Body odour aldehyde reduction by acetic acid bacterial extract including enzymes: alcohol dehydrogenase and aldehyde dehydrogenase

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Abstract

OBJECTIVE: The major causes of unpleasant human body odour are aldehydes produced by axillary-resident bacteria. There are many methods of body odour prevention; however, they all carry risks of destroying indigenous dermal bacteria that are necessary for the maintenance of the normal physical function of the skin. Furthermore, some methods cannot directly reduce the concentrations of substances that cause body odour. Therefore, a novel method of reducing body odour more safely and effectively is required. We focused on acetic acid bacterial enzymes, which can convert aldehydes into carboxylic acids, and investigated their effect on aldehydes and body odour.

METHODS: Subjects with strong body odour were recruited using screening questionnaires. Acetic acid bacterial extract including enzymes was applied to subjects’ skin, and their effects were evaluated by trained panellists and by quantitative aldehyde analysis using thermal detector gas chromatography/mass spectrometry.

RESULTS: Acetic acid bacterial extract including enzymes decreased the ratio of dilution to threshold and the concentration of body odour-producing aldehydes by up to 98.7%.

CONCLUSION: These results indicate that simply applying acetic acid bacterial enzymes on the skin can reduce the concentration of aldehydes that cause unpleasant body odour by directly converting them into carboxylic acids. Therefore, acetic acid bacterial enzymes can potentially be developed into new products that do not destroy indigenous bacteria and yet can effectively reduce unpleasant body odour.

Résumé

OBJECTIF: Les principales causes des odeurs corporelles humaines désagréables sont les aldehydes produits par les bactéries résidant sous les aisselles. Il existe de nombreuses méthodes de prévention des odeurs corporelles; cependant, elles prétendent toutes des risques de destruction des bactéries dermiques indigènes qui sont nécessaires au maintien de la fonction physique normale de la peau. En outre, certaines méthodes ne peuvent pas directement réduire les concentrations des substances à l’origine des odeurs corporelles. Par conséquent, une nouvelle méthode visant à réduire les odeurs corporelles de manière plus sûre et efficace est nécessaire. Nous nous sommes concentrés sur les enzymes bactériennes de l’acide acétique, qui peuvent convertir les aldehydes en acides carboxyliques, et nous avons étudié leurs effets sur les aldehydes et les odeurs corporelles.

MÉTHODES: Des sujets ayant une forte odeur corporelle ont été recrutés à l’aide de questionnaires de sélection. Un extrait d’acide acétique provenant de bactéries comprenant des enzymes a été appliqué sur la peau des sujets, et leurs effets ont été évalués par des experts qualifiés et par analyse quantitative des aldehydes par chromatographie en phase gazeuse/spectrométrie de masse à détecteur thermique.

RÉSULTATS: L’extrait d’acide acétique provenant de bactéries comprenant des enzymes a diminué le rapport de dilution jusqu’au seuil et la concentration d’aldehydes produisant les odeurs corporelles a chuté de 98,7 % au maximum.

CONCLUSION: Ces résultats indiquent que le simple fait d’appliquer des enzymes bactériennes de l’acide acétique sur la peau peut réduire la concentration des aldehydes à l’origine des odeurs corporelles désagréables en les convertissant directement en acides carboxyliques. Par conséquent, les enzymes bactériennes de l’acide acétique peuvent être potentiellement développées dans des nouveaux produits qui ne détruisent pas les bactéries indigènes et qui peuvent efficacement réduire les odeurs corporelles désagréables.

Introduction

Body odour is mainly caused by secreted sweat. Although sweat is almost odourless immediately after secretion, decomposition or denaturation of components contained in sweat by bacteria on the skin surface contributes to unpleasant body odour. Body odour is because of various substances and aldehydes are primarily detected in body odour [1–4]. A gas chromatographic/mass spectrometric analysis of changes in body odour components that occur with ageing revealed that 2-nonenal significantly increased above the age of 40 [5]. Furthermore, Seya et al. [6] showed that octanal and nonanal also formed body odour components. These aldehydes are produced by the oxidative degradation of fatty acids found in sebum [7, 8]. Therefore, we focused on aldehydes as the main cause of body odour in this study.

