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Effect of environmental factors (glucose, nitrate and nitrite) on the nitrite producing activity of oral *Actinomyces* species

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Introduction

Actinomyces sp.
Schaalia sp.
Veillonella sp.
Neisseria sp.
Streptococcus sp.
Rothia sp.

NO_3^- → NO_2^-

Contribution to Maintenance of health

Nitrates (NO_3^-) are readily taken into the oral cavity from foods such as green leafy vegetables, and some of the ingested nitrate is continuously secreted as salivary components. Some oral bacteria have the ability to reduce nitrate to produce nitrite (NO_2^-). Nitrite produced by oral bacteria is known to contribute to the maintenance of overall health by inhibiting the growth and metabolism of pathogenic bacteria such as *Streptococcus mutans*, *Porphyromonas gingivalis* and by normalizing blood pressure. Dominant bacteria of oral nitrite producers are *Actinomyces* species. The aim of this study was to elucidate nitrite-production and growth of oral *Actinomyces* species.

Material and methods

Actinomyces naeslundii

TYG(＋)
1.7% tryptone
0.3% yeast extract
0.5% NaCl
0.5% glucose
0.1% NH4HCO3
50 mM PPB(pH7.0)

With or without
1 mM potassium nitrate
or 0.1 mM potassium nitrite

Anaerobic culture at 37° C

Bacterial cell was collected, washed and resuspended in Wash Buffer

Adjust to OD at 660 nm = 10.0

Wash Buffer
75 mM KCl
5 mM MgCl2
75 mM NaCl
in 2 mM PPB(pH 7.0)

Reaction mixture

Bacterial cell suspension
(final OD=1 at 660 nm)
KNO_3
(final concentration :1 mM)
PPB (pH 7)
(final concentration :40 mM)
＋
environmental factors
(glucose / sodium lactate)

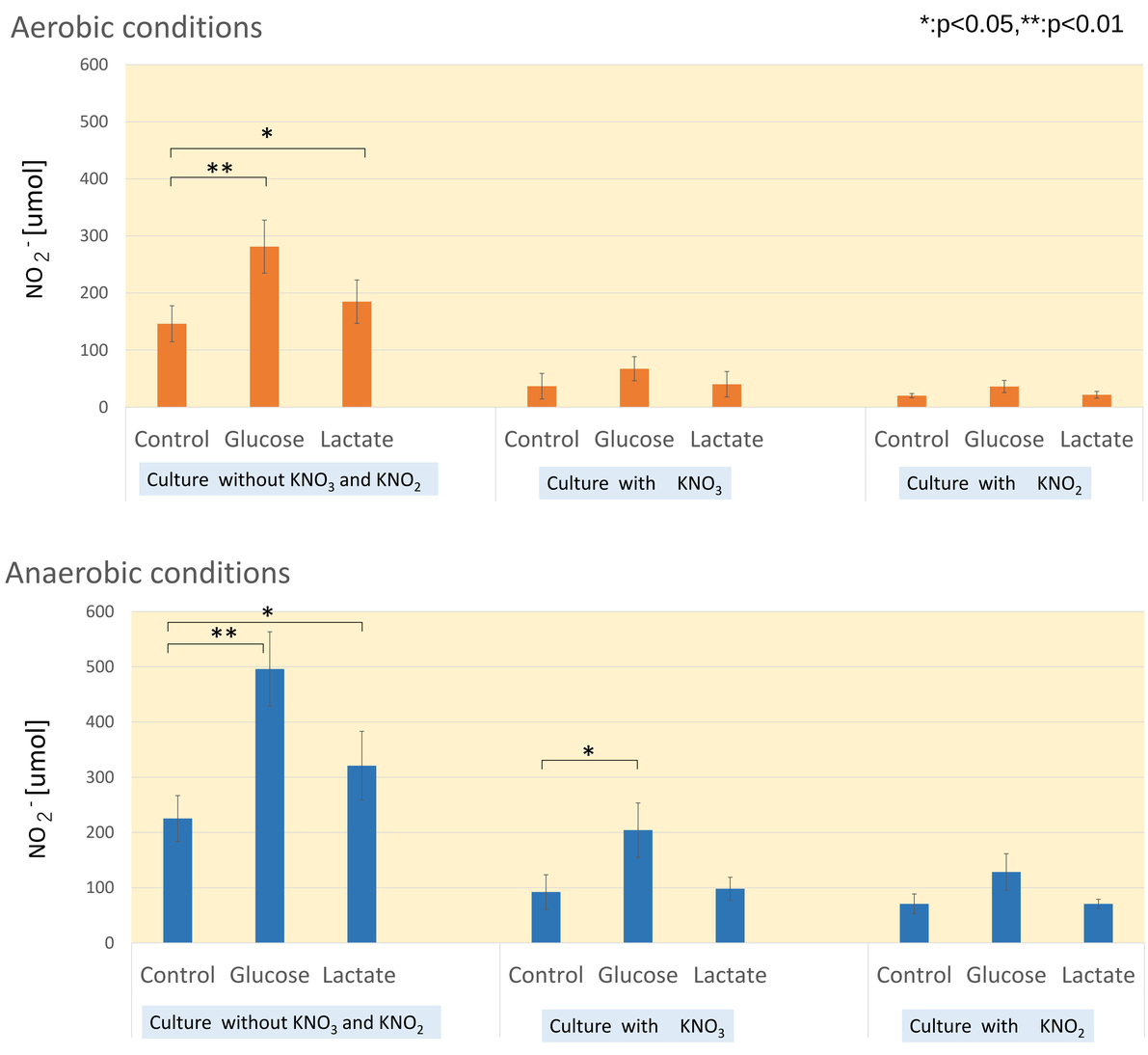
Incubation aerobically/anaerobically at 37°C for 30 min

