Egyptian Journal of Biology, 2010, Vol. 12, pp 1-5
© Printed in Egypt. **Egyptian British Biological Society (EBB Soc)**

Ethylene quantification in three rhizobacterial isolates from *Striga hermonthica*-infested maize and sorghum

Babalola OO

International Centre of Insect Physiology and Ecology, Nairobi, Kenya
Present address: Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
Correspondence address: Department of Microbiology, P.O.B 9536, UI HQ, Ibadan, Nigeria

Abstract

Volatiles from the headspace of three rhizobacteria (*Pseudomonas* sp. 4MKS8, *Klebsiella oxytoca* 10MKR7 and *Enterobacter sakazakii* 8MR5) isolated from sorghum and maize rhizospheres were analysed by gas chromatography and gas chromatography-mass spectrometry for ethylene. The ethylene released ranged from trace concentrations in *Pseudomonas* sp. 4MKS8, 72 nmoles/10^8 CFU/mL in *K. oxytoca* 10MKR7 to 210 nmoles/10^8 CFU/mL in *E. sakazakii* 8MR5. The level released by *E. sakazakii* 8MR5 was similar to the level required for stimulating suicidal germination in *Striga* seeds, suggesting the potential use of this rhizobacterium in the biological control of *Striga* spp.

Keywords: Bacteria, gas chromatography

Introduction

Ethylene is a plant hormone produced by various plants in response to physical, chemical and biological stresses (Reid 1995). It is biosynthesised in higher plants from methionine via *S*-adenosylmethionine and the enzyme 1-amino-cyclopropane-1-carboxylate. Although ethylene is involved in the regulation of numerous physiological processes in plants, it is also produced by microorganisms (Babalola 2002, Boiero *et al*. 2007, Perrig *et al.* 2007). Microbes have been known to produce ethylene via ethylene-forming enzymes (Araki *et al*. 2000).

Striga hermonthica (Del.) Benth. is a pest of maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] in sub-Saharan Africa (Babalola 2002). The grain losses of maize and sorghum, the two most important cereal crops in sub-Saharan Africa, due to *Striga* infestation is estimated at US$7 billion annually (Berner *et al.* 1995). *Striga* wounds the host plant outer root tissues and absorbs its supply of moisture, photosynthates, and minerals (Tenebe & Kamara 2002). For these reasons *Striga* control is very important to reduce or compensate for grin losses due to *Striga* infestation. One way of controlling the pest is to stimulate germination under conditions that will lead to total mortality of the seedlings, 'suicidal germination': ethylene is one substance that can do this.

Since agricultural production in Africa is centred mainly on resource-poor farmers, an eradication programme as carried out in the United States by direct soil injection with ethylene may not be practicable in Africa due to the high cost involved and technical limitations of African farmers. Ethylene-producing bacteria have been successfully used to stimulate *Striga* spp. seed germination in the laboratory (Berner *et al*. 1999), and in related screening experiments, several fluorescent pseudomonads suppressive to *S. hermonthica* seeds have been identified (Ahonsi *et al*. 2002). However, in these studies, the levels of ethylene produced by these and related rhizobacteria are not known. Therefore, the objective of this study was to quantify the levels of ethylene produced in the headspace of three rhizobacteria isolated from soils associated with maize and sorghum, with the aim of providing additional information to support the use of bacteria in the biocontrol of *S. hermonthica.*

Materials & Methods

Root samples of potted maize and sorghum were shaken vigorously to remove loosely adhering soil. Bacteria from the exorhizosphere and endorhizosphere were obtained from the root sample

Correspondence: tel +2347057845544 email : olubukola_babalola@yahoo.com

according to a standard protocol. Isolates were cultivated on tryptic soy agar medium in triplicate. After incubation for 24 h at 28 ^{o}C, representative types of colonies were further purified on tryptic soy agar and stored in 25% glycerol at –80 ^{o}C. *Pseudomonas* sp. 4MKS8 and *Klebsiella oxytoca* 10MKR7 were isolated from the rhizosphere of sorghum, while *Enterobacter sakazakii* 8MR5 was isolated from that of maize. Both soil samples were infested with *S. hermonthica*.