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Body odor reduction by acetic acid bacteria

N. Yoshioka et al.

Body odor greatly affects the first impression and there has been much consumer demand for controlling of body odor. There have been already some effective approaches for reducing body odor: (1) decreasing the number of bacteria on the skin surface using products that contain antimicrobial ingredients, (2) inactivating odor components with deodorant and (3) masking odor with fragrances [4]. However, the approach (1) has a possibility of destroying beneficial indigenous bacteria needlessly of people whose skin is sensitive that is necessary for the maintenance of the normal physical condition. Furthermore, the approach (3) does not directly reduce the levels of the actual substances that cause body odor and so does not address the root cause of the problem. Therefore, there currently exists a market demand for the development of better methods and products for the safe and effective reduction of body odor and we found a novel method with acetic acid bacterial enzymes that suppress body odor both safely and effectively suppress body odor.

Materials and methods

Acetic acid bacterial extract including enzymes

Acetic acid bacteria (Glucanacetobacter hansenii) were added to a medium consisting mainly of D (+)-glucose (Wako Pure Chemical Industries, Tokyo, Japan), yeast extract BSP (Oriental Yeast, Tokyo, Japan) and ethanol (99.5; Wako Pure Chemical Industries, Tokyo, Japan) and were cultured at 30°C with aeration. After cooling, the bacterial suspension was centrifuged for 10 min at 20,000 g and 5°C. The supernatant was discarded and the bacterial pellet collected and weighed. Citrate buffer solution (pH 5) was added ten times the weight of this pellet, and centrifugation was performed as previously described. After repeating this procedure twice, the final pellet was freeze-dried and used to produce acetic acid bacterial suspensions when needed.

Table 1  Thermal detector gas chromatography/mass spectrometry analytical conditions for aldehyde concentration determination in the preliminary and clinical tests

| Parameters | Preliminary test | Clinical test |
|------------|------------------|---------------|
| Thermal detector | UNITY Series 2 (MARKES, Llantrisant, U.K.) | | |
| Desorption temperature | 200°C | |
| Cold trap temperature | –15°C | |
| Gas chromatography/mass spectrometry system | 7890N/5975MSD (Agilent Technologies, CA, U.S.A.) | |
| Injector temperature | 250°C | |
| Carrier gas | He | |
| Gas chromatography column | DB-1301 (0.25 mm, 60 m, 1 μm) | HP-1 (0.25 mm, 60 m, 1 μm) |
| Column flow rate | 1.2 mL min⁻¹ | |
| Temperature programme | 40°C (5 min) → 5°C min⁻¹ (100°C) → 10°C min⁻¹ (290°C) → 290°C (1.5 min) | 40°C (5 min) → 5°C min⁻¹ (100°C) → 10°C min⁻¹ (290°C) → 290°C (1.5 min) |
| Mass spectrometry detector type | Mass spectrometer | |
| Mass spectrometry source temperature | 230°C | |
| Electron energy | 70 eV | |

Preliminary test

1 mL of acetic acid bacterial suspension [0.001 g mL⁻¹ acetic acid bacterial extract powder in 0.1% (w/v) saline] or 1 mL of 0.1% (w/w) saline alone was placed in two glass vials and 5 μL of 0.5% (v/v) acetaldehyde or 1 μL of 0.5% (v/v) 2-nonenal standard solution was added to both vials, respectively. Vials were heated for 10 min at 40°C and 2 mL of the headspace gas was collected from the void of glass vial with a gas-tight syringe (SGE Analytical Science, Melbourne, Vic., Australia). Acetaldehyde and 2-nonenal concentrations were determined by thermal detector gas chromatography/mass spectrometry (TD-GC/MS) and analytical conditions are shown in Table I.

