothår, Cirkulär bioekonomi

Table of contents

SLU	Swedish University of Agricultural Sciences
SMC	Spent Mushroom Compost
SMS	Spent Mushroom Substrate

Ash	Inorganic Compounds
Bioeconomy	Economy Based on Biomass
Physico-Chemical	Physical Chemistry
CFU	Colony Forming Unit
PGPR	Plant Growth Promoting Regulator
PGPB	Plant Growth Promoting Bacteria

1. Introduction

1.2. Background

Farmers and fruit growers are focusing more on improving the quality of the soil and soil health, which faces different challenges due to climate change, soil degradation, plant diseases and the continuous increases in fertilizer and diesel prices.

Improving soil quality with sustainable nutrient levels would lead to a soil with a greater nutrient availability, improved water holding capacity and plant resilience towards pathogens and reduction of weed occurrence in the orchard (Licznar-Małańczuk et al., 2021). Compost is used to improve soil health and the microflora present in the soil (Ahmad et al., 2021). The microbiota of composted materials has been shown to have suppressive abilities towards plant pathogens while positively influencing the plant growth and microflora in the soil (Ntougias et al., 2004, Klimas et al., 2016). Compost microbiota highly depends on the inputs of the compost and the material that is used during the process of composting (Blaya et al., 2016).

Spent mushroom compost (SMC) is used today in apple orchards as an additional fertilizer and as a general tool to improve soil structure due to its organic compounds. SMC should not be used fresh due to the high salinity level. The SMC needs to be weathered for a minimum of 6 months (Grimm et al., 2018). During this time, the salt levels in the compost decrease considerably due to leaching from the weathering process. The peak of the leaching starts around the sixth month of weathering and is stabilized after around twelve months, where most of the salt present in the SMC is released during the first year of the process. SMC consists of 60% water and 40 % dry matter, where half of the dry matter is ash (Uzun, 2004). This ash matter can be reduced with the help of weathering which reduces the ash content within 6 months where the inorganic compounds get converted to organic with the help of microbiota (Grimm et al., 2018). The SMC needs to be mixed with other organic inputs and aged between 6-18 months (Uzun, 2004).

The apple industry is dealing with losses in fruit waste due to bad fruits, storage rots and from the juicing industry (Gurpreet Singh et al., 2013). The apple material and its biodegradable organic matter content could improve physico-chemical properties, increase the microbial activity and act as bio-pesticide to control soil-borne diseases (Qin et al., 2021). Bioconversion of agro-industrial waste is a green biotechnology with minimal effects on the environment with renewable resources (Rana et al., 2021). Wood Chips from local foresting or cleared orchards are a great additional source of organic matter. Both waste materials are of great potential to be mixed and circulated back in composting material and to be used for improving the soil health.

1.3. Circular Bioeconomy

Bioeconomy is an important part of the agricultural industry that utilizes biobased material and renewable resources to elevate the usage of waste materials. Circular bioeconomy is recognized as an industrial process that is regenerative and restorative while recycling raw and waste material (Qin et al., 2021). Organic waste from apple orchards is used as fertilizer and as a tool to improve soil structure following bioconversion (Qin et al., 2021).

Farmers are experiencing challenges due to the increasing surge of food products while continuously dealing with losses due to bad fruits, storage rots and from the juicing industry (Gurpreet Singh et al., 2013). The juicing industry generates huge quantities of solids and liquid waste material. The solid waste residue usually consists of a mixture known as "apple pomace" that includes seeds, pulp and skin derived from the production of refined apple products such as jam and juice (Gurpreet Singh et al., 2013). Microorganisms, primarily fungal and bacterial species are being used as biological treatments in the agricultural industry. In this current study the compost is of origin from äpplerikets apple cultivation mixed with spent mushroom compost and local woodchips. This generates a bigger volume of nutrient rich compost which is a green sustainable way following bioconversion of materials that would otherwise be burned or transported away for other means.

