

Nitrogen Transformations *in Vitro* by some Soil Yeasts

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Abstract

Candida tropicalis, *Geotrichum capitatum*, *Geotrichum candidum* and *Rhodotorula minuta* were isolated from soils of Saudi Arabia. The ability of these soil yeasts to mediate nitrogen transformations *in vitro* was investigated. An incubation study was conducted to determine the ability of these soil yeasts to nitrify ammonium to nitrate via nitrite in Czapek Dox medium. The yeast of *R. minuta* formed the largest amount of nitrate (94 µg/ml) followed by *G. capitatum* (70 µg/ml). Moderate ammonium oxidation was exhibited by *G. candidum* (55 µg/ml). Ammonium nitrification was highly correlated with yeast biomass formed. The oxidation processes led to a marked reduction in the pH of the medium.

Key words: Nitrification; Yeasts; Biomass; Nitrite; Nitrate; soil.

Introduction

Nitrogen is essential nutrient for plant growth. Nitrogen undergoes a number of transformations in soil which together form the nitrogen cycle. These reactions are largely mediated by microorganisms (Killham, 1986). Nitrogen transformations in soil are integral parts of the overall nitrogen cycle in nature. Microbial activity in soil is important in the cycling of nitrogen. Nitrification in soil involves the oxidation of ammonium, via nitrite, to nitrate (Alexander, 1977). It is generally accepted that the dominant form of nitrification in most soils is chemoautotrophic, largely carried out by the Gram-negative bacteria *Nitrosomonas* and *Nitrobacter*.

Various common heterotrophic soil bacteria, actinomycetes and fungi are capable of oxidizing reduced forms of nitrogen in soil (Eylar and Schmidt, 1959; Killham, 1994, Mekki *et al.*, 2006). The ability of fungi and heterotrophic bacteria to nitrify ammonium has also been demonstrated (Alexander, 1977; Killham, 1986; Atlas and Bartha, 1993, Guest and Smith, 2002). Rezende *et al.*, (2004) mentioned an excellent potential for the use of yeast in the soil as a source of nitrate and available P for plant nutrition.

Although soils are known to contain yeasts, relatively little is known about their ecology and the

role which they play in mineral cycling. This lack of interest probably reflects the low population density and relatively small biomass of yeasts in most soils (Al-Falih and Wainwright 1995a). In fact the only soils which are likely to contain substantial number of yeasts are those receiving large quantities of soluble carbon sources, either naturally or as industrial or agricultural wastes. Al-Falih and Wainwright (1995a) reported that the soil yeast *Williopsis californica* is able to nitrify ammonium and generate nitrate.

The objective of this study is to investigate the role of selected soil yeasts, isolated from Saudi Arabian soil, on ammonium nitrification *in vitro* as an essential prerequisite to determining their role in mediating this transformation in soil.

Materials and Methods

Isolation of soil yeasts

Soil yeasts were isolated from a sandy soil (total C, 0.3%; total N, 0.%; pH, 7.2, obtained from Riyadh, central region, Saudi Arabia). The yeast strains included, *Candida tropicalis* (Cast.) Berkhout (9C), *Geotrichum candidum* Link (15D), *Geotrichum capitatum* (Diddens & Lodder) V. Arx (10A) and *Rhodotorula minuta* (Saito) Harrison var. *texensis* (8B), which were isolated and identified according to

the method described by Van der Walt (1970).

Media

The basal medium used in this study was Czapek-Dox liquid medium for the cultivation of soil yeasts. Suspensions (1 ml) containing 1.4×10^5 yeast cells were used to inoculate liquid Czapek Dox medium (100 ml in 250 ml capacity Erlenmeyer flasks), adjusted to pH 6.0 with 2N NaOH. The medium was amended with ammonium sulphate (final concentration 100 $\mu\text{g/ml}$).

