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Evaluation of rhizobacterial colonization and the ability to induce *Globodera pallida* hatch.

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Summary

Three bacterial isolates, SB13 (*Acinetobacter* sp.), SB14 (*Arthrobacter* sp.) and SB15 (*Bacillus* sp.) were previously isolated from the rhizosphere of sugar beet (*Beta vulgaris* ssp. *vulgaris*) plants and shown to increase hatch of potato cyst nematodes *in vitro*. In this study, the three isolates were assayed for rhizosphere competence. Each isolate was applied to seeds at each of four concentrations (10^5 – 10^8 CFU ml^{-1}), and the inoculated seeds were planted in plastic microcosms containing coarse sand. All three isolates were shown to colonise the rhizosphere, though to differing degrees, with the higher inoculation densities providing significantly better colonisation. The isolates increased sugar beet root and shoot dry weight. Isolates SB14 and SB15 were analysed for their ability to induce *in vivo* hatch of *Globodera pallida* in non-sterile soil planted with sugar beet. After 4 and 6 weeks, both isolates had induced significantly higher levels of percentage hatch compared to controls.

Keywords

Soil bacteria, plant pathogenic nematodes, plant growth promoting rhizobacteria, suicide hatch

Two species of potato cyst nematode (PCN) are major pests of the potato crop, namely *Globodera rostochiensis* (golden PCN) and *Globodera pallida* (white PCN). It has been estimated that, they cause a 9 % loss in total potato yield in the UK (Pickup, 2002) and, jointly, are considered some of the most economically important plant-parasitic nematodes (PPN) in the World (Nicol *et al*., 2011). PCN have spread from their centre of origin in the Andes region of South America and across Europe (Jones and Kempton, 1978), and are now present in most regions where potatoes are grown, with *G. rostochiensis* being more widespread than *G. pallida* (EPPO, 2007).

PCN, in common with most PPN, are generally controlled by one or more of chemical nematicides, cultural practices and the cultivation of resistant cultivars (Oka *et al*., 2000). However, there are major limitations with all of these techniques for the control of *G. pallida*, resulting in the emergence of *G. pallida* as the predominant PCN species in intensive potato growing areas (Minnis et al., 2000; Taylor and Hockland, 2010).

Previous work in this laboratory (Ryan and Jones, 2004) led to the isolation of PCN hatch-inducing bacteria from the rhizosphere of the non-host plant, sugar beet (*Beta vulgaris* ssp. *vulgaris*). These isolates were shown to increase hatch of both PCN species *in vitro*. Ryan and Jones (2004) proposed that some of the isolates that increased hatch had the potential to form part of a trap crop system once inoculated into the rhizosphere of sugar beet, if the effects could be confirmed *in vivo*.

Trap cropping is increasingly being examined for the management of PCN. It is a cultural control strategy that causes PCN to hatch but not

multiply by planting a hatch-inducing crop which does not support multiplication of PCN (Timmermans *et al*., 2006; Turner *et al*., 2006).

Previous studies have demonstrated the potential of rhizobacterial management of PCN. Jatala (1986) showed that some metabolites of *Paecilomyces lilacinus* could be used to induce hatch of *G. pallida* whereas Cronin *et al.* (1997) demonstrated the inhibition of *G. rostochiensis* hatch by chitinase-producing bacteria.

One of the most important factors when appraising bacterial inocula for the biocontrol of soil-borne plant pathogens is the ability of the inocula to colonise the plant rhizosphere (Parke, 1991). In this study, the ability of three isolates: an *Acinetobacter* species (SB13); an *Arthrobacter* species (SB14); and a *Bacillus* species (SB15), known to induce *in vitro* hatch of *Globodera pallida* (Ryan and Jones, 2004), to colonise the rhizosphere of sugar beet grown in microcosms was investigated, and the efficacy of two of the isolates to induce *in vivo* hatch of PCN in soil was examined.

Materials and Methods

Rhizosphere competence study

Isolation of rhizosphere bacteria from sugar beet roots

Ryan and Jones (2004) originally isolated bacteria from the rhizosphere of sugar beet (*Beta vulgaris* ssp. *vulgaris* cv. Crystal) grown from a soil in east Co. Cork, Ireland where potatoes had been grown the previous two years. Purified isolates were cultured in tryptic soy broth (TSB) and stored at –20 °C in 50 % (v/v) glycerol.

The sugar beet isolates SB13, SB14, SB15 were chosen for further study because of their significant effect on the hatch of PCN *in vitro* (Ryan and Jones, 2004).

PREPARATION OF RIFAMPICIN-RESISTANT ISOLATES FOR ROOT INOCULATION

The individual isolates were spread-plated onto tryptic soy agar (TSA) supplemented with the antibiotic rifampicin (75 μg/ml). Single colonies that grew were deemed rifampicin resistant (rif^R) and were re-streaked on TSA supplemented with rifampicin. Subsequent single colonies were transferred to TSB, which had also been supplemented with rifampicin. The colonies were grown in darkness at 16 °C for 48 hours and shaken continuously.

The culture medium was then centrifuged at 6440 x g for 10 minutes and the bacterial pellet washed and re-suspended in half-strength Ringer's solution (Ryan and Jones, 2003). The inoculum was adjusted to the required densities (10^5 CFU ml^{-1}, 10^6 CFU ml^{-1}, 10^7 CFU ml^{-1}, 10^8 CFU ml^{-1}) with half-strength Ringer's solution.

SUGAR BEET RHIZOSPHERE COLONISATION

Sugar beet cv. Crystal seedlings were germinated in non-sterile horticultural sand. When the root systems were approximately 2.5 cm long, the seedlings were transplanted into microcosms constructed from square plastic petri dishes (12 x 12 cm; Sarstedt, Dublin, Ireland) containing non-sterile horticultural sand. A hole was cut in one side of each dish to allow the seedlings to grow (Fig. 1). An aliquot (1 ml) of the

isolate suspension to be tested was pipetted onto the root system post-transplantation; the root system was then covered with sand and the dish sealed tightly. Each plate was covered with black plastic to prevent light reaching the root system and the plates were incubated together in a vertical position in a growth chamber under a 16-hour day at 16°C (day) and 10°C (night).

The experiment was designed so that plants were inoculated with three bacterial isolates (SB13, SB14, SB15) at five different inoculation densities, plus a half-strength Ringer's solution (negative) control, and the extent of colonisation was determined at each of five time intervals after inoculation (3, 7, 14, 21 and 28 days). For each treatment, six replicates were used. Including controls, this experimental design required a total of 540 plants.