Mushroom production has increased rapidly in the last few years, where the most cultivated species in Sweden include *Agaricus bisporus "*Champignon Mushroom", *Lentinula edodes* "Shiitake" and *Pleurotus* spp "oyster mushroom" (Persson, J., 2023). The *Agaricus bisporus* which grows on lignocellulose such as straw, sawdust or wood chips but is generally grown in compost which consists of straw, manure, gypsum, and nitrogen containing compounds (Grimm et al., 2018). The content in the mushroom substrate is greater in the fresh substrate than in the

SMC due to the fungal biodegradation during cultivation (Umor et al., 2021). When the mushrooms are harvested, the spent mushroom substrate residues can be reused to benefit the water holding capacity, soil aeration and to improve the structure of the soil with additional organic matter (Grimm et al., 2018). For every five kg of substrate, you get one kg of SMS (Umor et al., 2021). Numerous studies have shown that composted materials have a variety of beneficial properties which includes their potential disease reducing effects due to the high activity of microbiota. In apple orchards, SMC is used as a fertilizer, soil amendment or as an alternative pesticide (Uzun, 2004). Mineral fertilizer contains nutrients in the form nitrogen, phosphorus, and potassium, however in SMC, the nutrients are released slowly over a longer period which benefits the uptake of the nutrients from the plants (Uzun, 2004).

1.5 Role of microorganisms

The use of antagonistic microorganisms has been investigated for their potential to suppress root pathogens. In this study the microorganisms *Pseudomonas* spp and a general bacterial flora originated from the compost are of interest. *Pseudomonas* spp and *Enterobacter* spp have been widely investigated for their anatogonistic properties against root pathogens (Cernava et al., 2019, Xu et al., 2011). *Pseudomonas* spp produces secondary metabolites that have a suppressive effect on root pathogens such as *Phytophthora infestans* and *Phytophthora ramorum* (Xu et al., 2011).

Previous studies have shown anatognistic microogranisms such as *Trichoderma* and *Pseudomonas* spp from composted material have the potential to suppress a range of plant diseases (Dukare et al., 2011, Pot et al., 2021).

The usage of biocontrol agents towards *Phytophthora* spp is an environmentally friendly strategy to minimize the usage of chemical fungicides and have a low environmental impact on its control (Handelsman and Stabb, 1996, Blaya et al., 2016). Biocontrol agents usually produce several antibiotics which prevents resistance towards the agent due to the resistance to multiple antibiotics occur at a low frequency (Handelsman and Stabb, 1996).

The use of PGPBs in plant production have been widely studied for their potential to promote plant growth and general plant health (Abdelaal et al., 2021). PGPBs are phosphate-solubilizing and nitrogen-fixing microogranisms that can be applied as biofertilizers and increase the nutrient availability of the soil by improving microbial processes (Abdelaal et al., 2021). PGPBs play a vital role in soil nutrient management which improves plant growth and plant production. Phosphor is an essential element and important to plant growth and development through processes including photosynthesis and cell division. Organic acids are secreted from the bacteria by facilitating a release of the bound forms of phosphates by lowering the pH in the rhizosphere (Abdelaal et al., 2021). Applications of PGPBs in plant production include seed treatment, seedling dipping and application to the soil to obtain a greater yield and improving the soil health with a richness in valuable microflora and nutrient availability (Abdelaal et al., 2021). The use of PGPBs can also confer to the tolerance of the plants to various stresses (Abdelaal et al., 2021). PGPBs could be applied as a strategy towards reducing the environmental impacts by improving water and nutrient uptake and thereby increasing the environmental stress tolerance (Abdelaal et al., 2021). Among the various stresses induced by environmental changes, drought is one of the most harmful stresses for plant production (Abdelaal et al., 2021). PGPBs have been shown to positively influence plant growth under drought stress due to rhizobacteria producing exopolysaccharides such as alginate and cellulose (Abdelaal et al., 2021). Exopolysaccharides are responsible for the attachment between bacteria, root systems, and soil particles, thus may play a vital role under drought conditions for both plants and microbial populations (Abdelaal et al., 2021). Studies have shown that the use of *Pseudomonas* spp. as an inoculation agent in Soybean plants increased fresh weight and stem height under water deficit stress (Radhakrishnan et al., 2014). Furthermore, it has been shown that the application of *Pseudomonas* spp. in maize plants led to an improvement of agronomic including root elongation (Bhattacharyya and Jha., 2012). PGPBs have the potential to eliminate the harmful effects of stresses induced by environmental changes and drought (Abdelaal et al., 2021).