The flasks were incubated with shaking (100 rpm) at 25 °C for 4 weeks. After each week three flasks were removed and the contents filtered through pre-dried and pre-weighed Whatman No.1 filter papers. The weight of yeast cells retained by the filter papers was then determined (after drying to constant weight at 80 °C for 24 h) as a measure of yeast cell biomass. Flasks were set up in triplicate and uninoculated control flasks containing media plus ammonium sulphate were also included to account for any non-biological ammonium nitrification. Ammonium was determined according to the indophenol blue method (Wainwright and Pugh 1973). Nitrite and nitrate were also determined according to the method described by Hesse (1971) and Middleton (1959), respectively. The pH of the medium was determined with a glass electrode.

Results

Certain cultural characteristics of the yeasts are shown in Table (1). As can be seen, all of the yeast isolates could grow on a wide range of carbon and nitrogen sources, but fermented only D-glucose. While both of *G. candidum* and *G. capitatum* were able to ferment cellobiose in addition to D-glucose.

All of soil yeasts tested, with exception of *C. tropicalis*, transformed the ammonium sulphate to nitrate form, while nitrite was only formed transiently in trace amounts towards the end of the incubation period (Fig 1 and 4). *Rhodotorula minuta* was particularly active in this process forming 94 $\mu\text{g/ml}$ of nitrate at the end of the incubation period (Fig 4.) followed by *Geotrichum capitatum* with 70 $\mu\text{g/ml}$ of nitrate (Fig 1). Moderate oxidation of ammonium (55 $\mu\text{g/ml}$ of nitrate) was exhibited by *G. candidum* (Fig

Table 1. The cultural characteristics of the isolated soil yeasts.

Soil Yeasts				
Characteristics	<i>C. tropicalis</i>	<i>G. candidum</i>	<i>G. capitatum</i>	<i>R. minuta</i>
Assimilation				
D-Glucose	+	+	+	+
D-Galactose	-	-	-	-
L-Sorbose	-	+	+	-
Sucrose	+	+	+	+
Maltose	-	-	-	-
Cellobiose	+	+	+	-
Lactose	+	-	-	-
Melibiose	-	-	-	-
Raffinose	-	-	-	-
Melezitose	-	-	-	-
Soluble starch	-	-	-	-
Xylose	-	+	+	-
L-Arabinose	-	-	-	-
D-Ribose	-	-	-	+
Ethanol	-	+	+	-
Glycerol	+	+	+	+
Erythritol	-	-	-	-
Salicin	-	+	+	+
Lactic acid	+	+	+	+
Succinic acid	-	+	+	+
citric acid	+	-	-	-
Ammonium sulphate	+	+	+	+
Potassium nitrate	+	+	+	=
Ethylamine	+	+	+	+
Fermentation				
Maltose	-	-	-	-
D-Glucose	+	+	+	+
D-Galactose	-	-	-	-
Sucrose	-	-	-	-
Cellobiose	-	+	+	-
Lactose	-	-	-	-
Melibiose	-	-	-	-
Raffinose	-	-	-	-
Soluble starch	-	-	-	-

3). However, soil yeast *C. tropicalis* oxidised a slight amount of ammonium sulphate, such that there was no amount of nitrate detected in this treatment (Fig 2).

Figure 5 illustrates the yeasts biomass during nitrification process. The largest amount of biomass

(0.78 g/100 ml) was produced by *R. minuta* in a medium amended with ammonium sulphate (Fig. 5). While, the least amount of biomass was formed by *C. tropicalis* that was 0.2 g per 100 ml. Yeasts biomass were increased with time during ammonium nitrification process. Overall, with exception of *C. tropicalis*, yeasts biomass were found to be correlated with nitrogen ions. Nitrate production were highly correlated with yeasts biomass.

Changes in pH of medium inoculated with the soil yeasts after amendment of ammonium sulphate are shown in Table 2. The largest decline of pH values was observed in a medium inoculated with *R. minuta*, so at the end of incubation period the pH of medium was found to be 3.5. Both of *G. candidum* and *capitatum* decreased the pH of medium from 6.0 to 4.5 and 4.0 respectively after 4 weeks of incubation. The pH of medium decreased as the nitrate formed increased which correlated with biomass production of *G. capitatum*, *G. candidum* *C. tropicalis* and *R.*

Table 2. Changes in medium pH during nitrification by soil yeasts.

