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## Research Article

# Inhibition and Co-Regulation of Five Amino Acids Produced in *Alternaria alternata* Exposed to Acetaldehyde

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## Abstract

**Background and Objective:** Acetaldehyde is an inhibitor of growth of fungi including *Alternaria alternata* and alters biochemical processes. However, its effect on the regulation of the production of amino acids in *A. alternata* remained unknown. This study aimed to investigate the inhibition and co-regulation of five amino acids produced in *Alternaria alternata* exposed to acetaldehyde. **Materials and Methods:** To assess the regulation of the production of amino acids in *A. alternata* exposed to acetaldehyde colonies of this fungus were exposed this volatile compound at concentrations of 0, 5 and 10% over 24, 48, 96 and 120 h. **Results:** Results showed that acetaldehyde inhibited *A. alternata* mycelial growth in a dose-dependent manner. Moreover, evidence of suppression and co-regulation of the production of amino acids, leucine, isoleucine, proline, tyrosine and valine was discovered. **Conclusion:** From these results it could be concluded that acetaldehyde can suppress and co-regulate the production of the five amino acids.

**Key words:** Acetaldehyde *Alternaria alternata*, volatile compounds, mycelial growth, amino acids

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Alternaria alternata* (Fr.:Fr.) Keisel. is common fungal pathogen of plants which may cause severe symptoms both in the field and during storage. Control of fungal pathogens in storage may require fumigation with fungicidal reagents. The volatile compound acetaldehyde, which may occur in nature as a plant metabolite, was found to control *Monilinia laxa* in stone fruit<sup>1</sup>. It was also found to be inhibit the growth of *Botrytis cinerea* and *Rhizopus stolonifer*<sup>2,3</sup>, as well as inhibit growth, spore germination and development in *A. alternata*<sup>4</sup>. Recently, it was also found that apart from growth reduction acetaldehyde also reduces the production of adenosine 3', 5'-cyclic monophosphate in a dose-dependent manner in *A. alternata*<sup>5</sup>. Chemical groups which are derivatives of acetaldehyde are part of a patented fungicide product<sup>6</sup>. However, the influence of acetaldehyde on the production of the amino acids, leucine, isoleucine, proline, tyrosine and valine remained unknown as no study was conducted to assess relationships in the production of amino acids in *A. alternata* exposed to acetaldehyde. The work reported here in assessed the influence of acetaldehyde on five amino acids and it was found that acetaldehyde reduces the quantity of these amino acids in living *A. alternata* mycelia and the production of amino acids is co-regulated by this volatile compound.

## MATERIAL AND METHODS

The volatile liquid synthetic acetaldehyde (purity >99.5%) purchased from Sigma-Aldrich was used for this study and *Alternaria alternata* (strain PPRI 12409) was obtained from the Agricultural Research Council of South Africa. From fungal mycelia, actively growing on Potato Dextrose Agar, 3 mm plugs were cut with a cork borer, transferred mycelia down on Carrot Agar media and cultured for 24 h at 28°C. After the 24 h incubation, the fungal cultures were exposed to 0, 5 and 10% acetaldehyde diluted in distilled water. The acetaldehyde solution poured in eppendorf caps was enclosed with the fungi and sealed in 90 mm petri dishes. Two eppendorf caps were enclosed in each of the petri dishes and incubation continued for an additional 24 h for one set of petri dishes, for 48 h for another set, 96 and 120 h. The experiment was conducted in triplicate. Mycelia which had been exposed to acetaldehyde for 24 h were scrapped and primary metabolites were extracted using a solution of 75:25% (v/v) of methanol and water. The mycelia exposed to acetaldehyde for 24 h were probably the most reactive to acetaldehyde and would therefore show the biggest metabolite profile differences.

The extracts were analyzed using the LCMS-8040 triple quadrupole mass spectrometer (Shimadzu).