Headspace volatiles were allowed to equilibrate in 100 ml broth contained in 150 ml GIBCO reagent bottles (175 ml maximum capacity) for 6, 12, 24, 48 h. A gas chromatography (GC) syringe hole was drilled in each bottle cap, and then lined with sterile teflon-faced silicone rubber septa before closure. Samples of the headspace (100 µl) from the inoculated broth and an uninoculated control broth were analysed by GC and gas chromatography-mass spectrometry (GC-MS). GC analyses were performed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard capillary column (Ultra 1 Cross-linked methyl silicone, 50 m x 0.2 mm ID x 0.33 µm film thickness) using nitrogen as the carrier gas at a flow rate of 0.35 ml per min. The oven temperature was initially isothermal at 40 ^{o}C for 5 min, then programmed at 10 ^{o}C per min to reach 150 ^{o}C, and held there for 8 min. Chromatographic peaks were integrated using a Hewlett-Packard 3396 integrator. GC-MS analyses were carried out on a Fisons Instruments 8060 Series II chromatograph coupled to a Fisons Instruments VG Platform II MS (EI, 70eV), employing the same chromatographic conditions as described. Compounds were identified by comparing their spectral data with those of the library (National Institute of Standards and Technology Registry of Mass Spectral Data 1995) of the mass spectrometer. Authentication of the identity of the compounds was achieved by comparing the retention times of the headspace volatiles of the isolates with those of commercial samples of ethylene (Del Monte, Nairobi, Kenya) and 3-hydroxy-2-butanone (Aldrich, UK).

Fig 1: Total ion chromatograms of standard ethylene, BACT-8 (*E. sakazakii* 8MR5), BACT-10 (*K. oxytoca* 10MKR7) and BACT-44 (*Pseudomonas* 4MKS8) showing relatively strong ion fragments for carbon dioxide (m/z 44).

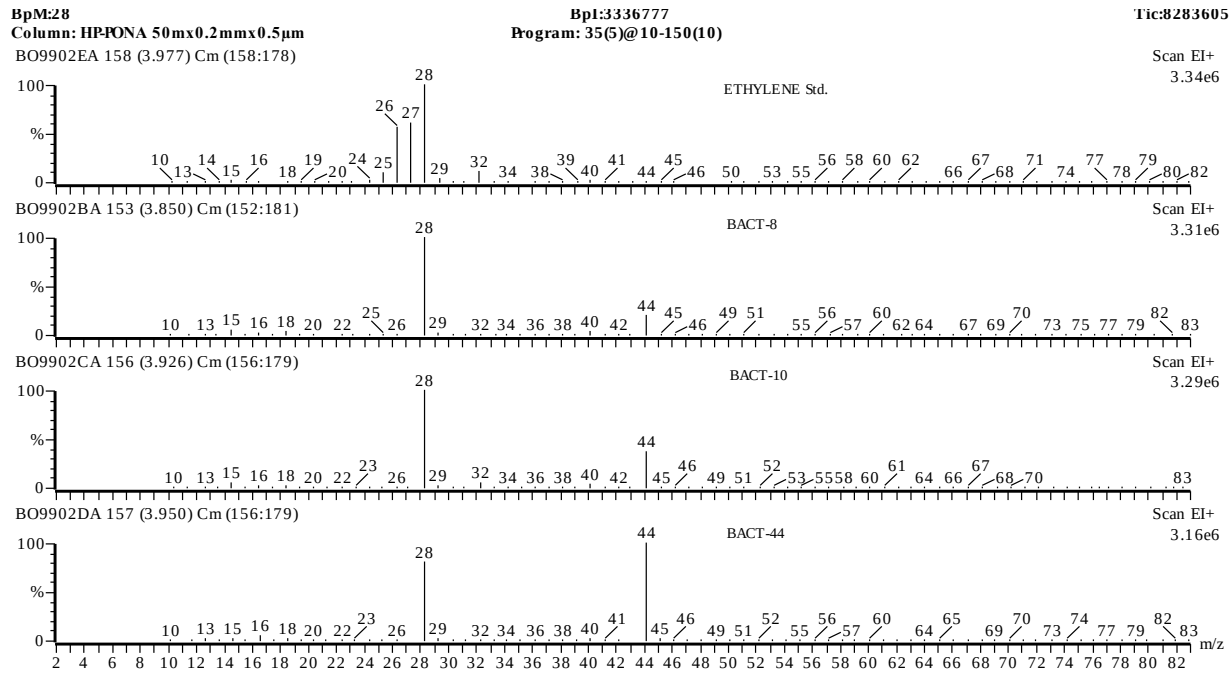


Fig 1 (contd.)

