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## Isolation of rhizobacteria associated with maize and assessment of their potential for use in *Striga hermonthica* (Del.) Benth. suicidal germination

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### Abstract

A screen-house pot experiment with commercial hybrid maize variety H511 was conducted at Kenya Sugar Research Foundation, Kisumu sub-station; Kenya. The experimental soil was infested with *Striga hermonthica* (Del.) Benth. at a rate of 1000 seeds/pot. The bacterial treatments were *Enterobacter sakazakii*, *Pseudomonas* sp., *Klebsiella oxytoca* and the uninoculated control in sterile soil. The plants were harvested at 78 days after planting and plant biometric parameters were determined. Except for *K. oxytoca* there was no significant differences among bacterial isolates for the number of days to *S. hermonthica* emergence compared to the uninoculated control. *K. oxytoca* was the major component of the total variation for *S. hermonthica* visual rating. *E. sakazakii* treated plants supported the largest amount of emerged *S. hermonthica* (2.82 *Striga* stems /pot) and the largest attached *S. hermonthica* (7.70 stem /pot). The result provided evidence that the application of any of these isolates could offer a better form of *S. hermonthica* biological control.

### Introduction

Parasitic witchweeds (*Striga* spp.) are a major uncontrolled weeds in the Sub-Saharan Africa decimating yields on cereals and legumes. *Striga hermonthica* (Del.) Benth. is of African origin and can infest maize, wheat, rice, millet, sorghum, teff and many grasses (Kim *et al.*, 1999) causing considerable yield losses throughout the seven-agro ecological zones of Sub-Saharan Africa (excluding mountainous and forested areas) (CIMMYT, 1998).

Maize is the dominant cereal crop in the moist savanna area of Sub-Saharan Africa (FAO, 1992) where the *S. hermonthica* problem has been most severe (Adetimirin *et al.*, 2000). It has been suggested that only an integrated method of control may effectively reduce *Striga* to non-economic levels. The need for indigenous microbes in the integrated control method can not be overemphasised. Natural enemies of *Striga* spp. have received more attention in recent years because of the success achieved in

the biological control of other weeds (Sauerborn *et al.*, 1991). The use of bacteria for biological control of *S. hermonthica* is much more recent as compared to the use of fungi. There have been few successful commercial releases of biocontrol agents, except for 'classical biocontrol' agents introduced from the center of origin of the particular pest, which had been lacking since the pest was introduced (Gressel, 2001). Studies by several workers in the search of biological control agent for *Striga* species (Abbasher *et al.*, 1995; Sauerborn *et al.* 1996; Abbasher *et al.*, 1998; Marley *et al.*, 1999; Ciotola *et al.*, 2000) have focused on fungi from the soil.

Mechanisms of plant growth-promotion by bacteria include: nitrogen fixation; synthesis of siderophores which can solubilize and sequester iron from the soil; production of phytohormones such as auxins and cytokinin, which can enhance plant growth; and solubilization of minerals such as phosphorus (Kloepper *et*

*al.*, 1989; Glick 1995; Patten and Glick 1996). A particular bacterium may use any one, or more, of these mechanisms (Burd *et al.*, 2000). Many plant growth-promoting bacteria possess several of these traits, a bacterium may utilize different traits at various times during the life cycle of the plant, and the impact of the bacterium on plant growth may vary depending upon the soil chemical and physical properties (Burd *et al.*, 2000). A diverse group of introduced microorganisms appears to have potential for biological control of soilborne diseases (Weller, 1988)

In Africa, cowpea is one of the commonly used trap crop in areas under *S. hermonthica* infestation. Trap crops (false hosts) are those plants which stimulate germination of *Striga* species seed without being parasitised and are consequently allowed to mature and produce a crop (Bebawi *et al.*, 1984). Some other trap crops of *S. hermonthica* documented include bean (*Phaseolus vulgaris*), groundnuts (*Arachis hypogea*), (Mumera, 1985; Kiriro, 1988) and soybean (*Glycine max* L.). Trap cropping is preferred by most farmers because of the yield obtained. However, it is hoped that if *S. hermonthica* stimulating-bacteria are introduced to the rhizosphere at cowpea planting this will augment the suicidal effect of cowpea on *S. hermonthica* and deplete the *S. hermonthica* seed bank in the soil.

This study evaluates the effects of bacteria on *S. hermonthica* germination in the presence of a host plant. The aim was to investigate the indigenous rhizobacteria of *S. hermonthica* infested maize and determine their potential for control of *S. hermonthica*.