Clinical test

Subjects

Questionnaires were given to men over the age of 40, and five subjects with strong body odor were recruited. After informing...
participants of the study purpose, procedures and risks, they signed consent forms. This study complied with the provisions of the Declaration of Helsinki and approved by the ethical committee of the medical corporation association of Shinkohkai, on 17 August 2015.

**Experimental design**

Subjects refrained from eating fatty foods and exercise for 1 day prior to the study. They then performed the following procedure. Upper bodies were first wiped with wet towels to remove sweat. After spraying 2–5 mL of water onto their underarms, chests and backs (from where body odour primarily emanates), they changed into T-shirts (GUNZE, Osaka, Japan) that had no odour components because of treatment with 50% ethanol [5]. Subjects then took two glucose tablets (Kabaya, Okayama, Japan) with 200 mL of hot water and rested for 30 min. After exercises (i.e. stressful calculations) were performed to promote sweating, T-shirts were placed in polyester bags for analysing odour (control samples). Subjects then rested for 30 min and then again wiped their upper bodies with wet towels. Next, the same test was conducted, but instead of water, 2–5 mL of the spray contained the acetic acid bacterial extract suspension [12% xanthan gum (DSP Gokyo Food & Chemical Co., Ltd., Osaka, Japan), 0.12% alpha corn starch (Tate & Lyle Food, PLC, London, U.K. & Industrial Ingredients, IN, U.S.A.) and 0.05% (w/w) acetic acid bacterial extract powder] (test samples). The sealed bags containing the T-shirts were filled with approximately 8.0 L of air and heated for 30 min at 40°C. Approximately 2.5 L of this air was collected in a thermal desorption tube for aldehyde concentration determination by TD-GC/MS. The remaining air in the bag was transferred to a fresh bag for sensory evaluation.

**Sensory evaluation**

The measurement of the ratio of dilution to threshold (i.e. odour concentration) was conducted using the triangle odour bag method [10], and odour evaluation was conducted blind by six trained olfactory panelists.

**Results**

**Preliminary tests**

The concentrations of acetaldehyde and 2-nonenal decreased by >99% in the presence of acetic acid bacterial extract including enzymes (Table II).

**Clinical tests**

Perceived odours in sensory evaluations decreased in the test samples for all subjects compared with the control samples. The control samples exhibited unpleasant odours, such as those associated with straw, stink bugs and sweat peculiar to medium-chain aldehydes, whereas these odours were seldom detected in the test samples (Table III).

Furthermore, the total concentrations of the five aldehydes (2-nonenal, n-hexanal, n-heptanal, n-octanal and n-nonanal) in control and test samples of five subjects (a, b, c, d and e) were determined by TD-GC/MS.

**Conclusion**

The results from this study suggest that acetic acid bacteria can decrease human body odour with respect to both sensory

| Table II | Measurements of acetaldehyde and 2-nonenal concentrations |
|----------------|---------------------------------|
| **Conc. (ppmv)** | **Reduction (%)** | **Conc. (ppmv)** | **Reduction (%)** |
| 0.1% saline | 23.7 | n/a | 13.8 | n/a |
| Acetic acid bacterial suspension | Below detection | >99 | 0.1 | >99 |

| Table III | Sensory evaluation of odour |
|----------------|-----------------------------|
| **Control samples** | **Test samples** |
| Unpleasant odours such as straw, stink bugs and sweat peculiar to medium-chain aldehydes | Few unpleasant odours |

**Figure 2** The total concentrations of five aldehydes (2-nonenal, n-hexanal, n-heptanal, n-octanal and n-nonanal) in control and test samples of five subjects (a, b, c, d and e) were determined by TD-GC/MS.
evaluation and aldehyde concentrations. This demonstrates that it is possible to directly degrade body odour-causing aldehydes by application of acetic acid bacterial enzymes to the skin surface. These findings may contribute to the development of improved methods for the safe and effective reduction of body odour.

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