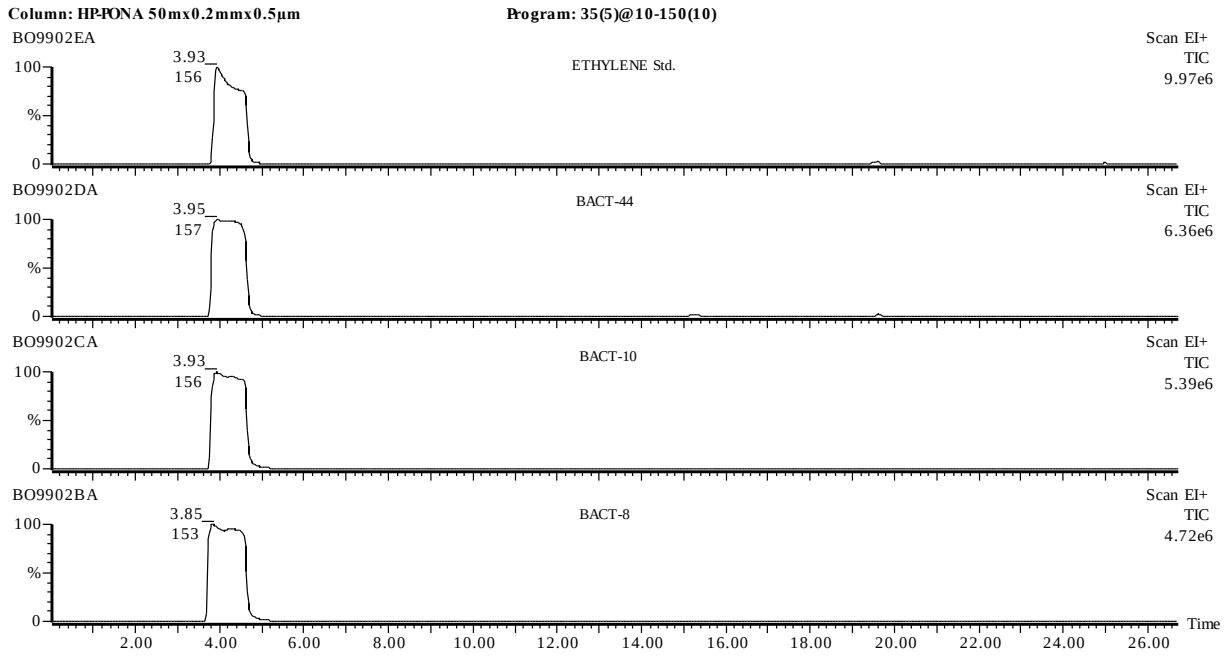
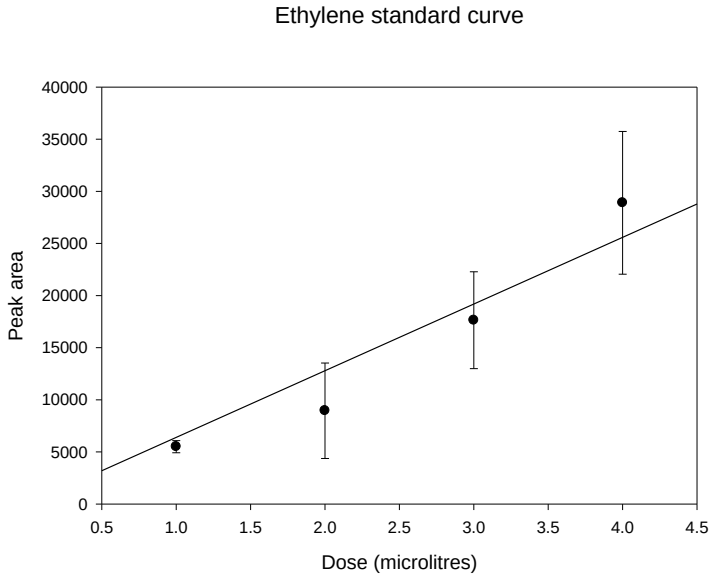


Fig 2: Standard curve to convert GC peak areas into ethylene concentrations.



Results

GC-MS analysis identified ethylene as the major component in the headspace volatiles of culture media of the three isolates after 48 h of incubation (Fig 1). Carbon dioxide (CO_2) was identified as a co-eluent with ethylene in all the analyses, while 3-hydroxy-2-butanone was identified as a trace component in the headspace volatiles of *Enterobacter sakazakii* 8MR5. Fig 2 shows the relationship between GC peak areas and volumes of ethylene. The combined concentration of ethylene with CO_2 was estimated for 100 µl headspace samples: *Pseudomonas* sp. 4MKS8 (trace), *K. oxytoca* 10MKR7 (72 nmoles per 10^8 CFU/ml), and *E. sakazakii* 8MR5 (210 nmoles per 10^8 CFU/ml). GC-MS analysis showed relatively strong fragment ions for CO_2 in the headspace volatiles of *Pseudomonas* sp. 4MKS8, with the

weakest in the volatiles of *E. sakazakii* 8MR5. Ethylene was not detected in media incubated for less than 48 h, and in the control media.