## Materials and Methods

**Pot Experiment.** Studies were conducted on screen-house benches at Kenya Sugar Research Foundation (KESREF). *Striga hermonthica* collected during the long rain, in the year 2000, was used to conduct the experiment. The experimental soil was pasteurized, allowed to cool overnight and artificially infested with *S. hermonthica* at a rate of approximately 1000 seeds/pot and left to precondition for 14 days. Pots were washed clean to prevent the carry-over of *S. hermonthica* from previous use. Clean *Striga*-free pots (10 × 10 × 14 cm) accommodating approximately 1 kg soil were filled with the experimental soil. Bacterial strains were grown on appropriate medium for 48 h and suspended in 5 ml of sterile, deionized water. A trough approximately 1.5 cm deep by 1 cm wide was made in the soil for bacteria inoculation and maize (hybrid, H511) planting. Only one strain of bacteria was added to the trough at a rate of 1.5 ml of  $1.4 \times 10^6$ cfu. Maize seeds were sown three to a pot at a depth of 1.5 cm and thinned to one plant per pot at 7 days after planting. Pots were watered twice daily with tap water within the retention capacity of the pot. The potted soils were re-inoculated with respective bacteria for 3-weeks on a weekly bases with same inoculum dosage as stated above, except for those pots that served as the control which received 1.5 ml of sterile water.

**Bacterial Culture Media.** King's medium B (KB) for pseudomonad (King *et al.*, 1954; Palleroni, 1986) was solidified with 1.5 % agar (BDH Laboratory supplies), Tryptone Soy Agar (TSA) was used for the two other bacterial isolates. Subsequently, one distinct colony of each of the three isolates was grown in either KB broth or TSB for use in the screen-house experiments. Incubation was done for 24 h at 28°C in a

rotary shaker. Representative colonies were stored in 25 % glycerol at  $-80^{\circ}\text{C}$ .

**Isolation of Rhizobacteria.** Potential biocontrol bacteria were originally isolated from maize roots in screenhouse pot experiment in an earlier study (Babalola, 2002). To isolate bacteria from the endorhizosphere, roots were washed in tap water to remove soil, and 1 g roots (Mawdsley and Burns, 1994) was picked from ten randomly selected pots. Surface sterilisation of the root sample (effected by immersion in 70% ethanol for 30sec and in 4% NaOCl for 3 min) was carried out to ensure that the isolates to be cultured were from the endorhizosphere. The collective macerated root systems were shaken for 10 min on a wrist-action shaker in 100 ml of phosphate buffer solution (PBS). The suspension was plated in appropriate cooled molten agar. Bacteria from the exorhizosphere were isolates from firmly adhering soil.

**Identification of Rhizobacteria.** Fluorescent colonies were identified at long (366 nm) wavelength under UV light. After purification by subculturing, biochemical identifications were done using 'Appareils et Procédés d'identification' (API) technique (API Systems, Biomerieux, SA, France). The API technique has advantages over the conventional methods. The API 20E is a standard identification system that uses 23 miniaturized biochemical tests of a database. The API 20E strip consists of microtubes containing dehydrated substrates. These tests were inoculated with a bacterial suspension, which reconstitutes the media. During incubation, metabolism produces colour changes that were either spontaneous or revealed by the addition of reagents.

**Data collection.** For each pot, the number of days to first *S. hermonthica* emergence

was recorded. Upon emergence, counting of emerged *S. hermonthica* seedlings was done weekly until harvest. Visual rating of *S. hermonthica* growth (Vrsg) was based on a scale of 0-6 (0 = no emergence; 1 = small plants, no flowering; 2 = medium plants, no flowering; 3 = medium plants, some flowering; 4 = large plants, full flowering; 5 = large plants, some capsules; 6 = large plants, full capsules) at harvest. Emerged *S. hermonthica* plants were monitored likewise. For the numbers of attached *S. hermonthica*, plants were cut at the base and the pot was submerged in water. After several gentle washings, the attached *S. hermonthica* were counted

**Experimental design and statistical analysis.** The experiment was laid out in Complete Randomized Design and repeated four times with five replicates on each occasion. *S. hermonthica* emergence counts were determined by the number of *S. hermonthica* plants per experimental pot. The *S. hermonthica* count data were scale transformed to square root before analysis. Data generated from bacterial types were analysed for differences in their ability to effect suicidal germination by analysis of variance (ANOVA) described in SAS (1998). Mean separation was done by Tukey's Studentized Range (HSD) Test at 5% level of significance.

