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Full Length Research Paper

Study on the effect of bacterial and chemical volatiles on the growth of the fungus *Aureobasidium pullulans*

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The aim of this study was firstly to determine if volatiles produced by a range of bacteria influences the growth of the fungus *Aureobasdium pullulans* and secondly, to determine if this fungus can use organic volatiles hydrocarbons as a sole carbon source. The results show that a) bacteria produce volatiles which influence the growth of the fungus *A. pullulans*, b) that the response to such volatiles can be inhibitory or stimulatory and that these effects are seen when the fungus is grown under both nutrient-rich and nutrient-poor conditions, and c) that *A. pullulans* can grow oligotrophically in saturated atmospheres of acetone, benzene and ammonium hydroxide, these volatiles being used as nutrient sources.

Key words: Fungi, bacteria, volatiles, bacterial volatiles, chemical volatiles.

INTRODUCTION

It is a well-established fact that volatiles (that is, so-called volatile organic compounds or VOCs) from a variety of source influence the growth of bacteria and fungi. Exposure to bacterial VOCs is known to reduce the growth of fungi, while fungal spore stimulation can be stimulated by VOCs produced by species of soil *Streptomyces* (Wheatley et al., 1996; Mackie and Wheatley, 1999; Kai et al., 2007; Kai et al., 2008). *Aureobasdium pullulans* is a fungus which commonly grows on indoor surfaces and is therefore likely to be exposed to a wide range of volatiles produced by bacteria, plants and those present in the domestic environment (Cooke, 1959; Depshande et al., 1992). As a result, it was chosen here as the test organism for use in the development of a simple method for determining the effect of bacterial, and other, VOCs on fungal growth. In addition to testing the effects of VOCs produced by bacteria, the effect of saturated concentrations of a range of common solvents on the growth of this fungus was also determined.

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MATERIALS AND METHODS

Determination of the effects of VOCs produced by individual bacteria on the growth of *A. pullulans*

All micro-organisms used in this study were obtained from the Department of Molecular Biology and Biotechnology Culture Collection, Sheffield, UK. All results were subjected to an analysis of variance using Anova.

The technique used to determine the effect of bacterial VOCs on the growth of *Aureobasdium pullulans* is shown in Figure 1. A thin layer of nutrient agar (Oxoid) was poured into the base of a large Petri- dish (150 × 15 mm, Falcon). A circle of one of the following bacteria was spread over the surface of the nutrient agar; *Escherichia coli, Serratia marcescens* and *Mycobacterium phlei*. A central disc of this inoculated nutrient agar was then removed with the aid of a flame-sterilized scalpel. Czapek Dox medium (Oxoid)

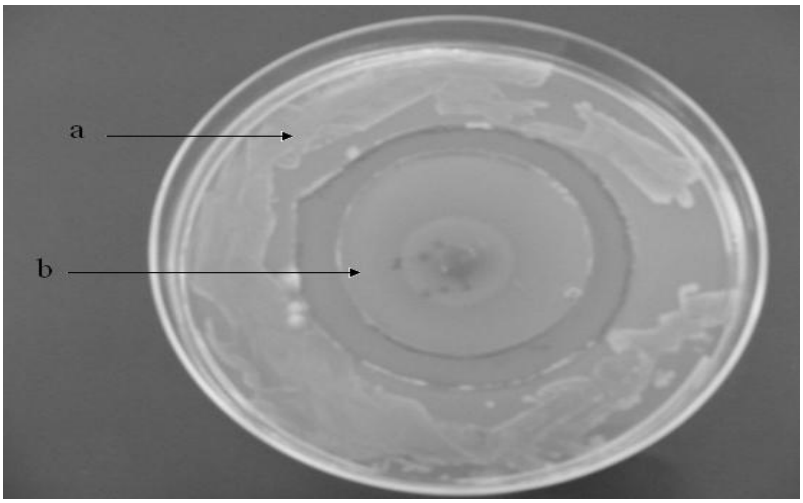

Figure 1. Method used to study effect of bacterial volatiles on the growth of *A. pullulans*. The fungus was inoculated on the central medium (a) (Czapek Dox), while the bacterium was inoculated onto the outer (Nutrient agar) disc (b).