Discussion

Several recent research findings (Boiero *et al*. 2007; Perrig *et al*. 2007) have found that ethylene is one of the compounds synthesised in the culture supernatant of certain rhizobacteria strains. Berner *et al.* (1999) reported that ethylene-producing bacteria are as effective in stimulating seed germination of *Striga* spp. as are soil injections of ethylene gas. The results here reveal that the rhizobacteria *Pseudomonas* sp. 4MKS8, *K. oxytoca* 10MKR7 and *Ent. sakazakii* 8MR5 all emit ethylene, but at varying concentrations and co-eluting with CO_2. Ethylene was detected in the headspace of the three rhizobacterial isolates after 48 h, which suggests that they have a high potential for use in the biological control of *Striga* seeds. Our studies show clearly that CO_2 co-elutes with ethylene in the GC-MS analysis.

Acknowledgements

The use of facilities at the International Centre of Insect Physiology and Ecology, Nairobi, Kenya is gratefully acknowledged. This research was supported by Third World Organization for Women in Science Postgraduate fellowship.

References

Ahonsi MO, Berner DK, Emechebe AM & Lagoke ST (2002) Selection of rhizobacterial strains for suppression of germination of *Striga hermonthica* (Del.) Benth. *Biological Control* 24: 143-152

Araki S, Matsuoka M, Tanaka M & Ogawa T (2000) Ethylene formation and phenotypic analysis of transgenic tobacco plants expressing a bacterial ethylene-forming enzyme. *Plant Cell Physiology* 41: 327-334

Babalola OO (2002) Interactions between *Striga hermonthica* (Del.) Benth. and rhizosphere bacteria of *Zea mays*, L. and *Sorghum bicolor* L. Moench for *Striga* suicidal germination In *Vigna unguiculata*. Ph.D. thesis. University of Ibadan, Ibadan, Nigeria.

Berner DK, Kling JG & Singh BB (1995) *Striga* research and control-A perspective from Africa. *Plant Disease* 79: 652-660.

Berner DK, Schaad NW & Volksch B (1999) Use of ethylene producing bacteria for stimulation of *Striga* sp. seed germination. *Biological Control* 15: 274-282.

Boiero L, Perrig D, Masciarelli O, Penna C, Cassan F & Luna V (2007) Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. *Applied Microbiology & Biotechnology* 74: 874-880

NIST: Registry of Mass Spectral Data (1995) Mass Lynx Libraries, Wiley Version 6.0V.G. Organic, England.

Perrig D, Boiero ML, Masciarelli OA, Penna C, Ruiz OA, Cassan FD & Luna MV (2007) Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. *Applied Microbiology & Biotechnology* 75: 1143-1150

Reid MS (1995) Ethylene in plant growth, development, and senescence, pp 486-508 *in* P.J. Davies (ed.) *Plant hormones*. 2nd edition. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Tenebe VA & Kamara HM (2002) Effect of *Striga hermonthica* on the growth characteristics of sorghum intercropped with groundnut varieties. *Journal of Agronomy & Crop Science* 188: 376-381.

الملخص العربي

تقدير قيمة الإيثلين فى ثلاث عزلات لبكتريا الريزومات المعزولة من ستريجا هيرمونثيكا والتى تصيب الذرة والشعير

بابالولا وو
المركز الدولى للبيئة والفسيولوجى – نيروبى – كينيا
العنوان الحالى: قسم الميكروبيولوجى – جامعة ولابيسى ونابانجو – آجولويى - مدينة وجين – نيجيريا

تم خلال هذا البحث تحليل الإيثلين المعزول من ثلاثة أنواع من البكتريا وهى: بسيبومونوس، كليبسيلا إوكسيتوكا، إنتيروباكتر ساكازاكى، والمصاحبة لريزمات نبات الذرة والشعير وذلك بإستخدام جهاز الفصل الكروماتوجرافى الغازى والمتعدد الطيف. أوضحت النتائج أن الإيثلين المنبعث من بسيبومونوس كان يقدر بكميات قليلة للغاية، بينما قدرت كميتها بحوالى 72 نانومولز/10^{8} س ف ى/ميلىلتر فى بكتريا كليبسيلا إوكسيتوكا ، وكانت قيمتها 210 نانومولز/10^{8} س ف ى/ ميلىلتر فى بكتريا إنتروباكتر ساكازاكى. أيضا أوضحت الدراسة أن الكمية المنبعثة من بكتريا إنتروباكتر ساكازاكى كانت مشابهة للمستوى المطلوب لإحداث الإنبات فى بذور نبات ستريجا، مما جعلنا نقترح إمكانية إستخدام هذا النوع من البكتريا فى المقاومة البيولوجيةِ لنبات ستريجا.