ESTIMATION OF EXTENT OF ROOT COLONISATION

The population of rif^R bacteria was quantified at each of five time intervals after inoculation. At each date, six replicates per treatment (a total of 90 plants per date) were removed from the growth chamber. The entire root system was washed in 10 ml Ringer's solution and a six-fold logarithmic dilution was conducted on each of the root washes. Aliquots (100 μl) of each dilution were spread plated on TSA supplemented with rifampicin. Plates were incubated at 16 °C for 48 hours in darkness before the number of CFU ml^{-1} was counted.

Root and shoot dry weights

At each harvest date, once the root wash had been performed, the plant was divided into root and shoot tissue by cutting the seedling at the point at which the radicle joined the hypocotyl. Roots and shoots were dried at 60 °C for 48 hours. The root dry weight and shoot dry weight were determined for each plant.

Hatch Study

Nematode material

G. pallida pathotype Pa2/3 was cultured on susceptible potato varieties. Cysts were recovered using a Wye Washer elutriator and stored dry at room temperature. Thus, one-year old, single-generation cysts of *G. pallida* pathotype Pa2/3 were used for all bioassays. Prior to use, cysts were rehydrated by soaking on filter paper saturated with distilled water within a darkened and closed plastic petri dish (Sarstedt, Dublin, Ireland) at 22 °C for 7 days.

Inoculation of seed

The bacterial inoculum was shake-cultured on TSB supplemented with rifampicin (75 μg/ml) in darkness at 16 °C for 48 hours. The TSB was then centrifuged at 6440 x g for 10 minutes and the bacterial pellet washed and re-suspended in half-strength Ringer's solution. This wash was repeated twice to ensure all residual medium was removed. The inoculum density of the bacterial suspension was adjusted to the

appropriate densities (10^7 or 10^8 CFU ml^{-1}) with half-strength Ringer's solution.

Non-dressed sugar beet seeds (*Beta vulgaris* ssp. *vulgaris* cv. Crystal) were sterilised in 10 % (v/v) sodium hypochlorite solution for 10 minutes followed by six washes in sterile distilled water to remove any residual sodium hypochlorite solution from the seed coat.

Seeds were inoculated by soaking in the bacterial inoculum suspension for five minutes on a shaker (200 rpm; 1 g seeds per 10 ml suspension).

CYST EXPOSURE TO TREATED *B. VULGARIS*

Tissue-embedding cassettes (dimensions 4.0 x 2.5 x 0.5 cm; Fisher Scientific UK Ltd. Loughborough, UK) were prepared by part filling with autoclaved horticultural sand and attaching a 20 cm long cord to each to facilitate recovery. These cassettes were porous, to allow cyst interaction with the rhizosphere, whilst retaining them for recovery and further analysis. Each cassette was further filled with sand containing 50 rehydrated cysts. The addition of sand ensured that cysts were not lost from the cassette during the experiment. This system had already been used successfully by this research group (e.g. Ryan and Devine, 2005).

Plastic pots (17.5 cm diameter) were part filled with non-sterile topsoil up to 6 cm below the rim. A prepared cassette of cysts was placed on the soil and covered with a layer of soil (c. 4 cm). Inoculated seeds (three per pot) were planted directly above the cassette and covered with a 2 cm layer of topsoil. The cassette cord was positioned to hang over the rim of

the pot for ease of recovery. Seedlings were thinned to one plant per pot after germination.

DETERMINATION OF PERCENTAGE HATCH

Cassettes were recovered at four or six weeks after planting. At each date, the content of each cassette was dried at room temperature for seven days and the cysts were separated from sand by hand rolling across a sheet of paper. Cysts rolled off the paper quickly, while irregularly shaped grains of sand were largely retained. Three random sub-samples, each of 5 cysts, were taken from the cysts recovered from each cassette (Ryan and Devine, 2005). To distinguish between viable and non-viable embryos (eggs), the cysts were soaked in 100 µl Meldola's Blue stain (0.05 % w/v) in an eppendorf tube for one week and then soaked in 100 µl of water for 24 hours (Ryan *et al*., 2000). Each set of five stained cysts was then placed in a 1 ml eppendorf tube (Eppendorf, UK) and crushed gently using a polypropylene homogeniser (Sigma Aldrich, Dublin, Ireland). The tube was vortexed and immediately three aliquots (20 µl each) of the suspension were taken and examined under a light microscope. For each sub-sample, the numbers of stained eggs (non-viable embryos; A), unstained, unhatched eggs (viable embryos; B) and empty eggshells (each representing a hatched juvenile; C) were counted. The percentage hatch of the viable eggs are given by the formula (adapted from that described by Ryan *et al*., 2000):

$$\% \text{ Viable egg hatch } = \frac{C}{(C + B + A)} \text{ x } 100$$

STATISTICAL ANALYSIS

All experiments were repeated once. No statistical differences were found between the two sets of experiments (data not shown) and data from the first iteration of each experiment are presented here. Root competence data were checked for normality. All approximated to normality (with/without transformation) and parametric interaction ANOVAs were carried out using DataDesk (Version 6; Data Description, Inc., NY, USA) with multiple comparisons conducted using the Tukey test. Dry weight and percentage hatch data were analysed using the parametric t-test.

Results

ROOT COLONISATION IN MICROCOSMS

Using 3-way interaction ANOVA with isolate, density and time being the main effects, there was no significant difference ($F[2,300]= 0.520$; $P>0.05$) in the ability of the three isolates (SB13, SB14, SB15) to colonise the sugar beet rhizosphere in sand microcosms, as measured by log CFU ml^{-1}recovered. There was, however, a significant difference ($F[3,300] = 147.246$; $P<0.01$) in colonisation between the different initial inoculum densities, with an initial inoculum density of 10^5 CFU ml^{-1}resulting in significantly lower levels of colonisation than other densities throughout the experiment and an initial inoculum density of 10^8 CFU ml^{-1}resulting in significantly higher levels of colonisation throughout the experiment compared to other inoculums densities.

Additionally, there was a significant difference ($F[4,300] = 16.463$; $P<0.01$) in the levels of colonisation between the different time intervals with mean colonisation levels at the final time interval (28 days) being significantly lower than that after 3 days.

For isolate SB13 specifically (Fig. 2a), there was a significant ($F[19,100]= 6.415$; $P<0.01$) reduction in the number of CFU $ml^{-1}$of rif^R bacteria recovered at the end of the experiment (28 days) compared to that at 3 days, when the inoculum densities were 10^5, 10^6 and 10^8 but not at 10^7 CFU ml^{-1}. There was no significant difference between the numbers of CFU ml^{-1} at the end of the experiment between the different inoculum densities.