1.6 Aim

The purpose of the study is to examine the impact of bacterial strains isolated from compost containing different fractions of SMC, apple material and wood chips on root growth and root hairs.

1.7 Research questions

- Does the microbial content of the compost contribute positive effects on seed germination and root growth of basil seeds.
- Do the compost fractions have the potential to enhance the growth and development of root hairs.

1.8 Hypothesis

The composted material is of good microbial quality and contributes to positive effects on the germination potential of seeds, root growth and the presence of root hairs.

2. Material and Method

2.1. Compost materials and treatments

The compost used in this study has been aged for 12 months and then divided into three different fractions and labeled G1-G3. 1 sample of 5 grams from each fraction was collected.

G1- 33% apple material: 33% SMC: 33% wood chips
G2- 25% apple material: 50% SMC: 25% wood chips
G3- 40% apple material: 20% SMC: 40% wood chips
Control – 100% Peat

2.2. Enumeration of microorganism

The enumeration of the microflora from the compost fractions was performed as described by Khalil and Alsanius (2011). The samples of 5g compost and of the control were collected and added to 12,5 ml of detergent solution (200 ml H_2O, 1,7 g NaCl) and shaked at 200 rpm for 20 minutes at room temperature. The enumeration of the microflora was performed on selective media for the targeted microorganisms using dilution series.

For the dilution, a series of test tubes were marked with one series per treatment and filled with 450 µl of sterile NaCl and 5 µl in each test tube. The dilution series were performed from the initial suspension of 100 up to 10^5 for enumeration of pseudomonas on Kings B Agar media (KB) and of bacterial flora on Tryptic Soy Agar media (TSA). For the enumeration of fungal flora, a suspension of 100 up to 10^3 were performed on Malt Extract Agar media (MA). A number of two plates per dilution and treatment were used and incubated at 25 C for 24 hours for TSA and KB.

2.2.1. Isolation and pure Culture

After the growth on selective media, a number of five colonies from the respective selected medium and treatment were picked with an inoculation loop and inoculated in test tubes containing 5 ml Tryptic Soy Broth (TSB). 15 test tubes each for TSA and KB. The test tubes where put on a shaker for 34 hours at 150 mot/min. After 24 hours, the cryo culture took part where the tubes where centrifuged at 3000 rpm for 30 minutes. The solution was discharged, and a 1 ml freezing buffer was added to the test tubes. The culture was then transferred to 2 ml Eppendorf tubes and kept in the freezer at -80°C.

2.4.3 Evaluation of root growth

Water agar (*Tab.1, appendix* 1) was mixed and autoclaved, one plate per colony, 30 plates in total with the control group. The basil seeds were inoculated with bacterial isolates from KB and TSA with a control group in sterile water and were put on a shaker for 24 hours at 180 mot/min. After they had been shaken the solution was discarded, and 4 seeds from each container were put on the water agar plates to grow for 2 weeks. Germination was recorded after 24 hours and at the end of the study. Root length measured after 2 weeks, and observation of the root hairs and growth were done under a microscope. For the observation of root hairs, they got categorized into different grades of root hair growth (scale 0-2) as follows:

0 = no root hair growth/little hair growth
1 = medium hair growth (less than half of the root)
2 = root hair growth on more than half of the root

3. Results

3.1. The enumeration of microorganism

The highest amount of CFU was shown in fraction G1 in the TSA group with the lowest CFU count recorded in the KB media, in fraction G3 (fig. 1). Fraction G2 and G3 in the MA group exceeded the control group. Furthermore, the amount of colony forming units in the MA group had the highest CFU count in the G3 fraction (fig. 1). In the KB treatment, no fraction exceeded the control group and in the TSA group both G1 and G2 exceeded the control group. Fractions G1 and G3 contained higher amounts of microorganisms compared to the G2 fraction with high levels of general bacteria, *Pseudomonas* spp. and fungal flora (fig. 1).