was then poured into a normal sized petri dish and a circle of this medium was removed and transferred to the centre of the large petri dish such that it replaced the circle of inoculated nutrient agar which had previously been removed. The size of this inner circle was such that the inner Czapek Dox circle of agar was well separated from the outer circle of nutrient agar which had been inoculated with the bacterium under investigation; as a result, there was no physical contact between the inner circle of Czapek Dox medium and the outer circle of nutrient agar (Figure 1). A disc (0.5 cm) was then removed from a culture of *A. pullulans* grown for 7 days on Czapek Dox medium and placed, culture surface down, in the centre of the Czapek Dox medium contained in the large petri dish. The large petri dish was then incubated at 25°C; three replicates were used for each bacterium tested. Mycelial extension from this central disk was then determined. The effects of any VOCs produced by the bacterial culture on the growth of the fungus was then determined at intervals for 2 weeks.

Determination of the effect of bacteria on the growth of *A. pullulans* when inoculated onto plain agar

The aforementioned experiment was repeated, except that the circle of Czapek Dox medium in the centre of the large petri dish was replaced by plain agar (Oxoid).

Determination of the effects of saturated volatiles on the growth of *A. pullulans* on nutrient-free silica gel

Nutrient-free silica gel medium was poured into the large petri dishes earlier mentioned. Two holes were then cut in the agar using a flame-sterilized scalpel and an autoclaved, sterile plastic cap from a Universal bottle was inserted in the resulting hole. One of the following volatile substances (2 ml) was then added to the plastic cap; ammonium hydroxide, benzene, and acetone. The plate was then sealed using waterproof tape. Controls containing sterile deionized water were also included. A culture disc of *A. pullulans* (grown at nutrient free silica gel medium at 25°C for 10 days) was then placed, culture- surface down, in the centre of the agar, equidistant from the two plastic caps and the plates were then incubated in triplicate plates in a fume cupboard at 25°C.

RESULTS AND DISCUSSION

The method used here (Figure 1) provided a simple and effective means of determining the effects of bacterial volatiles on the growth of *A. pullulans*. Since no contact was made between the bacterial inoculants and the culture of *A. pullulans*, any observed effects on growth of the latter must have been due to the presence of volatiles. Figure 1 shows that the effect of bacterial volatiles on the growth of *A. pullulans* varied with the bacterium used. The presence of *E. coli* had a statistically significant stimulatory effect on the growth of the fungus over the incubation period (Figure 2a). In contrast, growth of the fungus was statistically inhibited by *M. phlei*. (Figure 2b); finally a marked stimulation of the growth of *A. pullulans* was produced by *S. marcescens* (Figure 2c) producing the most marked stimulatory effect. The fact that bacteria produce volatiles which differentially influence the growth of fungi has been observed before by Dennis and Webster (1971), Moore-Landecker and Stotzky (1972), Fiddaman and Rossall (1993), Mackie and Wheatley (1999), Bruce et al. (2003), Barr (2006); Kai et al. (2007), (2008), Vesperman et al. (2007) and Welwei et al. (2008). Some of these authors assume that the same effects relating the production volatiles observed in laboratory experiments will also be seen in the environment, notably soils. However, it should be remembered that, since the cultures used in such experiments are enclosed, the concentration of bacterial volatiles present will likely be much higher than it occurred in the environment. Another criticism is that, most workers employ nutrient-rich media in their studies and since most natural environments are oligotrophic, that is, contain low levels of microbially-available nutrients; such experiments can be seen as being artificial and not reflecting the true nutrient situation likely