For isolate SB14 (Fig. 2b), at all time intervals, the number of CFU ml^{-1} of rif^R bacteria recovered from plants inoculated with the lowest inoculum density (10^5 CFU ml^{-1}) was significantly lower ($F[19,100]= 19.95$; $P<0.01$) than that from the other three densities. For these higher three densities, there was no significant difference in the number of CFU ml^{-1} recovered between day 3 and day 28 indicating that, while the isolate remained in the rhizosphere, it did not multiply significantly.

For isolate SB15 (Fig. 2c), there was no significant difference in the number of rif^R CFU ml^{-1} recovered from plants treated with the different inoculum densities. The number of CFU $ml^{-1}$of rif^R bacteria recovered from plants treated with an initial inoculum density of 10^8 CFU ml^{-1} of SB15 did not differ significantly over the course of the experiment.

For isolates SB13 and SB14, there were significant interactions ($F[12,100] = 2.47$; $P<0.01$ and $F[12,100] = 5.39$; $P<0.01$, respectively)

between the inoculum density and incubation time main effects. Hence, the significant differences between the levels of main effects could not be fully analysed by multiple analysis. There was no significant interaction ($F[12,100] = 0.76$; $P>0.05$) between the main effects for isolate SB15, so the levels of main effects were analysed.

Levels of rhizosphere competence (measured as log CFU ml^{-1} rif^R SB15 bacteria recovered from the rhizosphere) were significantly higher ($F[3,119]= 39.9$; $P<0.01$) when treated with the highest inoculum density (10^8 CFU ml^{-1}) compared to all other inoculum densities. Rhizosphere competence of bacteria inoculated at 10^7 CFU ml^{-1} was significantly greater than that of bacteria inoculated at 10^6 and 10^5 CFU ml^{-1}. There was no significant difference between rhizosphere competence of bacteria inoculated at the two lowest inoculum densities.

There were significant differences ($F[4,119] = 7.57$; $P<0.01$) between levels of rhizosphere competence of SB15 at different harvest times, with rhizosphere competence at the end of the experiment being significantly lower than at the start.

Effect of isolates on root and shoot dry weight

All of the isolates had a positive effect on the growth of sugar beet in the sand microcosms as measured by dry weight of root (Fig. 3a) or shoot (Fig. 3b) tissue after 28 days at the highest inoculum density.

Isolates SB13 and SB15 increased the root dry weight of plants by 50 %. This was a significant ($t= 1.5809$; 10df; $P<0.05$) increase in the case of SB15 but not significant in the case of SB13 for the highest inoculums

density (t= 0.6957; 10df; P>0.05). The most noticeable difference was the effect of SB14 on root dry weight, which showed a highly significant (t= 3.6756; 10df; P<0.01) increase of 140% compared to the uninoculated control.

Isolates SB13 (t= 2.2625; 10df; P<0.01), SB14 (t= 2.3527; 10df; P<0.01), and SB15 (t= 3.3397; 10df; P<0.01) significantly increased the shoot dry weight by 81 %, 70 % and 66 % respectively, when applied at the highest inoculum density.

THE EFFECT OF ISOLATES SB14 AND SB15 ON PCN HATCH *IN VIVO*

Due to the slow growth of SB13 *in vitro*, and its relatively poor performance as a coloniser of the sugar beet rhizosphere and its minor effect on root dry weight (Fig. 3a) in the sand microcosms, only isolates SB14 and SB15 were used for the pot trials, to assess the effect of colonisation on PCN hatch.

When the level of *in vivo* hatch was assessed after 28 days (Fig. B.4.a), at both inoculum densities, hatch was significantly higher than the control hatch (corresponding to unplanted soil). There was no significant difference between the hatch induced by the control and that induced by sugar beet alone. There was no significant difference between hatch induced by SB14 and SB15 at any inoculum density.

After 42 days (Fig. 4b), the level of hatch had increased in all treatments. Sugar beet inoculated with both isolates, at both inoculum

densities, induced PCN hatch that was significantly greater than that induced by the control.

Overall, there was an average of 92 % decline in the *G. pallida* population after four weeks with the SB15-inoculated sugar beet plants and an average of 89 % decline with the SB14-inoculated sugar beet plants.

Discussion

The results from this study have shown that the application of bacterial isolates from the sugar beet rhizosphere as a seed dressing to a sugar beet crop can induce significantly greater hatch of *G. pallida* than the planting of sugar beet alone. Any future work may utilise either isolate (SB14 or SB15) or a mixture of both. Most biocontrol systems, including those for PCN control, have used single biocontrol agents as antagonists to the pest or pathogen (Kerry, 2000). However, some mixtures of plant growth-promoting rhizobacteria (PGPR) have been shown to provide increased growth promotion and disease suppression compared to single isolates alone (Raupach and Kloepper, 1998; Siddiqui *et al*., 2000; Whipps, 2001). Further trials with PGPR mixtures are required.

Given that sugar beet does not induce significant levels of PCN hatch on its own, the hatch under the control is, as expected, broadly in line with spontaneous hatch in fallow soil. Turner (1996) noted that the rate of spontaneous hatch is at its highest in PCN populations during the first and second year after production. Devine *et al*. (1999) showed that PCN juveniles declined by 57 % in the first year and by 40.3 % of the

remainder in the second year when infested plots were left fallow. However, Evans (1993) reported that the decline rate of *G. pallida* in fallow soil might be as low as 15 % per annum. Here, G. pallida hatch (in 1-year old cysts) after 28 days was 56%.

The levels of hatch achieved under this current putative trap crop system compare well with other trap crops and other more conventional PCN control methods. Scholte (2000) showed that *G. pallida* could be controlled using a trap crop system based on potato plants that were destroyed after an eight-week growing period with the systemic herbicide glyphosate. Also, Turner *et al*. (2006) showed *G. pallida* field populations could be reduced using selected wild Solanaceae potato clones.

However, these two trials utilised potato plants or wild potato clones, and there are disadvantages of using such plants as trap crops, such as the necessity to use a systemic herbicide to destroy the crop if the trap crop is not fully resistant, the critical timing of herbicide treatment and the lack of a financial return for the farmer (Scholte, 2000).

In light of these disadvantages, a trap crop system utilising non-host plants would be preferable. Work in Wageningen University and Research Centre, The Netherlands, has shown that *Solanum sisymbriifolium* can reduce the population density of *G. pallida* in pot trials by 47 % after 44 days and 75 % after 150 days (Timmermans *et al.*, 2006). This includes levels of spontaneous, in-soil hatch. The plant is slow to establish, however, and is an invasive species in warmer climates such as South Africa, Australia and India (Olckers and Hulley, 1995). *Solanum sisymbriifolium* is now available commercially and is marketed

as both DeCyst™ (Greenvale AP Ltd., UK) and Sis Foil™ (Branstons Ltd., UK).