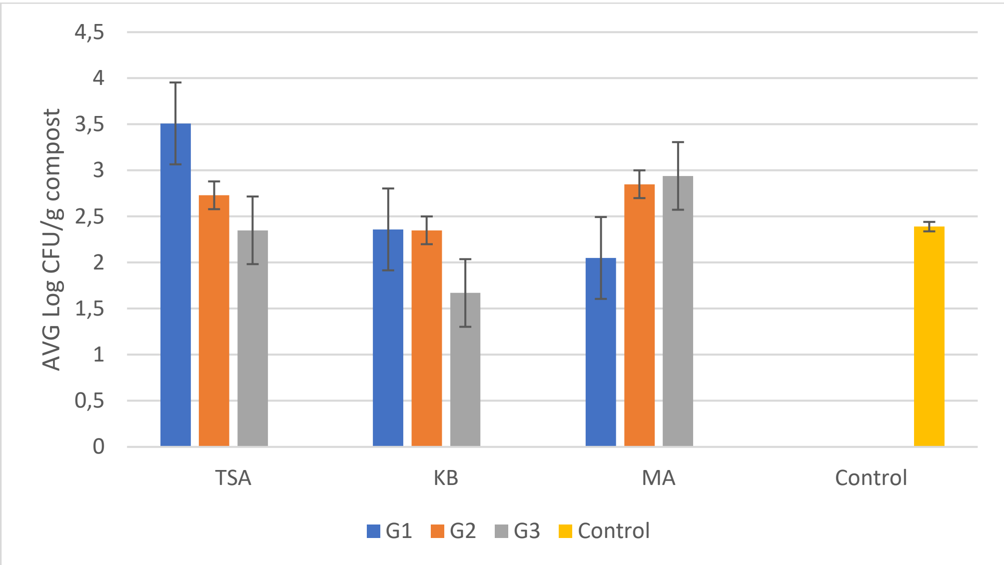

Figure 1: Average log CFU/g compost in treatments, G1 = apple material (33%), SMC (33%), woodchips (33%). G2 = apple material (25%), SMC (50%), woodchips (25%). G3 = apple material (40%), SMC (20%), wood chips (40%), Control = Peat (100%).

3.3 Germination

Seed germination was recorded after 24 hours and at the end of the study (table. 1 & 2. The control group had a 75% germination rate (table. 2) with 0 % root hair growth and non-viable roots at the end of the study (fig. 3 & 4). KB treatment fraction G1 had 80% germination after 24 hours and 85 % germination rate at the end of the study (table. 1 & 2). TSA treatment fraction G1 had a 65% germination after 24 hours and 80% germination rate at the end of the study (table. 1 & 2). G2 fraction in the different media did not have a big noticeable difference in germination after 24 hours and at the end of the experiment. Fraction G2 in the TSA media had an increase of 5% in germination at the end of the study (table. 2). The G3 fraction in both TSA and KB had the biggest increase in germination at the end of the study with a 40% increase in TSA and 25% increase in KB (table. 1 & 2). Fraction G1 had the highest germination rate in both media and the highest germination percentage was recorded in fraction G3 in the TSA treatment (table. 2).