**Statistical analysis:** The data was statistically analyzed by Mean  $\pm$  SD and regression analysis.

## RESULTS AND DISCUSSION

Volatile phase acetaldehyde concentrations caused subdued growth and development of *Alternaria alternata* colonies in Carrot Agar in 96 h at 29 °C as shown in Fig. 1. This finding is similar to that of Soylu and Kose<sup>7</sup> who found essential oils to reduce growth of *Botrytis cineria*. The LCMS-8040 triple quadrupole mass spectrometer profiles of primary metabolites showed that isoleucine, leucine, proline, tyrosine and valine production was reduced by acetaldehyde (Fig. 2) and was also co-regulated as shown by

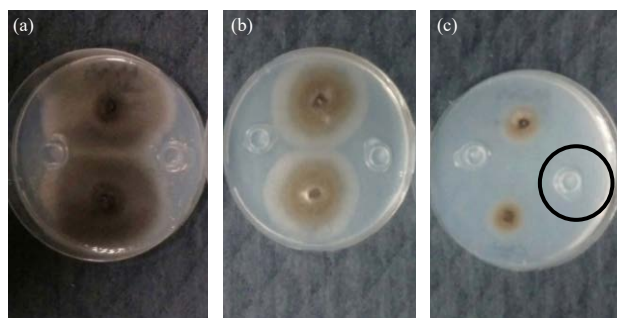


Fig. 1(a-c): Sample cultures of *Alternaria alternata* growing on Carrot Agar observed 96 h after exposure to acetaldehyde, (a) Control (not exposed to acetaldehyde), (b) Exposed to 5% acetaldehyde and (c) Exposed to 10% acetaldehyde  
Black circle shows eppendorf tube cap which contained the solution of acetaldehyde, two caps were included in each plate

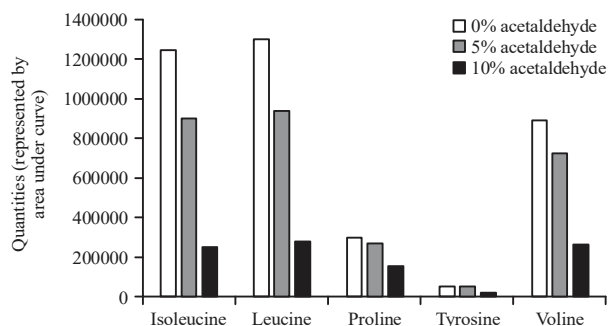


Fig. 2:Quantities of isoleucine, leucine, proline, tyrosine and valine in *Alternaria alternata* mycelia exposed to acetaldehyde for 24 h  
Three observations per treatment were done

Table 1: Correlation matrix of isoleucine, leucine, tyrosine and valine with each other

Compounds	Correlated with	Correlation coefficient
Isoleucine	Leucine	0.99997
Isoleucine	Proline	0.98527
Leucine	Proline	0.983905
Proline	Tyrosine	0.985543
Leucine	Tyrosine	0.939405
Isoleucine	Tyrosine	0.942052
Tyrosine	Valine	0.967996
Proline	Valine	0.996522
Isoleucine	Valine	0.996093
Leucine	Valine	0.995374

The closer to one is the correlation coefficient the greater is the co-regulation of the amino acids with each other

high correlation coefficients between the quantities of the different amino acids in cultures exposed to 0, 5 and 10% acetaldehyde for 24 h (Table 1).

The results in Table 1 showed near perfect correlation of 0.99997 between isoleucine and leucine indicates their strong biochemical relationship. However, it was assumed in a study by Kaback and Miller<sup>8</sup> that the uptake of isoleucine, leucine, tyrosine and valine may be stimulated by a single action of the uptake of D-lactate. This explains the high correlation coefficients between these amino acids. The synchronic increase in the five amino acids was also discovered in Chardonnay grapes infected with the fungi *Phaeoemoniella chlamydospora* or *Phaeoacremonium minimum*<sup>9</sup> and therefore supports the results obtained in this study that the production of these amino acids may be co-regulated.

### CONCLUSION

Apart from inhibiting the growth of *A. alternata* exposure of this fungus to this volatile compound revealed that the production of five amino acids leucine, isoleucine, proline, tyrosine and valine is co-regulated and reduced by acetaldehyde. However, additional work is necessary to ascertain co-regulation of production of these five amino acids so that findings of this preliminary work may be strengthened.

### SIGNIFICANCE STATEMENT

Acetaldehyde is a toxin volatile which reduces the growth of various fungal plant pathogens including *A. alternata*. This

study discovered that apart from inhibiting the growth of *A. alternata*, acetaldehyde reduces the quantities of amino acids, leucine, isoleucine, proline, tyrosine and valine (in a dose-dependent manner) in fungal cells. Moreover, the production of this amino acids was found to be co-regulated in *A. alternata*.

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