The ability of the putative sugar beet/rhizobacterial isolate trap crop system to induce early hatch of *G. pallida* (after 28 days) is another advantage of this system. It means that the isolate needs only to colonise the roots for a short time to be effective. Brodie (1982) has shown a 92 % decline in PCN population after 42 days using potato as a trap crop, but Whitehead (1977) and Turner *et al*. (2006) have both shown that eight weeks is too long a growing period with potato trap crops and results in PCN multiplication on the host. With non-host crops (such as sugar beet), the timing is not as critical given the inability of the PCN to multiply on the sugar beet roots. However, economic constraints on the farmer dictate that time under a trap crop should be as short as possible unless a viable crop will be forthcoming. Sugar beet has the advantage of producing a viable crop in European countries (IIRB, 2004).

The ability of this potential trap crop to increase the percentage hatch of *G. pallida* is notable, given the emergence of the species as the more important PCN species in major potato growing regions due to ineffective control techniques used against *G. pallida* (Den Nijs, 1992; Evans & Haydock, 2000).

Of the two isolates tested for hatching ability, one was identified (using a range of diagnostic tests) as an *Arthrobacter* sp. (SB14) and the other was a *Bacillus* sp. (SB15; Ryan and Jones, 2004). Both isolates, in addition to SB13 (an *Acintobacter* sp.), can be classified as plant growth-promoting rhizobacteria (PGPR) due to their effects on root and shoot dry

weight. The two can also be classified as plant health-promoting rhizobacteria (PHPR) because of their ability to reduce the effects of a plant pest or pathogen (Sikora, 1988). Cyst nematodes are optimum targets for biocontrol with PHPR due to the reliance of these nematodes on root-produced hatching factors (Sikora, 1991). In the EU, PGPR and PHPR are not specifically regulated but are considered as biocontrol agents within the legal framework (Malusá and Vassilev, 2014).

The exact mode of action of the isolates in increasing PCN hatch remains to be determined. Natural potato root leachate has been shown to contain a variety of hatch-influencing chemicals (Devine and Jones, 2000a). These include hatching factors (HF) that induce the hatch of PCN (Devine *et al*., 1996); hatching inhibitors (HI) which counteract the effect of hatching factors (Byrne *et al*., 1998) and hatching factor stimulants (HS) which stimulate HF-induced hatch but are themselves hatch neutral (Byrne *et al*., 1998). Due to the hatch of PCN in this study in the absence of a host source of HFs, it is hypothesised that isolates SB14 and SB15 are producing bacterially-derived HFs which stimulate the hatch of PCN. Further study is needed to test this hypothesis.

The application of natural hatching factors to fallow soil has previously been shown to induce “suicide hatch” of PCN (Devine and Jones, 2000b), much like that exhibited in this study. This resulted in a 50 % reduction in the population size of *G. rostochiensis* that was due to a combination of suicide hatch and increased in-egg mortality. The strategy described in this study demonstrates that a plant-bacterium delivery system can be used to influence *G. pallida* hatching, presents evidence that natural,

bacteria-derived hatching factors are involved. Interaction of bacteria and root may be a further source of hatching factors. Further identification of source and nature of the factors will be important to the advancement of this strategy.

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Tables and Figures

Fig. 1. Microcosm designed for rhizosphere competence experiments. Once prepared, the dish is wrapped in black plastic to prevent light penetration to roots.

Fig. 2(a). Root competence of the rhizobacterial isolate SB13 after inoculation with one of four inoculum densities. Any samples which share a common letter are not significantly different using the Tukey test (P >0.05).
Anova Summary Table:

Source	Df	SS	MS	F	P
Sample	19	68.63	3.61	6.42	≤ 0.01
I. Density	3	14.79	4.93	8.76	≤ 0.01
Time	4	37.15	9.29	16.49	≤ 0.01
Interaction	12	16.69	1.39	2.47	≤ 0.01
Error	100	56.31	0.56		
Total	119	124.95			

Fig. 2(b). Root competence of the rhizobacterial isolate SB14 after inoculation with one of four inoculum densities. Any samples which share a common letter are not significantly different using the Tukey test (P >0.05).
Anova Summary Table:

Source	Df	SS	MS	F	P
Sample	19	379.08	2034.42	5881.6	≤ 0.01
I. Density	3	330.97	110.32	318.95	≤ 0.01
Time	4	17.25	4.31	12.47	≤ 0.01
Interaction	12	22.36	1.86	5.39	≤ 0.01
Error	100	34.24	0.35		
Total	119				

Fig. 2(c). Root competence of the rhizobacterial isolate SB15 after inoculation with one of four inoculum densities. Any samples which share a common letter are not significantly different using the Tukey test (P >0.05).
Anova Summary Table:

Source	Df	SS	MS	F	P
Sample	19	313.48	1922.95	977.13	≤ 0.01
I. Density	3	235.86	78.62	39.95	≤ 0.01
Time	4	59.59	14.90	7.57	≤ 0.01
Interaction	12	18.03	1.50	0.76	0.6862
Error	100	196.80	1.97		
Total	119	510.27			

Fig. 3. The effects of rhizobacterial isolates (SB13, SB14 and SB15) from the sugar beet rhizosphere on (a) sugar beet root and (b) shoot dry weight 28 days after root inoculation at four inoculum densities. Significant differences from the corresponding controls are denoted by: * (P<0.05) and ** (P<0.01), following square-root transformation.

Fig. 4. Percentage *in vivo* hatch of *G. pallida* in the presence of sugar beet (a) 28 days and (b) 42 days after inoculation with hatch-inducing bacteria. The letters a and b within the graphs indicate inoculum densities used, 1.0×10^7 (a) and 1.0×10^8 (b) CFU ml^{-1}, respectively. 'Sugar beet' indicates uninoculated sugar beet plants only. 'Control' indicates hatch in the absence of plant and bacteria. Asterisks indicate inoculated plants resulting in significantly different (P<0.05) hatch from that of the corresponding uninoculated sugar beet plants.