Table 1: Percentage of germination in the different fractions after 24 hours in Kings B Agar media (KB) and Tryptic Soy Agar media (TSA)

Percentage of germination after 24 hours		
Fraction	KB	TSA
G1	80,0%	65,0%
G2	60,0%	70,0%
G3	30,0%	45,0%
Control	75,0%	75,0%

Table 2: Percentage of germination in the different fractions at the end of the study in Kings B Agar media (KB) and Tryptic Soy Agar media (TSA)

Percentage of germination at the end of the study		
Fraction	KB	TSA
G1	85,0%	80,0%
G2	60,0%	75,0%
G3	55,0%	85,0%
Control	75,0%	75,0%

3.2. Evaluation of root growth

In the evaluation of root growth, TSA treatment with bacterial isolates from fraction G2 exceeded the control group in length (fig 2). Seeds inoculated with isolates in both KB and TSA treatments showed a greater growth length than the control (fig. 2). Seeds in the control group had a greater length than fraction G1 and G3 in the

TSA treatment group. In the group with Kings B agar, seeds inoculated with *Pseudomonas* spp. from the G1 proportion showed a greater root growth than the control group (fig. 2). Seeds in the control group exceeded the length of fraction G2 and G3 in the KB treatment group (fig 2). Furthermore, the G3 treatment showed a statistically significant difference in root length between G3 treatment and the control group (P-value 0.0037).

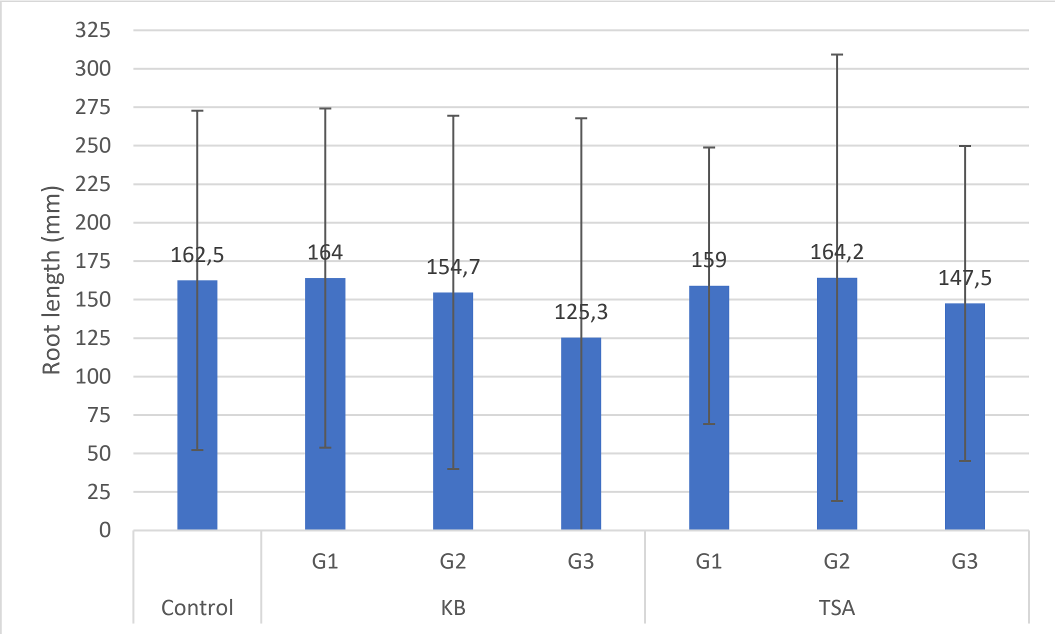

Figure 2: The effects of bacterial strains isolated from different compost fractions consisted of Spent mushroom compost, apple waste and wood chips on root length of basil plants. The bacterial strains were isolated from selective media for Pseudomonas spp., Kings B agar (KB), for general bacterial flora Tryptic Soy Agar (TSA) and a control group that consisted of peat. Statistical analyses where performed using the ANOVA method.