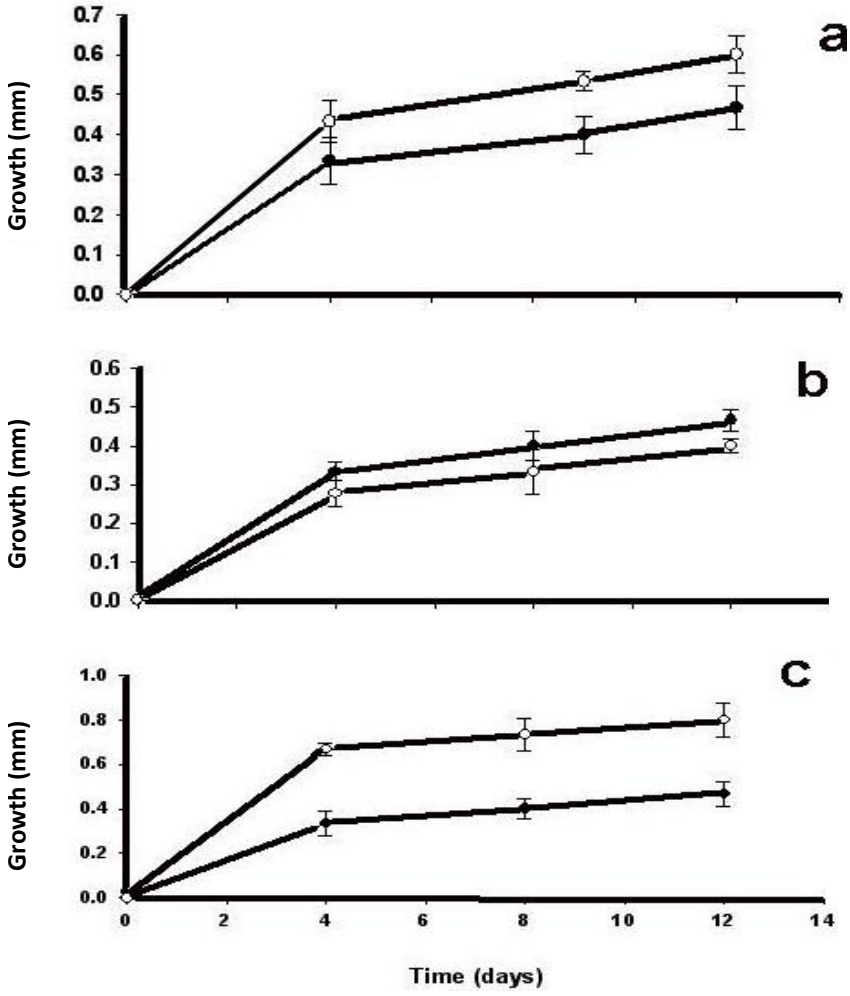

Figure 2. a) Effect of *E.coli* on the growth of *A.pullulans*, b). Effect of *M. phlei* on growth of *A.pullulans,* c) *S.marcescens* on the growth of *A.pullulans* (All changes are statistically significant at p=0.05. Control results are represented by closed circles, and treatment results by open circles).

to be present in soils and other natural environments. To avoid this problem, we repeated the aforementioned experiments but used plain agar instead of (nutrient-rich) Czapak Dox as the fungal growth medium. Not surprisingly, fungal growth was much reduced on plain agar compared with Czapek Dox medium (Table 1). However, the same fungal growth responses to bacteria-produced volatiles were seen, namely inhibition of fungal growth by *E. coli* and growth stimulation by *M. phlei* and *S. marcescens* (Table 1). Table 1 also shows that the percentage stimulation or inhibition of growth produced by bacterial volatiles was essentially the same, independent of whether the fungus was grown on Czapek Dox or Plain Agar, that is, the same response to bacterial volatiles on fungal growth is seen under both nutrient-rich and nutrient poor conditions. This suggests that the method used here can be used with confidence to study the effects of bacterial volatiles on fungal growth.