Figure 2a
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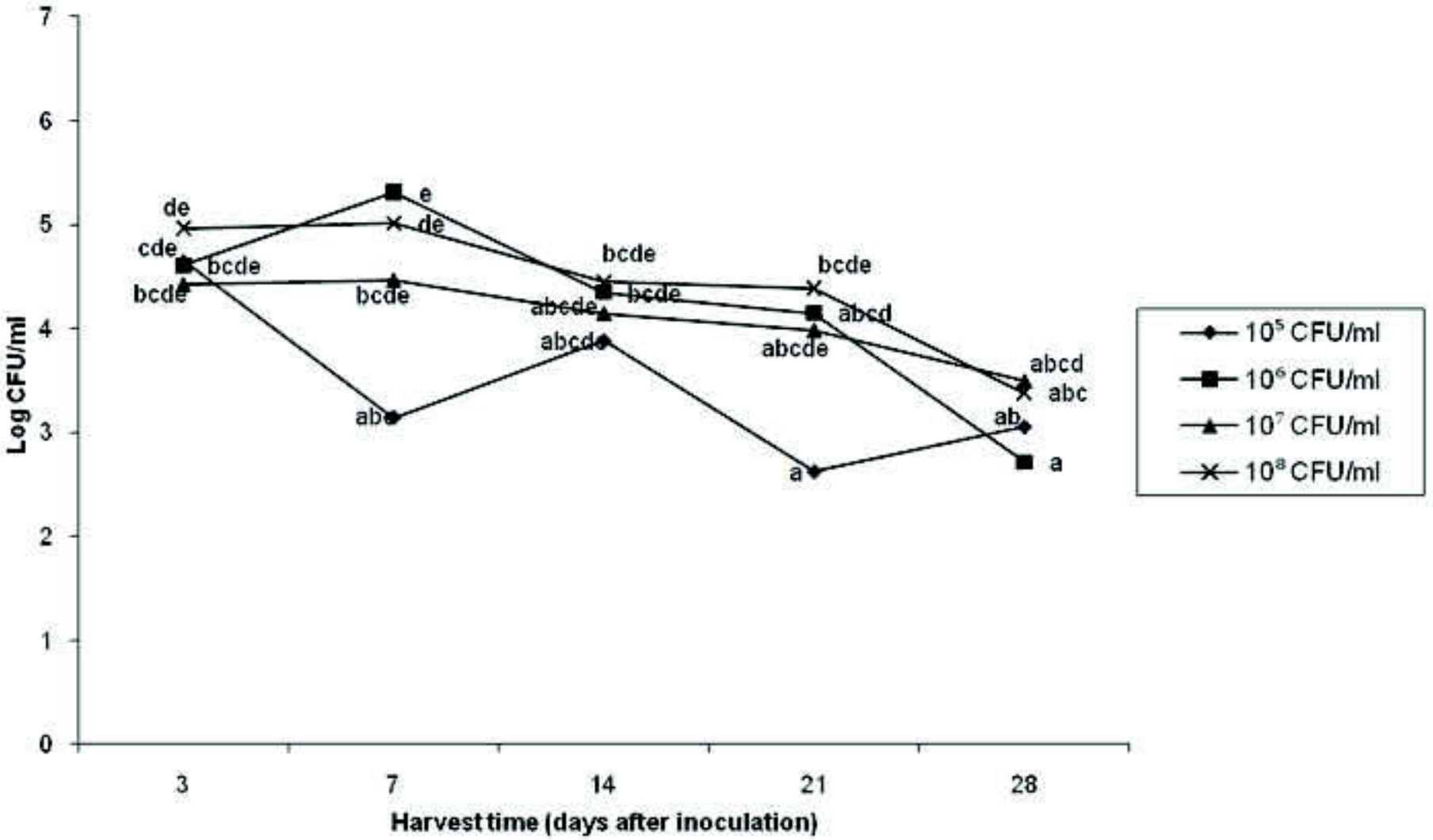


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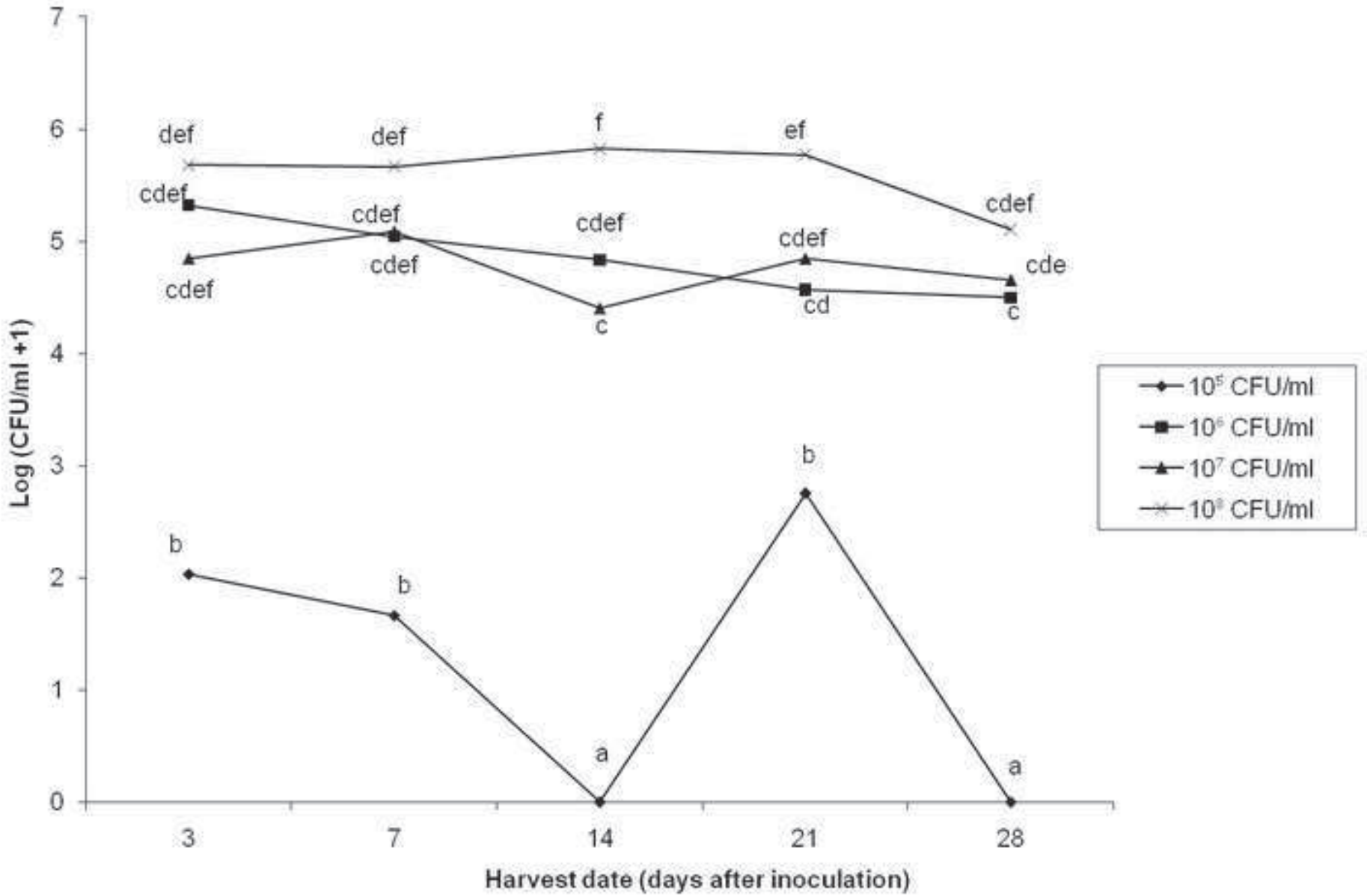