Yeast	Incubation period (in weeks)			
	1	2	3	4
<i>C. tropicalis</i>	5.6 ± 0.0	5.0 ± 0.1	5.1 ± 0.5	5.0 ± 0.2
<i>G. candidum</i>	5.3 ± 0.4	5.0 ± 0.2	4.9 ± 0.3	4.5 ± 0.3
<i>G. capitatum</i>	5.6 ± 0.1	5.6 ± 0.3	4.3 ± 0.2	4.0 ± 0.5
<i>R. minuta</i>	4.0 ± 0.5	3.4 ± 0.6	3.5 ± 0.1	3.5 ± 0.0

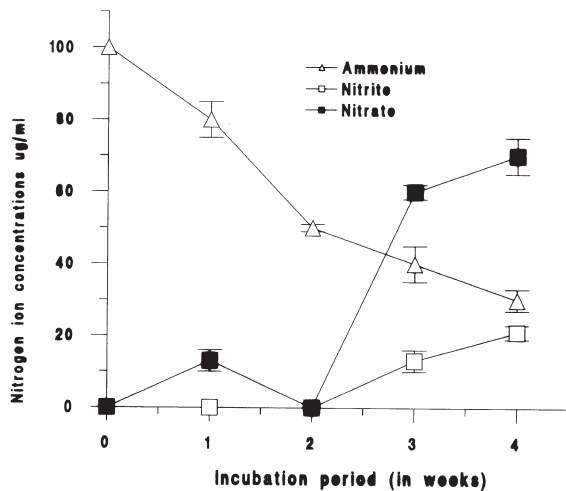


Fig 1. Ammonium nitrification by *Geotrichum capitatum*.

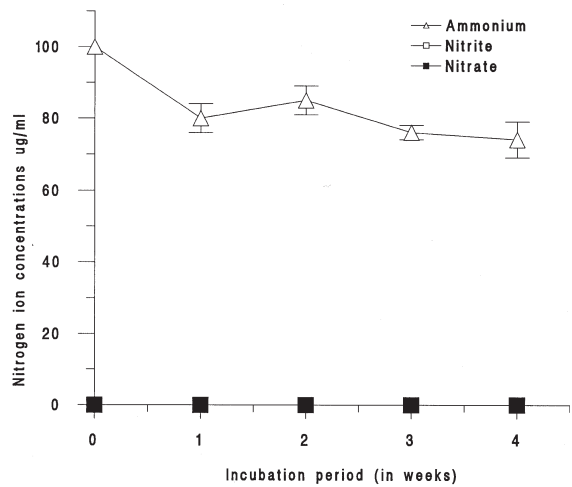


Fig 2. Ammonium nitrification by *Candida tropicalis*.

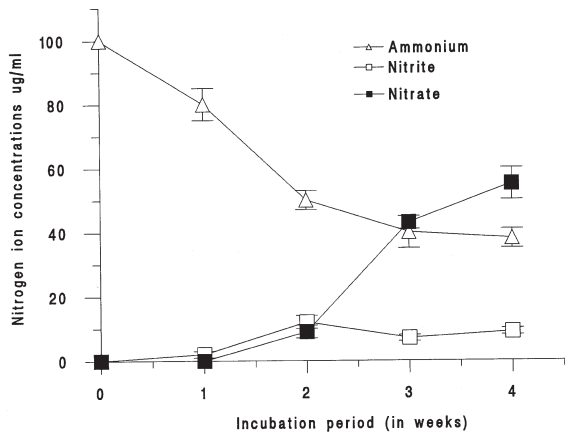


Fig 3. Ammonium nitrification by *Geotrichum candidum*.

minuta increased.

On the other hand nitrification process was highly correlated with yeast biomass formed (Fig 6). When the amount of nitrate produced is expressed in relation to biomass production however, *G. candidum* appeared to be the most active nitrifier followed by *G. capitatum* and *R. minuta*, respectively (Fig 6).