## Results

### Bacteria isolation and identification.

Three rhizobacterial isolates, 4MKS8 8MR5 and 10MKR7 were used in this study were selected after laboratory screening (Babalola, 2002) from a total of 180 bacterial isolates. The bacterial isolates were examined under a Zeiss light microscope. Photomicrographs were taken with an Olympus camera, using a kodak ektachrome film. The cell morphology of Isolate 4MKS8 (Plate 1) is presented. The isolate 4MKS8 was conventionally

observed and found to be straight or slightly curved rods. They could grow aerobically, acidified sugar-containing media only weakly and showed no obvious differences in acid production from sugars. Isolate 4MKS8 is gram-negative, oxidase and catalase positive, indicating a member

of the *Pseudomonas* species as outlined in Palleroni (1986). Table 1 presents the interpretation table of the 'Appareils et Procédes d'identification' (API) test. Isolate 8MR5 and 10MKR7 were identified as *Enterobacter sakazakii*, and *Klebsiella oxytoca* respectively.

Table 1. Biochemical characterisation of isolates using API kit

Tests	Substrates	Reactions/Enzymes	Bacterial isolates		
ONPG	Ortho-nitro-phenyl-galactoside	Beta-galactosidase	+	+	-
ADH	Arginine	Arginine dihydrolase	+	-	+
LDC	Lysine	Lysine decarboxylase	-	+	-
ODC	Ornithine	Ornithine decarboxylase	+	-	-
CIT	Sodium citrate	Citrate utilization	+	+	+
H <sub>2</sub> S	Sodium thiosulphate	H <sub>2</sub> S production	-	-	-
URE	Urea	Urease	-	+	+
TDA	Tryptophane	Tryptophane desaminase	-	-	-
IND	Tryptophane	Indole production	+	+	-
VP	Sodium pyruvate	Acetoin production	+	+	-
GEL	Kohn's gelatine	Gelatinase	-	-	+
GLU	Glucose	Fermentation/oxidation	+	+	+
MAN	Mannitol	Fermentation/oxidation	+	+	+
INO	Inositol	Fermentation/oxidation	+	+	-
SOR	Sorbitol	Fermentation/oxidation	-	+	-
RHA	Rhamnose	Fermentation/oxidation	+	+	-
SAC	Sucrose	Fermentation/oxidation	+	+	-
MEL	Melibiose	Fermentation/oxidation	+	+	-
AMY	Amygdalin	Fermentation/oxidation	+	+	-
ARA	Arabinose	Fermentation/oxidation	+	+	-
Identification			<i>E. sakazakii</i>	<i>K. oxytoca</i>	<i>Pseudomonas</i> sp.

Table 2. Days to first *S. hermonthica* emergence and *S. hermonthica* infection indices of four bacterial treatment 78 days after maize planting ( $\pm$  SE).

Bacterial treatments	Mean		
	Days to first <i>S. hermonthica</i> emergence (DAP)	Visual rating of <i>S. hermonthica</i> growth (0-6) <sup>a</sup>	Number of attached underground <i>S. hermonthica</i> <sup>b</sup>
Uninoculated control	48.55 $\pm$ 2.30	1.20 $\pm$ 0.20	4.35ab $\pm$ 1.00
<i>E. sakazakii</i>	46.17 $\pm$ 2.49	1.28 $\pm$ 0.20	7.70a $\pm$ 1.48
<i>Pseudomonas</i> sp	46.18 $\pm$ 3.03	1.34 $\pm$ 0.21	3.58ab $\pm$ 0.87
<i>K. oxytoca</i>	52.28 $\pm$ 2.92	0.93 $\pm$ 0.17	3.55ab $\pm$ 0.94

<sup>a</sup>*Striga* visual rating: 0=no emergence, 6=mature plant full capsule)

<sup>b</sup>Means in column followed by the same letter are not significantly different at  $p < 0.05$  by Tukey's studentized range test

Table 3. Mean number of emerged *S. hermonthica* shoots per greenhouse potted soil at four treatments levels ( $\pm$  SE).