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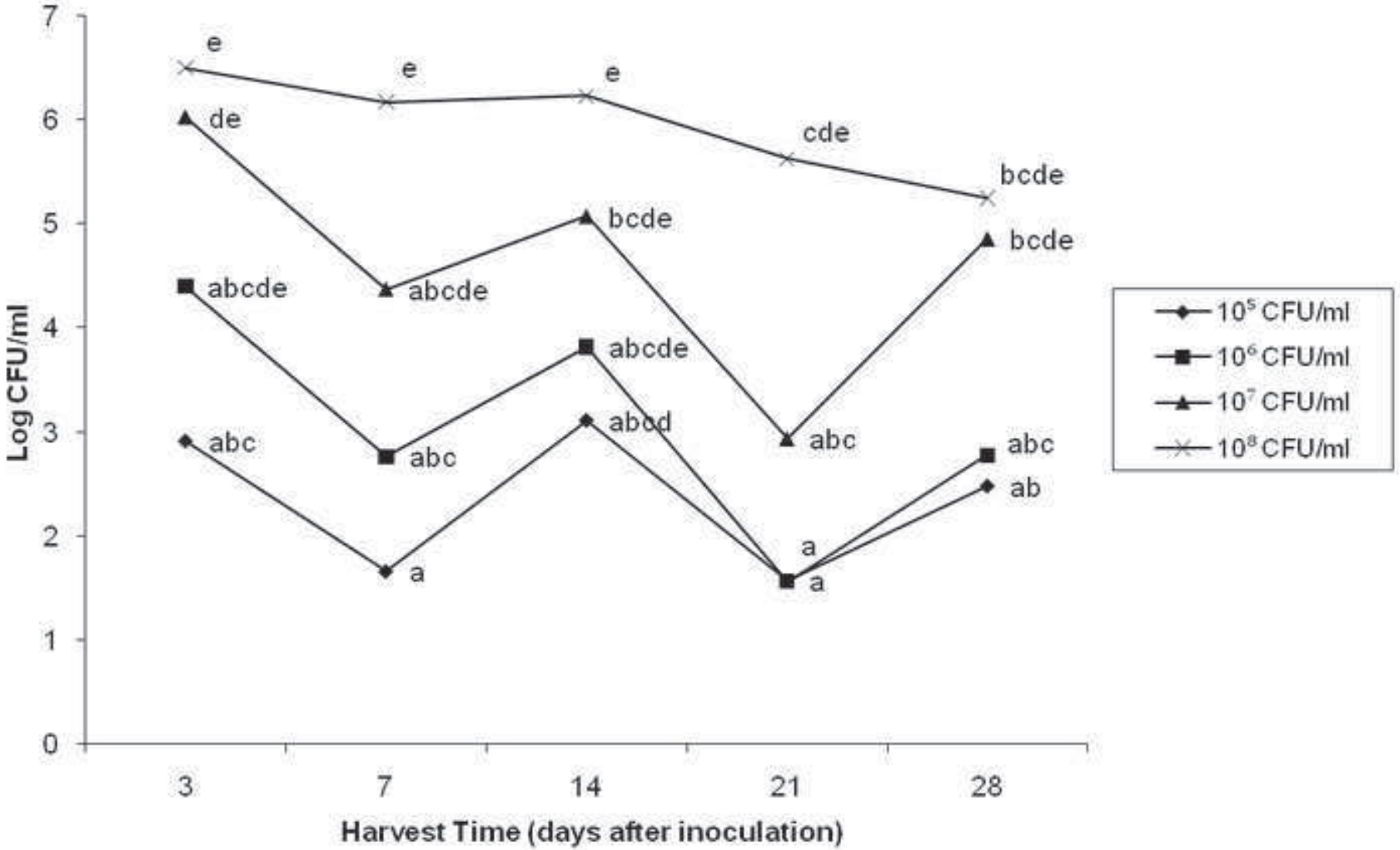


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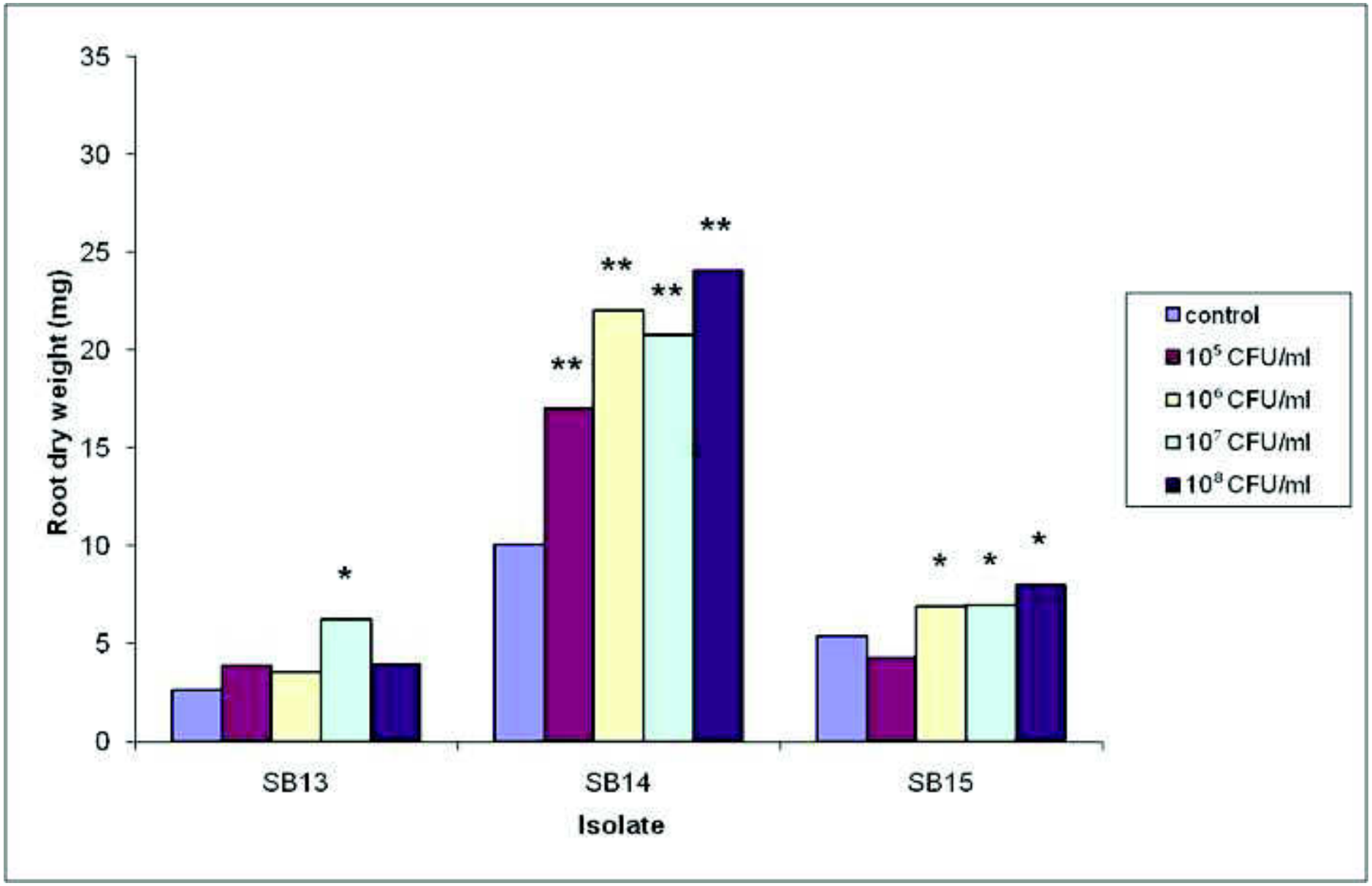