Centrifugation at 10,000 rpm for 3 minutes at 4° C.

NO_2^- production in the supernatant was measured with Griess reagent kit.

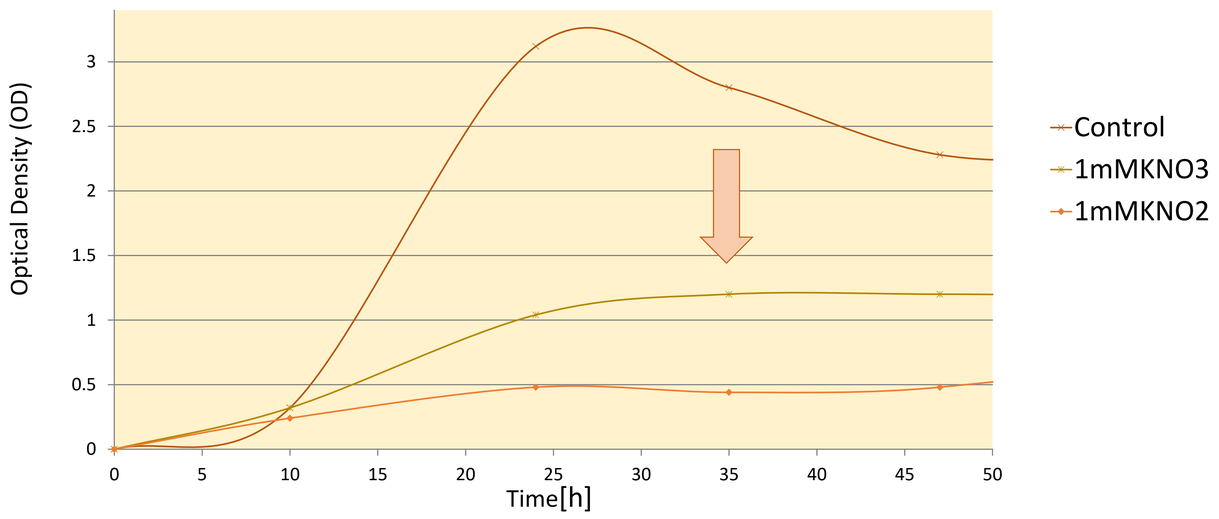
Results

Fig.1 NO_2 production activity of *Actinomyces naeslundii*



The activity of nitrite production (Fig.1) was increased in anaerobic conditions compared to aerobic conditions. The addition of glucose or lactate to the reaction mixture significantly increased the activity in both cases compared to the control. Nitrate or nitrite in the growth medium significantly decreased the activity of nitrite production. Nitrite was more effective than nitrate.
In a previous study, nitrite-producing activity of *Veillonella* species was increased by nitrate or nitrite in the growth medium, which was opposite to the present results.

Fig.2 Bacterial growth curve



Bacterial growth curve (Fig.2) showed that potassium nitrate (KNO_3) and potassium nitrite (KNO_2) inhibited bacterial growth, even though *Actinomyces naeslundii* produces nitrite.

Discussion

Our results show that not only glucose but also lactate that is produced by the bacterial carbohydrate metabolism increased nitrite producing activity, and that this activity is further enhanced under anaerobic conditions and decreased when grown in the presence of nitrate or nitrate.
These characteristics are totally opposite to those of oral *Veillonella*, another dominant nitrite producer, suggesting that oral *Actinomyces* can utilize reducing power derived from both glucose and lactate, and reduce nitrate to nitrite, which feeds back negatively to inhibit nitrite-producing enzymes.