3.4 Root hair growth

For the examination of root hair growth, seeds were categorized into different grades of growth (scale 0-2). In both KB and TSA treatments there was an abundance of root hair growth. Seedlings in the KB group showed a positive response to inoculation of the isolate with 100% of roots with root hairs, 13% roots with medium root hair coverage and 87 % of roots with root hair on more than half of the root (table 3). Seedlings in both treatments started forming side roots with majority of side roots forming in the KB treatment group (fig. 7, fig. 8, fig. 9). TSA group had 20% of roots with medium root hairs and 80% of roots with root hairs on more than half of the root (table 3). TSA group had a bigger percentage of roots with medium root hairs compared to KB treatment and a smaller percentage of roots with hairs on more than half of the root (table 3). The control group had a 0% root hair formation (fig. 3 & 4). Both TSA and KB treatments had a bigger percentage than the control group with 0 % in the different grades of growth (table 3).

Figure 3: Control Group with no root hairs (0)

Figure 4: Control group with no root hairs (0)

Figure 5: Root from the TSA treatment with root hairs on more than half of the root (2)

Figure 6: Root from the TSA treatment with root hairs on more than half of the root (2)

Figure 7: Root hair KB treatment with hairs on more than half of the root (2)

Figure 8: KB treatment with side root and root hairs on less than half of the root (1)

Figure 9: KB treatment with side root and root hair on less than half of the root (1)

Table 3: Root hair growth percentage distribution with the different grades of growth in Tryptic Soy Agar media (TSA), Kings B Agar media (KB) and control group.

Evaluation of root hair growth percentage	TSA	KB	Con-trol
Percent root growth with root hairs	100%	100%	0%
Percent of root growth with medium hairs (1)	20%	13%	0%
Percent root growth with hairs on more than half of the root (2)	80%	87%	0%

4. Discussion

The results in the study indicate that SMC, apple material and wood chips are of great potential after bioconversion to enhance the root growth and development of root hairs. The proportions play a vital role in the richness of microorganisms in the compost, which affects the seeds development and plant growth promoting microorganisms.

The germination of the control group had a 75% germination rate at the beginning and at the end of the experiment. In the KB and TSA media there was differences in germination after 24 hours and at the end of the experiment. The germination percentage in G2 did not show big noticeable differences after 24 hours and at the end of the experiment. KB treatment fraction G1 had the highest percentage of germination out of the 3 fractions with the lowest percentage in G3. Fraction G3 in the KB treatment group also had the lowest CFU and the lowest percentage of germination followed by fraction G2. In the TSA treatment fraction G3 had a 40% increase and had the highest germination percentage at the end of the study. The microbiota of compost highly depends on the inputs of the compost and the material used during the composting process (Blaya et al., 2016). The microbiota of compost has been shown to positively influence the plant growth and the microflora in the soil (Ntougias et al., 2004, Klimas et al., 2016). The materials used in the compost of this study came from circulated side streams and were rich in microorganisms, which had positive effects on germination and root growth. The difference in germination indicates that bacterial strains isolated from compost influence germination.

The effect of general bacteria and *pseudomonas* spp. on root growth investigated in previous studies (Pečenková, 2017). *Pseudomonas* spp. and other PGPRs promote plant growth by producing secondary metabolites such as plant growth-stimulating hormones, indole acetic acid (IAA) and siderophores (Sah et al., 2021). KB treatment inoculated with *Pseudomonas* spp. expressed root hairs with the formation of side roots. The percentage of root growth with root hairs in KB treatment was 100 % with a bigger percentage of side roots compared to TSA treatment. Seeds in the TSA treatment inoculated with general bacterial flora expressed mostly root hairs and not a big percentage of side roots. Roots with root hairs on more than half of the roots were 80% for the TSA group and 87% for the KB group. Phyto-hormone producing bacteria have been widely studied for their effects towards germination, plant growth, water uptake and nutrient availability (Sah et al., 2021). IAA, which is synthesised from the amino acid tryptophan, stimulates rapid cell elongation, cell division, differentiation and promote a better uptake of nutrients (Sah et al., 2021). Root hairs play a vital role in absorption of minerals, uptake of water, anchoring in the soil (Cai and Ahmed, 2022) which is out of interest for farmers where the climate is getting warmer and dryer. Promoting root growth and the formation of root hairs could lead to a healthier crop and better yields as the plant could penetrate deeper into the soil for the search of water and nutrients. Young tree saplings in orchards would have a better survival potential with the help of a strong root system and a big percentage of root hairs. Climate change affects the farmer and farming practices, longer periods of drought with minimal to no rain makes it harder for the farmer to stay afloat. This leads to a bigger financial burden to the farmer in means of watering, management, and loss in yield. Compost in plant production is of great potential as a soil amendment or fertilizer to promote plant growth, root length water uptake and to promote general plant health.