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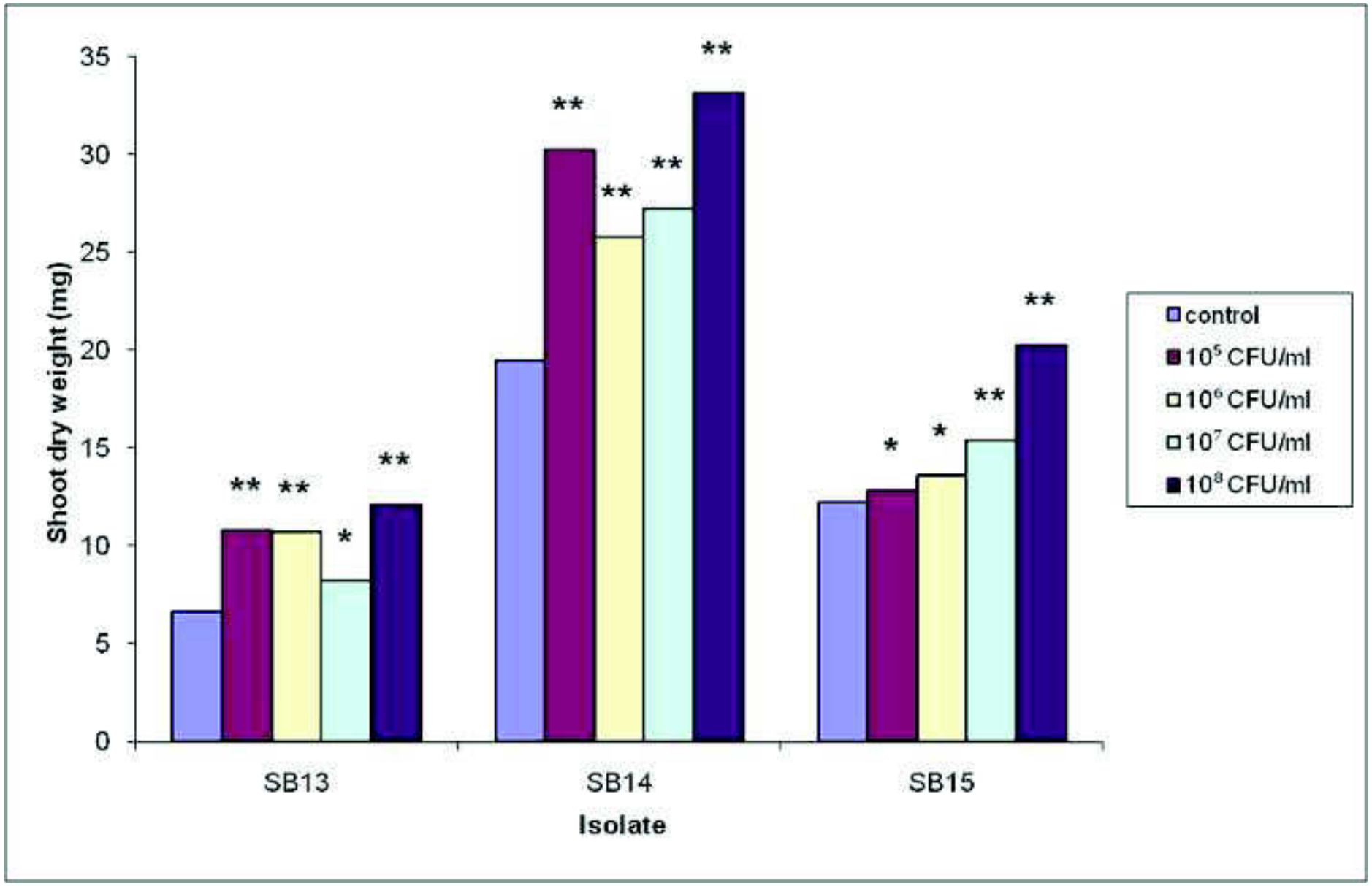