Discussion

The soil yeasts isolated from Saudi Arabian soils were identified as *Candida tropicalis*, *Geotrichum candidum*, *Geotrichum capitatum*, *Rhodotorula*

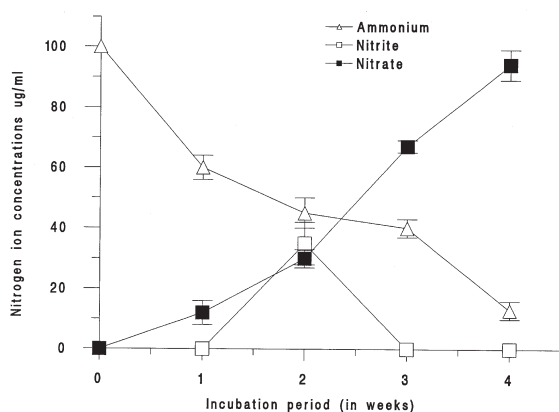


Fig 4. Ammonium nitrification by *Rhodotorula minuta*.

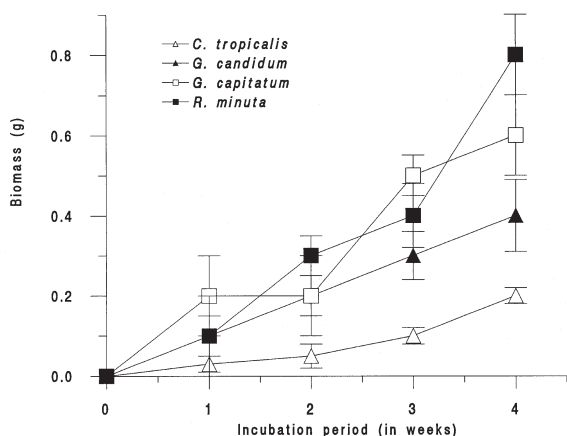


Fig 5. Biomass of the soil yeasts during nitrification.

minuta. On broth, colonies of the yeasts were cream in colour, with exception of *R. minuta* that was pink, and formed a white climbing pellicle and ring with a non-flocculent deposit. Colonies growing on potato dextrose agar were creamy white and shiny smooth; *pseudomycelium* was formed.

The soil yeasts isolated oxidized ammonium sulphate in vitro leading to the formation of large amounts of nitrate. *C. tropicalis*, oxidised a slight amount of ammonium sulphate and failed to form nitrate.

The role of yeasts in biological heterotrophic nitrification has been neglected, for example in reviews, by Eylar and Schmidt (1959); Alexander (1977) and Killham (1986), of heterotrophs capable of participating in the process no mention is made of yeasts. However, this study clearly showed that at least three soil yeasts can achieve substantial rates of

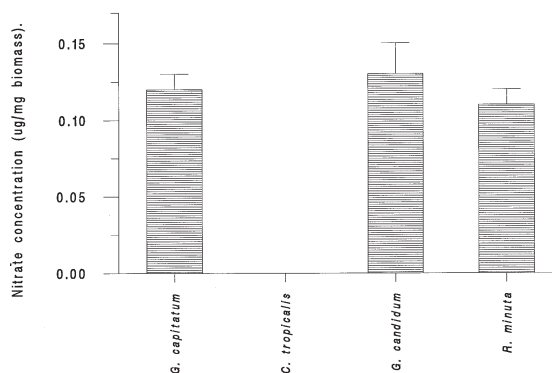


Fig 6. Nitration (net nitrate production) per mg biomass of yeasts over the 4 weeks incubation period.

nitrification *in vitro*.

However, in all yeasts tested the concentration of nitrite ions never exceeded the concentration of nitrate formed, consistent with the findings of previous studies (Al-Falih and Wainwright 1995a, Al-Falih and Wainwright 1995b). Results showed a small amount of nitrite in all yeasts with some exception. The nitrite was totally absent from the *C. tropicalis* yeast treatment. Nitrite ion is usually considered to be intermediates and rarely exceed the concentration of nitrate. As a result, nitrite ion was only formed transiently in trace amounts towards the end of the incubation period.