Finally, we used this simple method used here to study the effect of acetone, benzene and ammonium hydroxide on the growth of *A. pullulans* when inoculated onto nutrient-free silica gel medium. Figure 3 shows that the fungus not only managed to grow, but that its growth increased in a saturated atmosphere of these compounds. The most marked stimulatory effect at the end of the growth period was produced by benzene followed by acetone and ammonium hydroxide. Presumably, acetone and benzene acted as a source of carbon, on this nutrient-free medium, for the growth of *A. pullulans*, while ammonium hydroxide provided a source of nitrogen. At first sight, it is perhaps surprising that *A. pullulans* can grow in a saturated atmosphere of acetone and benzene, both of which are generally regarded as inhibitory to microbial growth. However, this fungus is well known for its ability to grow and cause deterioration to all kinds of materials, often in environments where

Table 1. Effect of bacteria on *Aureobasidium pullulans* growing on oligotrophic (Plain agar) and nutrient-rich (Czapek Dox) media.

Variable	Control	Treatment	% Change over control
E. coli			
Plain agar	0.20	0.15	-25.0
Czapek Dox	0.47	0.35	-26.0
M. phlei			
Plain agar	0.16	0.30	+87.5
Czapek Dox	0.31	0.59	+90.3
S. marcescens			
Plain agar	0.12	0.25	+108
Czapek Dox	0.39	0.79	+102

Means of triplicates, All difference between control and treatment were significant (p = 0.05). This means that the percentage changes are roughly similar independent of whether plain agar or Czepek Dox agar is used.

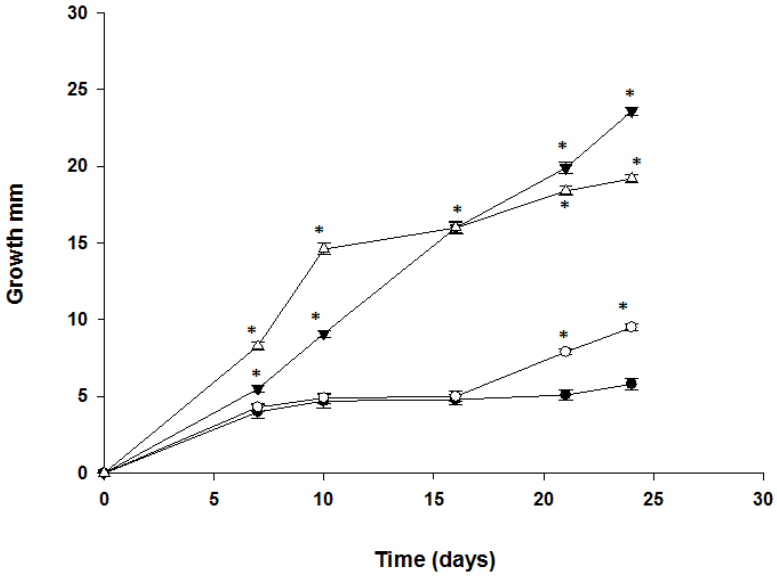

Figure 3. Effect of saturated concentration of various volatiles on the growth of *A. pullulans* .Benzene, closed triangles; acetone, open triangles; ammonia, open circles; control, closed squares). (*Significantly different from control, p=0.05).

nutrients appear to be in short supply (Cooke, 1959); it does so by having the ability like many other fungi, to sequester traces of carbon and nitrogen from the atmosphere, that is, by growing oligotrophically under low nutrient conditions (Wainwright et al., 1991).

In conclusion, by using a simple experimental approach we have shown that a) bacteria produce volatiles which influence the growth of the fungus, *A. pullulans*, b) that the response can be inhibitory or stimulatory and that these effects are seen when the fungus is grown under both nutrient-rich and nutrient-poor conditions. Finally, *A. pullulans* was shown to be capable of growing oligotrophically in saturated atmospheres of acetone, benzene and ammonium hydroxide, these volatiles being used as nutrient sources.

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