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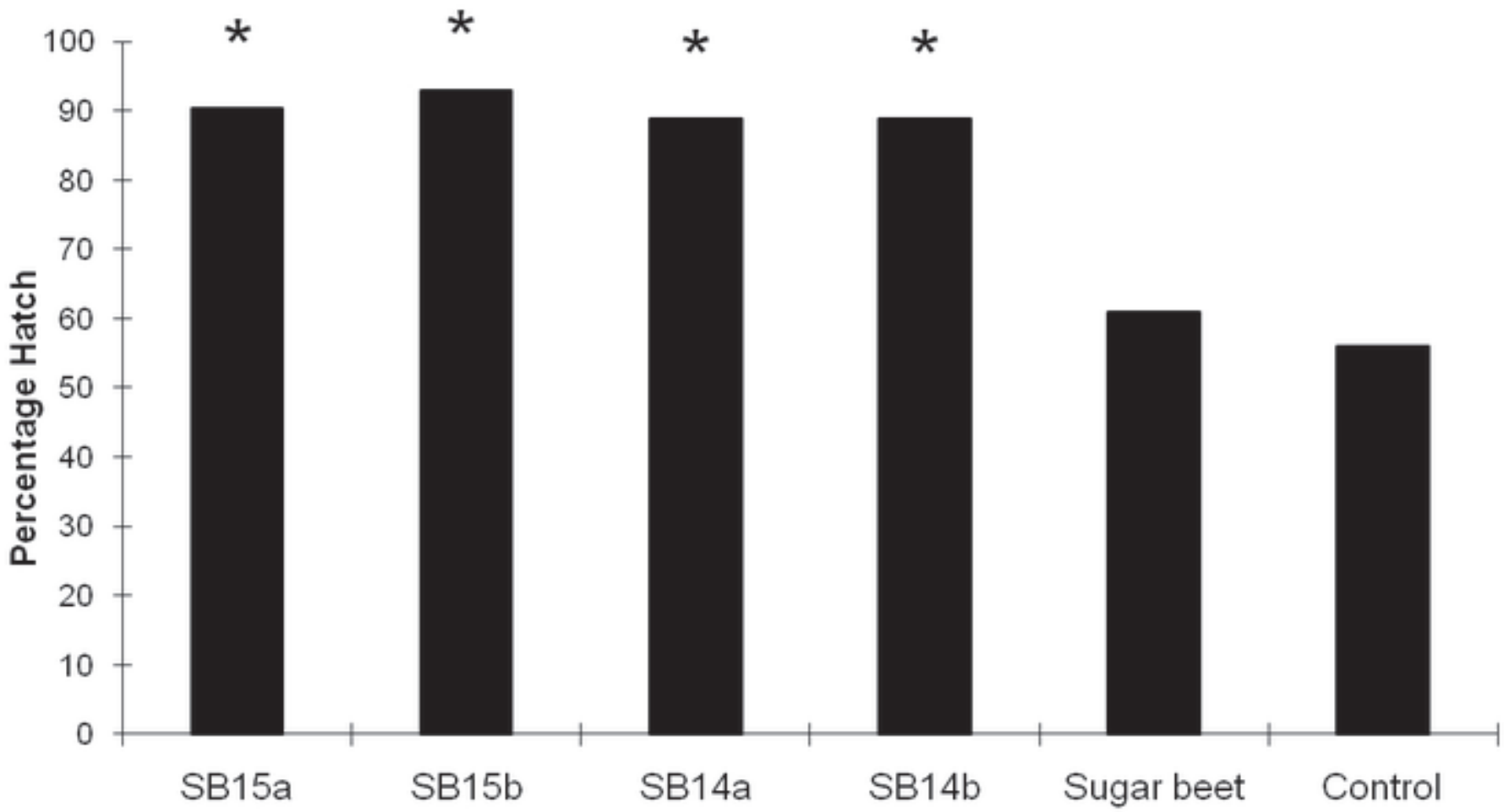


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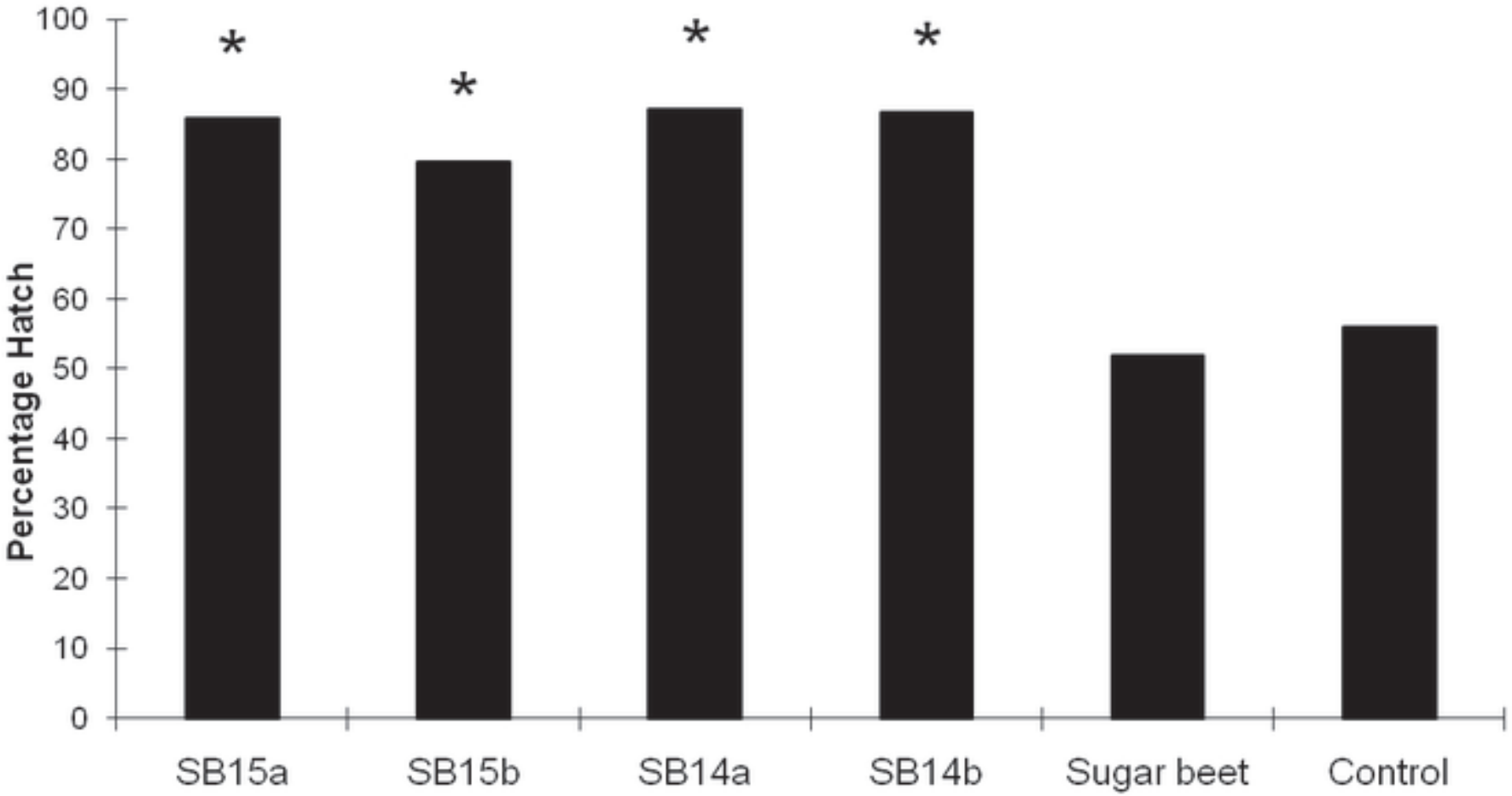


Figure 1
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