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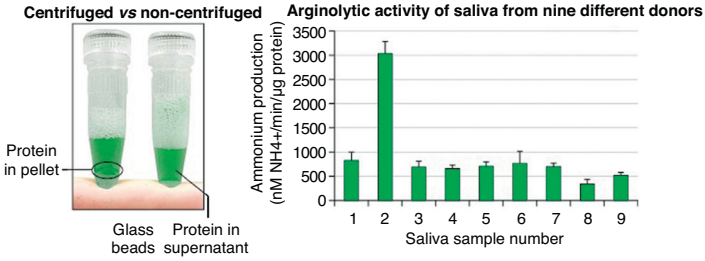
Determination of arginine catabolism by salivary pellet☆

M.A. Hoogenkamp *, J.M. ten Cate

Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Free University Amsterdam, Department of Preventive Dentistry, Gustav Mahlerlaan 3004, 1081 LA Amsterdam, The Netherlands

GRAPHICAL ABSTRACT



ABSTRACT

To determine the formation of ammonium from arginine by oral bacteria residing in saliva and dental plaque, an arginolytic activity assay based on the work described by Nascimento et al. [2] was developed. Following the original methodology, insufficient ammonium production could be determined.

To improve the method for our research goal, the following modifications were made to the original protocols:

- The following changes were made to the arginine catabolism assay resulting in a 1000-fold increase in sensitivity: (i) the salivary pellet was washed and concentrated five times resulting in the removal of low density compounds interfering with the assay, (ii) the pH of the Tris–maleate buffer was increased from 6.0 to 7.5 resulting in a better conversion of arginine to ammonium and (iii) the incubation time was increased to 3 h to ensure that non-responders and salivary pellets low in cell numbers could yield detectable levels of ammonium.
- Removal of a centrifuge step from the protein determination resulted in a higher protein yield improving the accuracy of the assay.
- Changing from the use of the toxic, environmentally hazardous, mercury containing Nessler's reagent to a colorimetric enzyme assay achieved a safer and greener determination of ammonium concentration.

* Corresponding author.
E-mail address: M.Hoogenkamp@acta.nl (M.A. Hoogenkamp).

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Method details

In short, the assessment of the arginolytic potential involved the following steps: (i) preparation of the salivary pellet to concentrate the oral bacteria present in the saliva; (ii) performing the arginolytic activity assay to assess the arginine catabolism; (iii) determination of the amount of ammonium produced from arginine; and (iv) determination of the protein weight of the salivary pellet. The final concentrations of ammonium were corrected per time unit and protein weight.

Preparation of the cell suspension

While optimizing the arginolytic activity assay, the pH of the Tris–maleate buffer was raised from pH 6.0 to pH 7.5. This was done as the arginolytic activity of a range of oral micro-organisms and the salivary pellet significantly produced more ammonium under pH 7.5 as compared to pH 6.0. The cell suspensions were prepared as follows:

1. Stimulated human saliva from various donors was thawed.
2. Four milliliter of each saliva sample was centrifuged for 10 min at 4000 × *g* at 4 °C.
3. The salivary pellet was washed once with 800 μl 10 mM Tris–maleate buffer, pH 7.5 (Sigma) to remove low density salivary components.
4. The pellet was re-suspended in a final volume of 800 μl Tris–maleate buffer.

Arginolytic activity assay

In comparison to the original protocol the incubation time was lengthened to three hours. This was done to ensure that ammonium formation, in salivary pellets, low in cell amounts and saliva from non-responders, was still measurable.

To assess the arginolytic activity of the prepared cell suspensions, the following protocol was followed:

1. Of each cell suspension 237.5 μl was pipetted, in duplicate, into a non-skirted 96-well PCR plate (Corning) and pre-incubated at 37 °C in a thermocycler (Eppendorf).
2. The remaining cell suspension was stored at −20 °C for protein determination.
3. The arginolytic activity assay was started by adding 12.5 μl 1 M arginine to a final concentration of 50 mM (Sigma) to each cell suspension.
4. The plate with the cell suspensions was incubated at 37 °C for 3 h.
5. Immediately after the addition of the arginine, and after the three-hour incubation period, 50 μl samples were taken, transferred to a PCR plate (VWR) and placed on ice for 5 min.
6. After cooling, the samples were heat inactivated for 5 min at 80 °C, to stop all enzymatic reactions.
7. The PCR plate was centrifuged for 10 min at 1509 × *g* at 4 °C.
8. The supernatants were transferred to a microtiter plate (Greiner), the plate was sealed and stored at −20 °C until further analysis.

Ammonium determination

To determine the amount of ammonium produced from the catalysis of arginine, an assay was used as based on the method of Da Fonseca et al. [1].