Bacterial treatments	<i>S. hermonthica</i> emergence count at:					
	6WAP	7WAP	8WAP	9WAP	10WAP	11WAP
Uninoculated control	0.70 $\pm$ 0.5	1.75 $\pm$ 0.6	2.80 $\pm$ 0.7	4.85 $\pm$ 0.9	7.75 $\pm$ 1.3	9.95 $\pm$ 1.3
<i>E. sakazakii</i>	0.52 $\pm$ 0.2	0.90 $\pm$ 0.3	1.45 $\pm$ 0.5	2.00 $\pm$ 0.7	2.50 $\pm$ 0.8	2.82 $\pm$ 0.8
<i>Pseudomonas</i> sp.	0.50 $\pm$ 0.2	0.87 $\pm$ 0.3	1.36 $\pm$ 0.4	1.87 $\pm$ 0.6	2.41 $\pm$ 0.7	2.71 $\pm$ 0.8
<i>K. oxytoca</i>	0.36 $\pm$ 0.1	0.71 $\pm$ 0.2	1.47 $\pm$ 0.4	1.78 $\pm$ 0.7	2.40 $\pm$ 0.7	2.79 $\pm$ 0.8

$p < 0.05$  by Tukey's studentized range test

### Effect of treatments on *S. hermonthica*.

*K. oxytoca* significantly affect emergence. The variation in number of *S. hermonthica* plants among treatments was not significant (Table 2). Mean squares of *S. hermonthica* ratings by *S. hermonthica* growth for bacteria inoculated per pot were marginally significant for *K. oxytoca* (Table 2). Among the isolates, *K. oxytoca* was the major component of the total variation (Table 2). The number of attached underground *S. hermonthica* was significantly increased by *E. sakazakii*, however, *Pseudomonas* sp. has the highest visual rating of *S. hermonthica* growth. The results of four experiments showed that bacterial treatments did not affect the *S. hermonthica* emergence count (Table 3).

### Discussion

During the 'Appareils et Procédes d'identification' (API) test, isolate 4MKS8 is *Pseudomonas* sp. However, it was not identified to species level. Perfect identification of isolate 8MR5 (*Enterobacter sakazakii*), and 10MKR7 (*Klebsiella oxytoca*) was achieved. The endorhizosphere can be defined as intercellular spaces accessible to microorganisms (Klyuchnikov and Kozhevin, 1991). It appears that *K. oxytoca* 10MKR7 thrive and stimulate because iw was from inside the roots and when reintroduced to the soil still colonize the rhizosphere

effectively. The stimulating ability of *K. oxytoca* 10MKR7, and *Pseudomonas* 4MKS8 throughout the period of this study shows that they are soil-borne rather than contaminants from air, water, or seed. These isolates are more importantly envisioned to have good growth rate which supports their colonization capacity.

The fact that *E. sakazakii* and *Pseudomonas* sp. have visual rating of *S. hermonthica* growth greater than water (control) is a likely confirmation that they are stimulating bacteria. As presented in Table 2 for VRSG, *Pseudomonas* sp. will offer a superior suicidal germination over the control by stimulation of *S. hermonthica* during integration with a nonhost of *S. hermonthica*. Several factors could be responsible for the unpredictable performance of the introduced bacteria on *S. hermonthica* emergence count. Among such factors is competition with the other rhizosphere bacteria, each of which is likely to have a different biotic potential. Ambiguous results obtained may be due to the environmental-resistant factors which might have retarded the carrying capacity of the isolates and thereby interfered with their profound need to survive. It should be noted as propounded by Kim (1991; 1994) that *S. hermonthica* emergence count data only give a partial view of complex interactions of factors. Besides, a great spatial variability in *S. hermonthica* seed concentrations in soil could have

compounded the problem. Hoffmann (1996) that worked on *Fusarium nygamai* for the biocontrol of *S. hermonthica* in maize also found an improvement in maize vigour, grain and biomass yield and (as observed in our study), any differences were not statistically significant.

Earlier studies by Beauchamp *et al.*, (1993) have shown that the number of bacteria and competition with other rhizosphere microorganisms affected the sensitivity of the technique used. In the present work, an innovative method of bacterial application was employed. However, whichever method was adopted, the trend of results in this work is similar to the findings of Loper *et al.*, (1984); Weller, (1984); Bahme and Schroth, (1987), namely that introduced microorganisms do not become widely disseminated. The colonization pattern changed from dispersed to aggregated within 3 days of inoculation (Bilal *et al.*, 1993). This result shows that the bacteria behave as a plant growth promoter and is consistent with the previous observation by Burd *et al.*, (2000) that in many cases where the bacterium appeared to have a positive effect on plant growth, statistical analysis indicated that the observed effect was not always statistically significant.

It is most likely that the bacteria did not exert any harmful effect on the *S. hermonthica*, but the host plant was able to withstand the debilitating effect of *S. hermonthica* with increased microbial activity than when the microbial activity in the rhizosphere was minimal. However, the available results envisage the feasibility of bacteria as one of the future bioherbicide researches. The effect will be for long-term control, as the endemic conditions of *S. hermonthica* in Africa soil cannot be combated within a short period of years. Further studies are being carried out in the field.

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