Bacterial strains isolated from compost did not have significant differences. Fraction G3 showed the least amount of root growth in the TSA treatment but had a higher germination percentage than fraction G2 (fig 2). Similar numbers were obtained from root length in the TSA treatment where fraction G2 stood out with the biggest root length out of the three different fractions. The results indicate that fractions G1 and G2 are of interest for plant growth and the formation of root hairs. The amount of fungal flora recorded in the G3 treatment (apple material 40%, SMC 20% and wood chips 40%) indicates that a smaller proportion of SMC mixed in the compost could benefit the fungal flora.

The use of compost originating from circulated side streams are of great potential, to be used after bioconversion. The circulated side streams generate a bigger volume of nutrient rich compost, which is a sustainable following bioconversion of materials that would otherwise be burned or transported away for other means.

5. Conclusion

Microbial strains isolated from compost are of great potential to promote root growth and the formation of root hairs. The usage of compost as a soil amendment or fertilizer in plant production could lead to a better water uptake, healthier crop, and better tolerance towards drought stress. The richness of microbiota in the compost plays a vital role in promoting root growth and formation of root hairs with PGPBs. The microbial content of the compost needs to be rich in microbiota to have the potential positive effects of PGPBs. Promoting root length and the formation of root hairs could lead to an enhanced water uptake, better nutrient availability, and resistance towards various environmental stresses. The use of PGPBs in plant production could lead to better general plant health by increasing the nutrient availability, thereby increasing the tolerance towards environmental stress factors while increasing the microbial processes in the soil. The use of *Pseudomonas* spp. in plant production are of great means for the targeted beneficiaries as shown in previous studies (Radhakrishnan et al., 2014; Bhattacharyya and Jha., 2012) and shown in this study on the effects of germination, root length and root hair formation. Compost originating from circulated side streams could lead to a better bioeconomy since it reduces waste materials and creates greater volumes of nutrient and microbial rich compost. The findings show that treatment G1 and G2 are out of interest for improving soil health and root growth but need further testing and evaluation.

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7. Appendix

7.1. Appendix 1

Table 2: Media recipes

Medium name	**Ingredients**	**Incubation temperature °C**	**Incubation time (hours)**
Tryptic Soy Agar (TSA)	(Tryptone soy agar (Difco): 2 g, Bacto™ agar (Difco): 7,5 g, H_2O: 500 ml)	25	48
Kings B Agar (KB)	(Peptone: 10 g, Aq: 500 ml, Bacto agar: 7,5 g, K_2HPO_4: 0,75 g, $MgSO_4 * 7H_2O$: 0,75 g, Glycerol (99%): 7,5 ml. For the enumeration of Pseudomonas Spp.	25	48
Malt Extract Agar (MA)	Malt extract: 15 g, Bacto™ agar (Difco) 10 g, H_2O: 500 ml. For the enumeration of fungal flora	25	72
Water agar	(Bacto™ agar: 20 g, H_2O: 1000 ml) For the evalutation of root growth and root hairs	25	336
TSB	(Tryptic soy broth: 6 g, H_2O: 200 ml) For the isolation and pure culture	22	24
V8	(Bacto™ agar: 7.5 g, $CaCO_3$: 1 g, V8 juice: 100 ml, H_2O: 400 ml)	22	168

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