In our previous study we found that the soil yeast *Williopsis californica* and *Saccharomyces cerevisiae* oxidized added ammonium forming 69 $\mu\text{g/ml}$ and 58 $\mu\text{g/ml}$ of nitrate respectively (Al-Falih and Wainwright 1995a). Therefore, it appears that both of *Rhodotorula minuta* and *Geotrichum capitatum* used here are highly effective in nitrifying ammonium. But when the amount of nitrate produced is expressed on the basis of ammonium oxidized per weight of yeast biomass however, *G. candidum* appeared to be the most active nitrifier.

It is difficult, using the currently available literature, to make meaningful comparisons of the relative activity of fungi in the processes studied here. This is because such reports are based on studies employing various growth media and incubation conditions. As a generalization however, it appears that the four yeasts used here are less effective at nitrifying (Wainwright and Grayston, 1987) *in vitro* than are filamentous

fungi such as species of *Aspergillus*, *Fusarium* and *Penicillium*.

Amendment of Czapek Dox medium with ammonium sulphate caused a marked decline in pH values in all cases that due to the formation of organic acids. Other workers reported that the medium pH fell to a lower value following ammonium amendment (Al-Falih and Wainwright, 1995b; Wainwright and Grayston, 1987). However no significant reduction in pH of *C. tropicalis* and *R. minuta* yeasts occurred as there was a small amount of biomass.

Results showed that yeasts are capable of oxidizing added ammonium sulphate using Czapek Dox as a nutrient source. Of particular interest is the role of yeasts in the process, since in the past only the role of mycelial fungi in nitrification has been emphasised. It is also noteworthy that a yeast of *C. tropicalis* failed to nitrify added ammonium. This observation could be useful in elucidating the biochemistry of fungi and yeasts nitrification, since it would be interesting to determine what biochemical features this yeast lack when compared with actively nitrifying fungi and yeasts.

In conclusion, the soil yeasts *Candida tropicalis*, *Geotrichum candidum*, *Geotrichum capitatum* and *Rhodotorula minuta* oxidized ammonium to nitrate. While such in vitro studies do not provide direct evidence that soil yeasts can mediate these processes when growing in soil, they indicate that yeasts have the potential to participate in such nutrient transformations. Although these *in vitro* results are of interest, they probably have little bearing on the relative nitrification ability of yeasts when growing in soil, rather than culture.

Since most environment contain only small amounts of carbon, it is unlikely that the rates of nitrification observed using Czapek Dox medium would be relevant to nitrification in, for example, soil. However, *in vitro* studies such as this do at least indicate the potential ability of yeasts to nitrify ammonium sulphate; a yeast which is incapable of nitrifying *in vitro* is unlikely to do so in the environment.

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تحويلات النيتروجين في المختبر بواسطة بعض خمائر التربة

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ملخص

في هذا البحث تم جمع عينات التربة من المملكة العربية السعودية وعزلت الخمائر التالية: *Candida tropicalis*, *Geotrichum capitatum*, *Geotrichum candidum* and *Rhodotorula minuta*. أجريت تجربة معملية لدراسة دور خمائر التربة في تحولات النيتروجين. حيث تم تقدير تحولات الأمونيوم إلى نيتريتات ثم إلى نترات بواسطة هذه الخمائر. وجد في هذه الدراسة ان أكبر كمية نترات (٩٤ ميكروجرام/مل) تكونت بواسطة الخميرة *Rhodotorula minuta*. وتليها خميرة *Geotrichum capitatum* (٧٠ ميكروجرام/مل) بينما لوحظ أكسدة الأمونيوم بمعدلات متوسطة عند استخدام خميرة *Geotrichum candidum* والتي بلغت ٥٥ ميكروجرام/مل في نهاية فترة التحضين. تشير الدراسة إلى أن حدوث عملية التآزت للأمونيوم مرتبطة بدرجة كبيرة بكمية الكتلة الحيوية المتكونة للخمائر. كما أدت تحولات النيتروجين إلى انخفاض في درجة حموضة الوسط البيئي.